

**Evaluation of Ozone Treatment, Pilot-Scale Wastewater  
Treatment Plant, and Nitrogen Budget for Blue Ridge  
Aquaculture**

by

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# **Evaluation of Ozone Treatment, Pilot-Scale Wastewater Treatment Plant, and Nitrogen Budget for Blue Ridge Aquaculture**

Simonel I. Sandu

(ABSTRACT)

Sustainable tilapia production at Blue Ridge Aquaculture (BRA) is constrained by availability of high quality replacement water. I developed a pilot-scale wastewater treatment system to treat and reuse effluent presently discharged. An initial study was conducted to determine the response of the BRA waste stream to ozone application. Dosages of 6.9, 4.8 and 2.4 g O<sub>3</sub> were applied for 30 minutes to 35 L of settled effluent. Optimum ozone dosage and reaction time, ozone transfer efficiency, ozone yield coefficient, degree of pollutant removal, and other ozone and water quality parameters were determined. Most results suggested that the maximum process feasibility limit for ozone contact time was approximately 9 minutes at an applied ozone concentration of 23g/m<sup>3</sup> (6.9 g O<sub>3</sub> dose). Formation of foam increased solids and COD removal up to three times. Poor removal or accumulation of DOC and TAN was observed, indicating the need for biological treatment following ozonation.

Next, I evaluated a pilot station treatment train including sedimentation, microscreen filtration, fluidized bed denitrification, ozonation, aerobic biological oxidation in a trickling filter, and jar-test chemical flocculation. Significant improvements were found regarding solids, COD, cBOD<sub>5</sub>, NO<sub>3</sub><sup>-</sup>-N, TKN, and turbidity. Removal of foam after ozonation improved ozonation efficacy and pollutant removal.

A nitrogen budget for the BRA facility was derived, indicating that 35% of the nitrogen applied in feed was assimilated in fish. I evaluated the possible impact of residual inorganic nitrogen forms from treated effluent upon fish in the recirculating systems. I found that less than 1% of the TAN produced would return the recovered stream, and that the existing biological contactors can remove it. Evaluation of TAN fate indicated that 84% was oxidized in biofilters, 14% was oxidized by passive nitrification,

and 1% was removed by water exchange. For  $\text{NO}_3\text{-N}$ , I determined that 56% was removed by passive denitrification and 44% by daily water exchange.

The pilot station design was effective for removing organics and nutrients, and can serve as the basis for scale-up for treating and reusing the entire BRA effluent stream.

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## Glossary

- $A^*$  = specific ozone adsorption ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )
- ACR = areal conversion rate ( $\text{mg m}^{-2} \text{ day}^{-1}$ )
- BRA = Blue Ridge Aquaculture
- BS = broodstock subsystem
- $C$  = concentration ( $\text{mg L}^{-1}$ )
- $C_0$  = initial concentration ( $\text{mg L}^{-1}$ )
- cBOD<sub>5</sub> = carbonaceous biological oxygen demand ( $\text{mg L}^{-1}$ )
- CF = chemical flocculation
- $C_{G_e}$  = effluent gas concentration ( $\text{mg L}^{-1}$ )
- $C_{G_0}$  = influent gas concentration
- $C_L$  = liquid concentration (in reactor) ( $\text{mg L}^{-1}$ )
- $C_{L_e}$  = effluent liquid concentration ( $\text{mg L}^{-1}$ )
- $C_{L_0}$  = influent liquid concentration ( $\text{mg L}^{-1}$ )
- $c(M)_e$  = effluent pollutant concentration ( $\text{mg L}^{-1}$ )
- $c(M)_0$  = influent pollutant concentration ( $\text{mg L}^{-1}$ )
- COD = chemical oxygen demand ( $\text{mg L}^{-1}$ )
- DOC dissolved organic carbon ( $\text{mg L}^{-1}$ )
- DR = denitrification reactor
- DRi = denitrification reactor influent (mixing basin)
- F = foam
- FBBR = fluidized bed biological reactor
- FDS = fixed dissolved solids ( $\text{mg L}^{-1}$ )
- FE = final effluent, after chemical flocculation
- $F(O_3)$  = ozone dose or feed rate ( $\text{mg L}^{-1} \text{ s}^{-1}$ )
- FS<sub>1</sub> = fingerlings subsystem 1
- FS<sub>1</sub> = fingerlings subsystem 1
- FSS = fixed suspended solids ( $\text{mg L}^{-1}$ )
- FTS = fixed total solids ( $\text{mg L}^{-1}$ )
- HS = hatchery subsystem
- $I^*$  = specific ozone dose or input ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )

$k$  = half order reaction rate coefficient ( $\text{mg}^{-1/2} \text{L}^{1/2} \text{min}^{-1}$ )  
 $k'$  = pseudo-first order reaction rate coefficient ( $\text{h}^{-1}$ )  
 $L_N$  = nitrogen loading to the water ( $\text{g N kg}^{-1} \text{fish}$ )  
 MB = mixing basin  
 MSe = microscreen filter effluent  
 MSF = microscreen filter  
 NTU = nephelometric turbidity units  
 $\text{NO}_3^- \text{-N}$  = nitrate-nitrogen ( $\text{mg L}^{-1}$ )  
 $\text{NO}_2^- \text{-N}$  = nitrite-nitrogen ( $\text{mg L}^{-1}$ )  
 $\text{OH}^\circ$  = hydroxyl radical  
 OR = ozonation reactor  
 ORe = ozonation reactor effluent (stripping chamber influent)  
 ORi = ozonation reactor influent (denitrification reactor effluent)  
 $P_{TAN}$  = ammonia production ( $\text{g N kg}^{-1} \text{fish}$ )  
 $Q_G$  = gas flow rate ( $\text{L s}^{-1}$ )  
 $r_A(\text{O}_3)$  = ozone absorption rate ( $\text{mg L}^{-1} \text{s}^{-1}$ )  
 $r(M)$  = pollutant removal rate ( $\text{mg L}^{-1} \text{s}^{-1}$ )  
 $r(\text{NO}_x\text{-N})$  = reduction rate of  $\text{NO}_3^- \text{-N} + \text{NO}_2^- \text{-N}$   
 $r(\text{O}_3) = r_L$  = ozone consumption rate ( $\text{mg L}^{-1} \text{s}^{-1}$ )  
 SB = sedimentation basin  
 SBe = sedimentation basin effluent  
 SBi = sedimentation basin influent (raw influent)  
 SC = ozone stripping chamber  
 SMF = sludge from microscreen filter  
 SS = settleable solids  
 SSB = sludge from sedimentation basin  
 TDS = total dissolved solids ( $\text{mg L}^{-1}$ )  
 $t_e$  = final time (s)  
 TF = trickling filter  
 TFe = trickling filter effluent  
 TFi = trickling filter influent (stripping chamber effluent)

$t_H$  or  $t$  = hydraulic retention time (min)  
TKN = total Kjeldahl Nitrogen  
 $TNI$  = total nitrogen input (kg)  
 $TNR$  = total nitrogen recovered  
 $TNUA$  = total nitrogen unaccounted for (kg)  
TOC = total organic carbon ( $\text{mg L}^{-1}$ )  
 $t_r$  = reaction time (s) =  $n$   
TS = total solids ( $\text{mg L}^{-1}$ )  
TSS = total suspended solids ( $\text{mg L}^{-1}$ )  
 $t_0$  = initial time (s)  
 $V_L$  = liquid volume ( $\text{m}^3$ )  
VDS = volatile dissolved solids ( $\text{mg L}^{-1}$ )  
VSS = volatile suspended solids ( $\text{mg L}^{-1}$ )  
VTS = volatile total solids ( $\text{mg L}^{-1}$ )  
 $Y(O_3/M)$  = ozone yield coefficient ( $\text{g O}_3 \text{ g}^{-1} \Delta M$ )  
 $Y_{COD}$  = biological yield with regard to COD  
 $Y_{NO_3^- - N}$  = biological yield with regard to  $\text{NO}_3^- - \text{N}$   
 $Y_{VSS}$  = biological yield with regard to VSS  
 $\eta(O_3)$  = ozone transfer efficiency (%)  
 $\eta(M)$  = degree of pollutant removal (%)  
 $\Delta t_i = t_e - t_0$

## **Chapter 1. GENERAL INTRODUCTION**

### **1.1. Background**

Current trends of expanding world population and increasing per capita consumption of seafood contrast with declining wild stocks, which diminish because of overharvest (New, 1991). The decline of natural fisheries has encouraged rapid expansion of aquaculture, which includes production of fishes, crustaceans, mollusks and plants. Aquaculture products are defined as those aquatic organisms (plant and animal) produced under controlled or semi-controlled environmental conditions. Environmental controls in aquaculture range from no control (typifying ocean net pen culture and sea ranching) to moderate control (typifying pond and raceway systems), to complete environmental control (obtained in recirculating systems) (Brazil, 2001).

The impacts of aquaculture using no-control methods on water quality, sediments, benthic invertebrates, zooplankton, phytoplankton and wild fish communities raise serious environmental and ecological concerns (Bardach, 1997; Ackefors, 1999; Pearson and Black, 2001). Traditional aquaculture production in ponds and raceways requires large quantities of water. It also poses environmental impacts, such as eutrophication of natural waterbodies receiving aquaculture effluents and depletion of groundwater. Despite these concerns, cage and pond culture remain the predominant methods of aquaculture. They are preferred because of relatively low energy requirements, and simplicity and low cost for construction and operation of such facilities. However, the aquaculture industry seeks to minimize environmental deterioration directly related to expansion of production. Increasing regulation of aquaculture effluents and the need to conserve water resources and energy has pushed the aquaculture industry to focus on development and refinement of water recycling technologies, particularly recirculating aquaculture systems, or RAS (Rosenthal, 1994).

## **1.2. Recirculating aquaculture systems**

RAS can be defined as an assemblage of parts used for the husbandry of aquatic organisms in which water is continuously cleaned and recycled (Libey, 1996). Water is cleaned via mechanical and biological filtration. Mechanical filtration removes particulate wastes, while biological filtration removes dissolved wastes via biochemical reactions that occur during bacterial metabolism (Hall, 1999). Through water treatment and reuse, RASs use a fraction of the water required by ponds to produce similar yields, making them suitable for areas where water of suitable quality is scarce or where water must be heated or cooled in order to ensure acceptable fish growth rate (Arbiv and van Rijn, 1995). In addition to water conservation, RAS allow greater control of water quality, allow location of production facilities close to markets, and increase growth on a year-round production schedule, avoiding seasonal limitations imposed in outdoor systems (Van Gorder, 1994). These benefits are achieved through water treatment processes and through significant reduction of effluent volume. From a water treatment perspective, reduced effluent volume is a major advantage of recirculating technology (Chen, 2001). Other considerations that might favor growth of aquaculture in reuse facilities are efficient use of space and labor (Losordo et al., 1998). Finally, because the environmental impact of aquaculture is of growing concern, in some areas, in order to conform to environmental regulations, fish culture in RAS is the option of choice (Weston, 1991, Ackefors and Enel, 1994).

## **1.3. Critical operational and design considerations of RAS**

A major challenge to aquaculturists is maintaining suitable water quality for the crop throughout the culture process (Hall, 1999). Critical environmental parameters include concentrations of dissolved oxygen, un-ionized ammonia nitrogen, nitrite-nitrogen, and carbon dioxide in the water of the culture system. Levels of waste solids, dissolved organics, nitrate-nitrogen concentration, pH, temperature, and alkalinity also can impair environmental quality (Losordo et al., 1998), and must be considered in design of water decontamination/recovery processes. Therefore, water quality control in such systems

requires more than biological management of water quality (van Rijn, 1996). In order to be operational and to maintain adequate life support, all RAS must remove solids, dissolved organics and carbon dioxide, oxidize ammonia and nitrite to nitrate, and reoxygenate water before returning it to the fish tank. Management strategies and design of system unit processes focus on improvement of diets and delivery methods, culture systems, oxygenation and circulation devices, filtration methods and monitoring devices (van Rijn, 1996), and are all significant contributors to maintenance of water quality.

### 1.3.1. Sludge removal

Effluent discharged from a recirculating system often is associated with high levels of solids. The resulting effluent has a higher concentration of suspended solids, dissolved organic matter and nutrients than the culture water. Thus, the effluent usually is called sludge. Comparing the waste production of fish culture systems with those of other animal production operations on a wet weight basis, aquaculture systems generate a greater sludge volume than most other animal operations (Chen, 2001).

Virtually all the wastage generated within a RAS originates from the feed, and solids production in RAS depends largely on the type of culture system, management practices, quality and composition of feed, and cultured species (van Rijn, 1996). At a crude level, pelleted feeds used in aquaculture production consist of protein, carbohydrates, fat, minerals and water. The portion not assimilated by fish is excreted as a highly organic waste (fecal solids), and along with the unconsumed feed and the biofloc formed in the RAS, contributes to the total solids (TS). According to APHA et al. (1998), “total solids” is the term applied to the material residue left in the vessel after evaporation of a sample. TS includes “total suspended solids” (TSS), the portion of total solids retained by a filter, and “total dissolved solids” (TDS), the portion that passes through the filter. Igniting the residue from any of these determinations at 550°C will result in estimation of total fixed solids (TFS), fixed suspended solids (FSS), and fixed dissolved solids (FDS), while the weight loss by ignition corresponds to the volatile solids (TVS, VSS, and VDS, respectively).

Selection of processes for treatment and management of sludge from RAS is determined by the characteristics of the sludge in terms of pollutant concentrations,

specific gravity and size distribution. Hence, when designing facilities for solids separation and concentration, tests must be conducted on the sludge at issue, characterizing oxygen consumption rate, settled sludge volume, and sludge volume index or specific gravity (APHA et al., 1998). The specific gravity is determined by the source of particles, while the size distribution is determined by the solids removal process used as well as the source of particles, fish size, temperature, and turbulence in the system (Speece, 1973).

Waste solids can be classified into particulate (settable, suspended and floatable) and dissolved solids. The nutrients and organics associated with particulates (TSS) can be converted to the dissolved form within the system as the particles decay with time. This process is associated with dissolved oxygen consumption and generation of ammonia-nitrogen (Spotte, 1979), which in its un-ionized form is highly toxic to the fish (Chen et al., 1993). In the particulate form, suspended solids can promote gill damage (Stickney, 1979; Wickins, 1980) and potentially can clog biofilters intended for nitrification (Muir, 1982). For this reason, waste solids should be removed from the system as quickly as possible. Settable solids are those that will generally settle out of the water within a one-hour period under quiescent conditions. Typically, they have diameters greater than 100  $\mu\text{m}$ . Settable solids are the easiest to remove, using sedimentation or swirl separation processes. The use of tube settlers (lamellar sedimentation) in the sedimentation tank can improve process efficiency.

Suspended solids are those colloidal solids (smaller than 100  $\mu\text{m}$ ) that will not settle to the bottom of the fish culture tank or settling basin. Screen filtration, foam fractionation, ozonation, and porous or granular media filtration can physically or mechanically remove those particles larger than 50 to 20  $\mu\text{m}$ , the efficiency being a function of the method employed (Chen et al., 1994). Ozonation removes smaller solids by aggregating them into bigger, filterable particles, and by direct action as a chemical oxidant (Summerfelt et al., 1997).

Fine and dissolved solids (< 30  $\mu\text{m}$ ) contribute more than 50 percent of the TSS in a RAS (Libey, 1993; Losordo et al., 1998). They can increase BOD<sub>5</sub> in the system and cause gill irritation and damage to fish (Chapman et al., 1987). Fine and dissolved solids can be removed by using foam fractionation (Timmons, 1994).

### 1.3.2. Nitrification

Total ammonia-nitrogen (TAN), consisting of un-ionized ( $\text{NH}_3$ ) and ionized ammonia ( $\text{NH}_4^+$ ), is a by-product of protein metabolism. Controlling the concentration of TAN (specifically,  $\text{NH}_3$ ) in the culture tank is a primary objective of recirculating treatment system design (Wheaton et al., 1994; Kamstra et al., 1998). TAN is excreted from the gills of fish as they metabolize protein and also is produced when bacteria decompose organic waste solids. Because the un-ionized form of ammonia-nitrogen is extremely toxic to fish, its concentration must be maintained below a low limit (Emmerson et al., 1975; Spotte, 1979; Losordo et al., 1998). The ionized/un-ionized ratio of ammonia is directly related to water pH (Huguenin and Colt, 1989; Wheaton et al., 1990).

Nitrite-nitrogen ( $\text{NO}_2^-$ ) is a product of biological oxidation of ammonia-nitrogen, resulting from the metabolism of a group of nitrifying bacteria that oxidize ammonia-nitrogen as an energy source for metabolism and growth. Nitrite-nitrogen can be a concern for fish health if it accumulates, but another group of nitrifying bacteria oxidizes nitrite as an energy source, thereby producing nitrate-nitrogen ( $\text{NO}_3^-$ ), a less toxic byproduct. Both groups are autotrophic aerobes, and the entire process of ammonia oxidation to nitrate is called nitrification. The accumulation of the final by-product from nitrification, nitrate-nitrogen, is moderated by routine water exchange during maintenance operations or by denitrification to  $\text{N}_2$  gas, and typically remains at levels that do not appear to be growth-inhibiting (Spotte, 1979; Wheaton et al., 1994).

Biological filtration (biofiltration) is the most used technology for promoting nitrification. In the biofiltration component of a RAS, ammonia is converted to nitrate by autotrophic bacteria in a biological fixed film process (Brune and Gunther, 1981). Biofilters provide a substrate with a large surface area where nitrifying and heterotrophic bacteria attach and grow, forming the biofilm. Gravel, sand, plastic beads and plastic rings are examples of media types suitable for biofiltration. The most common configurations for biological filters include rotating biological contactors, fixed film reactors, expanded media filters, and mixed bed reactors (Wheaton et al., 1990).

### 1.3.3. Biological oxidation of organics

In aerobic treatment of wastewater (including aquaculture effluent) in biofilters, dissolved organics are removed by conversion to heterotrophic bacteria biomass. Nitrification performance can be severely suppressed if the carbonaceous biochemical oxygen demand (cBOD<sub>5</sub>, representing the amount of readily biodegradable organics) (APHA et al., 1998) is high in the treated water, with nitrification ceasing completely as cBOD<sub>5</sub> concentrations exceed 20 mg/L (Wanner and Gujer, 1984). The phenomenon is due to competition between autotrophic and the heterotrophic bacteria for space on the biofilter surface, with heterotrophs growing faster than autotrophs if enough organic substrate (i.e., cBOD<sub>5</sub>) is available to them (Parker and Richards, 1986). Chemical oxygen demand (COD, which represents the total amount of organic matter) and the total organic carbon or dissolved organic carbon (TOC and DOC, respectively, both measured in order to determine the amount of organics that are converted to CO<sub>2</sub> during chemical oxidation) are also metrics for estimating the organic content of the waters. TOC and DOC are more convenient and direct expressions of the organic content than BOD<sub>5</sub> or COD. Organic nitrogen content is also important when analyses are performed to characterize the organic content of water. Organic nitrogen includes such natural materials as proteins and peptides, nucleic acids, urea, and numerous synthetic organic materials, and is measured as the total Kjeldahl nitrogen (TKN). TKN is defined functionally as organically bound nitrogen. The definition holds true if the ammonia nitrogen is removed initially from the sample (APHA et al., 1998).

### 1.3.4. Denitrification

Water quality control in a RAS can be achieved by combining treatment and reuse of water with water exchange. Water exchange can be minimized by removing NO<sub>3</sub><sup>-</sup> (a process called denitrification, or dissimilative nitrate reduction), and by advanced chemical oxidation (e.g., ozonation – see following section) for the elimination of non-biodegradable organic matter (Otte and Rosenthal, 1979).

Denitrification occurs when facultative or strictly anaerobic heterotrophic bacteria (e.g., bacteria from the genera *Pseudomonas*, *Achromobacter*, and *Bacillus*) metabolize nitrate-nitrogen to produce nitrogen gas, which is released to the atmosphere.

Denitrification starts with a dissimilative nitrate reduction (i.e., reduction of nitrate to nitrite through reactions coupled to the electron transport system, with nitrate as the electron acceptor), and involves further the reduction of nitrite to gaseous end products, primarily to molecular nitrogen (Moore and Schroeder, 1971). According to Payne (1973), the suggested pathways are:  $\text{NO}_3^- \rightarrow \text{NO}_2^-$  and  $\text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ . The rate of dissimilative nitrate reduction is considerably slower than the aerobic reactions and, consequently, does not take place in the presence of dissolved oxygen (Moore and Schroeder, 1971). According to Wiesmann (1994), only the catabolic use of nitrate is called denitrification. In some situations (e.g., when ammonia is missing as a nitrogen source), nitrate also may be removed by assimilative nitrate reduction to ammonia. Assimilative nitrate reduction involves the reduction of nitrate-nitrogen (valence = +5) to amino nitrogen (valence = -3) and the incorporation of this amino nitrogen into cellular material (Nason, 1956, cited by Moore and Schroeder, 1971).

Other requirements for the denitrification process are a carbon source for cell growth (electron donor), and a bacterial population capable of performing denitrification (Moore and Schroeder, 1971). The organic matter to nutrient ratio provides an indicator of the suitability of wastewater for biological nitrogen removal, indicating whether sufficient organic matter is available to denitrify the nitrate-N available (Grady et al., 1999).

Usually, aquaculture effluents are rich in nitrate and have a limited concentration of potential electron donors. To address this problem, a supplemental, exogenous source of carbon (e.g., methanol, sugars or volatile fatty acids) is added to the effluent to be treated (Semon et al., 1997; Jeris and Ovens, 1975; Coelho, et al., 1992; Brazil, 2001). Also, for the denitrification process to proceed efficiently, a current practice effects anoxic conditions by injecting nitrogen gas into a treated effluent that contains remnant dissolved oxygen. Nitrogen gas, being more soluble in water than molecular oxygen, removes the oxygen by displacement (Whitson et al., 1993).

Although many configurations for denitrification biofilters exist (e.g., downflow packed bed, upflow packed bed, or suspended growth series), fluidized bed biological reactors (FBBRs) have several distinct advantages, which make them preferable for aquaculture applications. First, they have a very high surface area to volume ratio, and hence produce a relatively small footprint for the ammonia or the nitrate removal they

achieve. By fluidizing the media, FBBRs make all surfaces of the media (e.g., sand) particles available for bacterial growth. Finally, they are reliable if operated properly, relatively inexpensive, and if properly designed, relatively easy to operate (Wheaton, 2002). Grady et al. (1999) described FBBRs as having the benefits of efficient biological oxidation, efficient mass transfer and highest volumetric loading rate. However, FBBRs do not remove particulate substrates very efficiently and depend for particulate removal on other forms of mechanical filtration.

### 1.3.5. Chemical oxidation

Oxidation, the major mechanism by which wastewater pollutants are controlled, occurs through a variety of biological, chemical, and physical-chemical processes. Chemical oxidation has the potential to remove from wastewater organic materials that are resistant to other treatment methods, whether those methods are biological or the longer trains of processes known as tertiary or advanced treatment (Evans 1975).

Oxidation-reduction or redox reactions are those in which the oxidation state of at least one reactant is raised while that of another is lowered. The increase in the positive valence (or decrease in the negative valence) with oxidation takes place simultaneously with reduction in chemically equivalent ratios. Therefore, in chemical oxidation processes, reaction mechanisms change the structural and chemical properties of the organic substances (Marco et al., 1997). Some oxidations proceed readily to CO<sub>2</sub>. In other cases, the oxidation is not carried as far, perhaps because of the dosage of the oxidant, the pH of the reaction medium, the oxidation potential of the oxidant, or the formation of stable intermediates. However, in any case, the oxidant itself is reduced (Rice and Browning, 1981). The parameters BOD<sub>5</sub>, COD, and TOC or DOC are used to quantify chemical oxidation, as they are used for biological oxidation. DOC is also an important indicator for disinfection performance.

The most popular chemical oxidants used for water and wastewater treatment are: chlorine, hydrogen peroxide, potassium permanganate, and ozone. Among them, ozone (O<sub>3</sub>), a gas, is clean and is safe, and has the highest chemical reactivity (Rice and Browning, 1981). Ozone is the most efficient chemical oxidant used in water and wastewater treatment. Because of the strong oxidizing properties of ozone, its

applications in aqueous solutions include removal of iron, manganese and other inorganics, color, taste, odor-causing components, algae, organics, suspended solids, microflocculation of dissolved organics, as well as pretreatment before biological processes, bacterial disinfection, and viral inactivation. Due to these remarkable properties, ozone has become an important agent for treatment of water and wastewaters resulting from various activities, including aquaculture (Rice, 1986).

The most widely-used method for ozonation is the diffusion of an ozone/oxygen or ozone/air mixture into bubble column reactors equipped with diffusers or venturi injectors, mostly operated in a reactor-in-series counter-current continuous mode (Masschelein, 1985; Xu and Liu, 1990; Bellamy et al., 1991; Gottschalk et al., 2000). When considering use of ozonation as part of a water or wastewater treatment scheme, the optimal use of ozone is determined by the rates of reaction of the impurities to be oxidized. Oxidation rates of refractory materials will be reaction rate controlled, while oxidation rates of readily oxidized materials will be controlled by the rate of mass transfer of ozone into solution (Rice and Browning, 1981). The gas source utilized for ozone generation is air or pure oxygen. Regardless of the special benefits for wastewater treatment, ozone production is highly energy-demanding, requires expensive equipment, and because of its instability, cannot be stored and requires onsite production. Despite these inconvenient features, recent research proves ozonation economically feasible, and it is included often in the design of various wastewater treatments (Bellamy et al., 1991; Gottschalk et al., 2000).

#### **1.4. Closed systems for intensive fish culture**

The continuous elimination of toxic metabolites and growth-inhibiting substances is an essential process when operating a closed system for intensive fish culture. Several experimental recirculating systems based on zero water-discharge have been described, using biological filters for degradation of accumulating organic compounds that originate from fish excretion and excessive feeding. Hirayama et al. (1988) used an aquarium equipped with only a mechanical (sand) filter, and demonstrated that the elements C, N, and P in organic substances accumulating in the culture water accounted for 3.2% of the

amounts added to the aquarium as food. They concluded that accumulation of organic substances may be the cause of suppression of fish growth in closed recirculation system.

Results of Otte and Rosenthal (1979), Spotte (1979), Lucchetti and Gray (1988), and Losordo et al. (1998) suggested that the final effect of the water treatment process in RAS needs to be similar to that of a flow-through system configuration. Further, when recovery and reuse of the effluent is intended, the effluent quality needs to be improved by employing additional water treatment strategies, such as denitrification, advanced chemical oxidation processes, supplemental gas exchange, and some form of disinfection.

Studies conducted by van Rijn and Rivera (1990) demonstrated that by employing biological oxidation and reduction of inorganic nitrogen (through nitrification by trickling filters and denitrification by fluidized bed filters, respectively), nitrite accumulation could be prevented. That was possible at the expense of adding an exogenous carbon source to sustain denitrification. They observed that denitrification in the fluidized bed filter was highly improved by the incorporation of a sedimentation basin, in which considerable degradation of organic matter took place, but their findings did not address to what extent this treatment system could be operated under different regimes (e.g., stocking densities, water composition, feed loading rate, different fish species). Recently, Gelfand et al. (2002) demonstrated in a marine RAS that, by using nitrification, denitrification, and sedimentation, an entire rearing cycle of 392 days was possible without water exchange, keeping an equilibrium in the system with regard to organics and nutrients. However, they observed low survival rates of tilapia *Oreochromis sp.* and gilthead seabream *Sparus aurata*, but suggested that factors other than sub-optimal environmental conditions triggered it. Zhu (1990) conducted studies on closed RAS and concluded that two different closed systems (biodrum and submerged biofilters, and biofilter channel, respectively), designed at an industrial scale (70 tons of fish per year), were not viable unless an exchange rate of 5 – 8% of water per day was employed.

The use of only biological oxidation in water recycling systems can be a disadvantage in that a substantial amount of water flow has to be make-up water, and a high proportion of the total volume of the system is occupied for the biological filter unit, leaving a smaller volume for fish cultivation (Otte and Rosenthal, 1979). Furthermore, using biological filtration only, the accumulation of poorly biodegradable substances cannot be

avoided. Searching for solutions to these problems, Rosenthal and Otte (1979) tested a system that included a trickling filter, settling tank, denitrification unit, and reaction tower for advanced oxidation with an ozonator and foam separator. In this system, 45% of the system volume was fish rearing tank and 55% was treatment units. With an ozone dose of 6.2 mg/L water, they demonstrated that water can be maintained with quality parameters suitable for fish and that only 0.7% of total water volume per day was introduced to replace that lost with sludge and by evaporation. Malone and DeLosReyes (1997) concluded that the major parameters limiting the development of biosecure, closed recirculating aquaculture filtration technology are cost-effective removal of particulates and nitrogen from the recirculating water.

Although partial recycling is not particularly problematic using biofilters only, a large-scale intensive aquaculture unit is still dependent on a sufficient source of high quality water. In order to utilize a limited water source on an economically viable scale and to be independent of local water supply, a high rate of recycling is required. To be suitable for reuse, the recycled waste stream needs to be nearly void of suspended solids, dissolved organics and nitrogenous compounds. Therefore, it is necessary to employ advanced techniques (e.g., a combination of biological filtration with ozonation) to treat the wastewater before reusing it.

### **1.5. Study system for wastewater recovery and reuse**

Blue Ridge Aquaculture (BRA) in Martinsville, Virginia breeds, hatches, and grows out hybrid tilapia for live shipment and sale in the domestic market. The current production level of the facility is about 1360 tons of fish per year. The facility operates three greenhouses (for spawning broodstock and for fingerling rearing), and 42 independent RAS for growout. Each RAS incorporates a rearing tank (215 m<sup>3</sup>), a sedimentation basin (37 m<sup>3</sup>), and a rotating biological contactor (RBC) filter with 13,366 m<sup>2</sup> active surface area. The exchange rates are 23% per day from the growout systems, a daily flushing/replacement of 90 – 100% of the volume from the fingerling systems, and a weekly exchange of about 50% from the broodstock basins. Growth in production at BRA is constrained by the availability of high quality water. At the same time, BRA is

discharging about 2290 m<sup>3</sup> of wastewater per day to the Martinsville municipal sewerage system. BRA would do well to recover water from its waste stream to be reused for production purposes. Significant reductions in solids, organic carbon and nitrate-nitrogen must be achieved for water to be reused. In order to achieve these goals, a wastewater treatment system for water recovery and reuse must be developed and placed on line. It will include many elements and techniques commonly used in domestic, and sometimes in industrial wastewater treatment. In this study, a treatment strategy was designed, built and evaluated at a pilot scale, along with identification of critical parameter values (e.g., ozone dose required, or the total amount of nutrients and organic compounds produced by the RAS that must be removed). The study also evaluated BRA effluent response to ozone and established the optimum dosage, estimated a nitrogen budget, and the system's maximum carrying capacity with respect to ammonia nitrogen.

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## Chapter 2. OZONATION TREATABILITY OF BLUE RIDGE AQUACULTURE EFFLUENT

### ABSTRACT

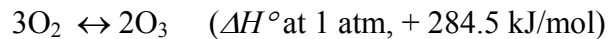
This study was conducted to evaluate the effects of ozone on Blue Ridge Aquaculture (BRA) wastewater effluent at various ozone dosages ( $D_1 = 6.9$ ,  $D_2 = 4.8$  and  $D_3 = 2.4$  g), in order to optimize treatment efficiency and to determine the feasibility of a single treatment process employing ozone. Between 63.2 and 84.8% of ozone reacted after 30 minutes. Ozone transfer efficiency  $\eta(O_3)$  decreased from 98.4% after the first 3 minutes to 45.2% during the last 10 minutes at dosage  $D_1$ . COD removal was much faster in the first 9 minutes, and declined thereafter. Large ozone dosages resulted in better COD removal, but also in more ozone wasted. The total mass COD removal was 61.0-77.4%, of which 25.5-40.4% was oxidized, with the balance removed in foam. Ozone yield  $Y(O_3/COD)$  for the oxidized fraction was between 1-6 g, but was greatly improved by foam removal from the reactor, to 0.3-2 g. Water biodegradability increased with the ozone consumption, by 24.5-34.9%. COD/DOC ratio decreased up to six-fold in 30 minutes, indicating an increase of the degree of oxidation of organics, as well as mineralization. From 66.7-77.1% of TSS was removed ozonation, with a greater effect on the volatile fraction. Turbidity was reduced by 77.7-85.6%, higher ozone dosages having the greater effect. TKN was reduced by 72.0-93.8%, most likely with foam. TAN increased during ozonation by 13.6-45.5%, following mineralization of nitrogen-containing organics. Alkalinity decreased by 15-30%, probably acting as scavenger on ozone. Hardness decreased by as much as 25%, becoming bonded with colloids and removed with foam.

Results of this study suggested that ozone application becomes limiting when high quality effluent is desired, in terms of the amount of ozone injected versus the amount transferred and reacting with the wastewater. It is recommended that ozonation of BRA effluent be approximately 9 minutes, and not exceed 15 minutes at the highest dosage tested. The ozonation should be followed by biological oxidation, rather using ozonation as a single treatment process.

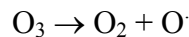
## 2.1. Literature review

### 2.1.1. Ozone

Ozone, a colorless gas at room temperature, is an allotropic form of oxygen, with a molecular formula  $O_3$  and molecular weight of 48.00 grams per mole. It can be generated from air or pure oxygen by different methods, among them corona discharge, which is the most widely-used procedure (Masschelein, 1994). The process involves the passage of air (or oxygen) between electrodes across which an alternating high-voltage potential is maintained, producing a uniform blue-violet glow discharge throughout the gas, ionizing some of the molecular oxygen (Diaper, 1975). Ozone is formed by recombination of ionized oxygen atoms and ionized molecular oxygen. In this process, only 4 – 12% of the energy supplied is used for the formation of ozone and the rest is transformed to heat (Ozonek et al., 1994). The overall reaction for ozone formation is described as an endothermic reaction (Bablon et al., 1991):



Ozone is an unstable gas under conditions normal to water and wastewater treatment, and hence, it cannot be manufactured, stored, or transported, and must be generated at its point of use (Rice, 1997). Ozone is thought to have a mechanism of oxidation described by the reaction:



where nascent oxygen produces a high-energy oxidation via a free radical reaction (Raiton, 1972). The polarized ozone can react with the substances by physical interaction. It can dimerize and chemically oxidize reactive molecules. Organic chemists have long made use of the ability of ozone to cleave carbon-carbon bonds in synthetic and structure-determination procedures (Rice and Browning, 1981). This reaction may induce increased solubility of the materials, biodegradability, and so on; oxidation is usually fast, and the oxidation by-products are not evidently toxic for the environment. As one of the most reactive gases known, ozone is recognized as the most powerful oxidizing agent available for the treatment of water and wastewater (Martin and Elmghari-Tabib, 1982; Rice, 1997). When molecular ozone dissolves into water, the molecule can remain as  $O_3$  or can decompose by various mechanisms, ultimately producing the hydroxyl free radical

(HO<sup>°</sup>), a stronger oxidizing agent than molecular ozone. Therefore, the chemical effects of ozone in water are a result of its direct reaction with dissolved compounds, its decomposition in reactive free radicals, or the subsequent reaction of these free radicals with solutes (Rice, 1997).

### 2.1.2. Ozone application with water and wastewater

Ozone treatment of drinking water has a history of more than 125 years. The first use of ozone was in 1893 for water sterilization at Oudshoorn, Holland. The first major application of ozone for public water supply sterilization was in 1905 at Nice, France (Evans, 1975). Due to its reputation as the most powerful oxidizing agent/disinfectant readily available for water and wastewater treatment, ozone is used extensively today for disinfection and viral inactivation, chemical oxidation and preoxidation in preparation for biological treatment of drinking water, domestic wastewater and industrial wastewater (Rice et al., 1986). On drinking water, ozone is very efficient for bacterial disinfection and viral inactivation, removal of soluble iron and manganese, removal of taste and odor, algae control, organics control, destruction of inorganics, microflocculation and removal of suspended solids (Rice et al., 1986).

In industrial and municipal wastewater, the most prominent application of ozone is disinfection, inactivation of viruses, microflocculation coagulation for removing suspended solids, removing BOD and COD, algae, and odors (Powell, 1980). The amount of ozone necessary to perform each of these functions depends upon a number of factors, but primarily upon the ozone demand of the constituents of the water/wastewater to be treated.

Kirk et al. (1975) found in a set of pilot plant studies of tertiary wastewater treatment with ozone that practically all of the COD can eventually be oxidized by ozone, and in the technical sense, there appears to be no fraction of the COD that is refractory to ozone treatment. However, such treatment can be achieved at the expense of up to 3 lb O<sub>3</sub>/1 lb COD removed. They also observed that the COD reduction rate is rapid in the first stages of treatment, but slows drastically in the latter stages after 50 – 70% of the COD has been removed. pH changes consistently toward neutrality during ozone treatment, whether the feed water is acidic or basic, and the change is greater for higher COD feeds. A

correlation was found between pH and organic pollutant removal; the higher the pH of the feed, the higher the COD removal. Chemical nitrification was found significant during ozone treatment only with high pH feeds. Turbidity reduction reached as high as 70%.

Beltran et al. (1997) showed the impact of chemical oxidation on biological treatment of a primary municipal wastewater. Pre-ozonating a primary municipal wastewater reduced levels of COD and improved biodegradability by 25% compared to non-ozonated wastewater. They concluded that: ozone is an appropriate technology to be combined with biological oxidation, and that there exists an optimum ozone dose for which both ozone efficiency and COD level reduction per ozone level are maximized. In subsequent studies, Beltran et al. (1999a, 1999b) tested a combination of chemical-biological treatment of domestic wastewater, and showed that pre-ozonation and post-ozonation were effective treatments for domestic sewage. Pre-ozonation followed by combined biological-chemical oxidation led to improved BOD and COD reductions of 88% and 66% respectively, compared with 80% and 47% without pre-ozonation. Pre-ozonation also improved the total Kjeldahl nitrogen (TKN) removal and supported better nitrification in the subsequent biological process. In the post-ozonation study, the results showed higher biodegradability of the treated wastewater. Reduction of 59.1% of COD was observed after the biological process, compared to 71% obtained when a post-ozonation step was included. Ozonation after the secondary treatment also allowed better nitrogen content reduction, with total nitrite elimination (Beltran et al., 1999b).

Lin and Wu (1996) investigated ammonia, nitrite and nitrate removal from aqueous solution using ozonation and ion exchange, finding that ozonation was able to completely convert nitrite to nitrate, but that ammonia removal was more limited. Partial ammonia removal was possible only above pH 8.

Walker et al. (2001) investigated the ozonation of a mixture of organic compounds dissolved in an aqueous solution having salt concentrations ranging from 0.0 to 2.0 *M*. They found that increasing ionic strength resulted in greater consumption of ozone (i.e., lower concentration in the off-gas) and that bubble size decreased with increasing salt concentration up to about 1*M* NaCl.

Weemaes et al. (2000) studied the effect of oxidative pretreatment employing ozone on anaerobic digestion of domestic sludge. They found that 29% of the organic matter was solubilized, 38% was removed, and the methane production increased by a factor of 1.8 after treatment with a dose of 0.1 g O<sub>3</sub>/g COD.

Paraskeva et al. (1998) examined the effects of ozone on a secondary municipal effluent and the influence of the ozonation conditions on the treatability of this effluent. Ozone reduced the COD, BOD<sub>5</sub>, and color concentrations. It also increased the dissolved oxygen concentration of the effluent dramatically, in most cases to saturation levels.

For industrial wastewater treatment, ozonation was found worthwhile in many applications, from removal of refractory (xenobiotic) and toxic residual compounds, to dyes from the textile industry. For example, Ciardelli et al. (2001) showed that using a combination of ozone with biological treatment, 95 – 99% of the residual dyes from textile production wastewater were removed, and that the recovered water could be reused in the industrial process.

However, some exceptions were also reported. Medley and Stovler (1983) assessed ozone's effect on the biodegradability of three organic pollutants that are difficult to biodegrade: acrylonitrile, 1,2-dichloropropane, and 2,4-dinitrophenol. While the last two compounds proved to be highly oxidized by ozone and easily biodegraded after ozonation, acrylonitrile was not oxidized readily enough for it to be economically removed through total oxidation by ozone. Additionally, ozone had an adverse effect on biodegradation. Hence, ozone is not a cure-all for difficult-to-biodegrade pollutants.

### 2.1.3. Ozone application in aquaculture

In addition to its traditional use in sterilize water supplies, ozone has been used in recirculating aquaculture systems (RAS) to improve recirculation stream quality (Otte and Rosenthal, 1979; Rosenthal and Otte, 1979; Williams et al., 1982; Rosenthal and Krunker, 1985; Paller and Lewis, 1988; Poston and Williams, 1988; Brazil et al., 1996; Bullock et al., 1997; Summerfelt et al., 1997).

Rosenthal and Krunker (1985) cited Scherb and Brawn (1971) as the first researchers to observe that not all effluent components concentrated in the recycling water of a nearly closed recycling system with only biological water treatment are reduced. Rosenthal and

Kruner (1985) incorporated ozone treatment in a RAS and demonstrated that concentrations of such components could be reduced effectively.

To reduce the volume of water utilized for the purification unit in brackish water RAS, Rosenthal and Otte (1979) showed that biological filtration with ozonation supported COD removal, cracked non-degradable substances and made them digestible to bacteria, and supported the aerobic oxidation of toxic nitrogen compounds (e.g., nitrite and ammonia).

Other studies also showed benefits of ozone application in intensive aquaculture systems with respect to other parameters. Colberg and Lingg (1978), Williams et al. (1982), and Paller and Lewis (1988) showed the efficacy of ozone in color and turbidity removal, although the effects of ozonation upon particle size was not clarified. Rosenthal (1981) found that ozone doses of approximately 7 – 10 mg/L in a RAS increased BOD<sub>5</sub>. At this dose, ozone was not sufficient to oxidize the organic compounds completely, but did break up large organic molecules into smaller, more easily biodegradable ones. Otte and Rosenthal (1979) and Rice (1986) showed that ozone chemically oxidized nitrite to nitrate, supported nitrification in the RAS, reduced organic substances that are not easily degraded by microorganisms in biofilters, caused suspended solids coagulation triggering turbidity decrease, destroyed bacteria, and inactivated viruses.

Rueter and Johnson (1995) studied the effects of ozonation on suspended solids removal from hatchery waters, and showed that ozonation improves flocculation and particle removal in aquacultural waters. They demonstrated improvement of up to 55% in solids removal, in addition to excellent disinfection and turbidity removal. The amount of improvement depended on raw water hardness and on initial suspended solids concentration. They assessed that the improvement was related to a decrease in particle stability following ozonation, and that some of the coagulation effects observed may be attributed to the decrease in pH as discussed by Edwards and Benjamin (1991). Edward et al. (1993) noted that ozone could act to remove carbon by oxidizing dissolved organic matter, or by improving coagulation of organic-containing particulates. They reported that ozonation improved particle removal at doses of less than 0.7 mg ozone per mg TOC.

In a recirculating rainbow trout culture system, Summerfelt et al. (1997) demonstrated that ozone addition reduced the mean concentration of TSS by 35%, COD by 36%, DOC by 17%, and color by 82% within the water entering the culture tanks. They also found ozone responsible for the reduction of mean nitrite concentration by 82% within the culture tanks, but it did not affect turbidity. Additionally, solids removal by microscreen filtration increased by 33% due to ozonation, and ozone doses between 0.025 and 0.039 kg per kilogram feed produced similar results. They speculated that the increased solids removal was caused by the smaller particles coalescing into larger, more easily filtered particles. In the same systems, Bullock et al. (1997) found that ozone prevented bacterial gill disease (BGD) outbreaks without other chemical treatments.

Monroe and Key (1980) used ozone to treat river-drawn makeup water entering freshwater hatcheries and fish farms, and showed ozone effective against bacteria even in the presence of suspended particles. Krumins et al. (2001a) found that part-day ozonation at a dose of 15 grams ozone per kilogram feed significantly reduced TOC, turbidity, and TAN compared with the control, but did not significantly reduce average nitrite concentration.

## **2.2. Problem statement**

With growing interest in ozonation in water and wastewater treatment, technical and scientific knowledge of this process is continuously expanding, making ozone technology a promising, viable water treatment alternative. However, given the large compositional diversity of waters or wastewaters needing treatment, a treatability study is required for each particular case (Bellamy et al., 1991). Such a treatability study determines the treatment characteristics of that specific water, indicating the appropriate location of ozone application, ozone dosage, contact time, rates of application and of pollutant removal, and other process variables affecting facility design and operation. Depending on the type of the water treated (i.e., drinking or wastewater), combinations of ozone with H<sub>2</sub>O<sub>2</sub> or UV light may also be considered in order to enhance pollutant removal. The ozone dosage required to meet the treatment objectives is usually the most important input for the design of the ozone generation and contact system. In all applications, ozone

is consumed by reaction with target compounds, non-target ozone-demanding substances, and spontaneous decomposition. Treatability studies are the only way of determining this dose requirement (Bellamy et al., 1991). Ozone treatability studies are designed and executed to determine design parameters that encompass all treatment objectives, including optimal contact time. Contact time is not important for fast ozonation reactions, but is a determining parameter for time-sensitive reactions. However, since short-circuiting or mixing may affect ozone contact at low detention time, ozone transfer efficiency needs to be determined (Bellamy et al., 1991).

Blue Ridge Aquaculture (BRA) from Martinsville, Virginia, produces around 1360 metrical tons of tilapia per year, and requires approximately 2290 m<sup>3</sup> of water daily. The wasted effluent is discharged to the City of Martinsville sewage system, and the replacement with fresh water rely on local phreatic resources, and on City water system for supplementation. Concerns are raised about the costs and reliability of the City water system and BRA desires to find an alternative solution, such as to treat and to reuse its wastewater. Previous work at a pilot station level intended to remove solids, dissolved organics and nitrogenous compounds from the waste stream by sedimentation and micro screening, and nitrate by biological filtration (Brazil, 2001, unpublished data). However, the final effluent from the tested treatment train was not of sufficiently high quality that recovered water could be used as make-up water for the fish tanks. Considering the multiple advantages of using ozone in wastewater treatment, a new design for the pilot scale treatment train was proposed in this study, including advanced chemical oxidation by ozonation. To design the ozonation process within the treatment train, a treatability study was conducted to determine the response of this specific waste stream to ozone, and to define the optimization between high levels of treatment and economic costs of ozonation. The ozone doses tested in this study, although they may appear high, were selected based on the preliminary determinations of organic and solids concentrations for the settled effluent. The possibility that the ozonated effluent would be further treated by nitrification, a process that requires low concentrations of organics affected the choice of ozone doses. This ozone treatability study was developed to address the following objectives.

### 2.3. Objectives

The goal of this study was to define the interaction between the BRA wastewater stream and ozone, i.e., to track the ozone and water quality parameters at different ozone dosages and residence times. The ozone dosages and residence times were varied in order to determine the effective ozone dosage for treating the effluent.

The specific objectives of this study were:

- 1) To evaluate the oxidation effect of ozone upon cBOD<sub>5</sub>, COD, DOC, TKN, TAN, NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total, suspended and dissolved solids, turbidity, alkalinity, hardness, and pH on BRA effluent water following sedimentation;
- 2) To determine ozonation and pollutant removal parameters under various operating conditions, in order to assess the ozone dosage and reaction time for which ozone efficiency and pollutant level reduction are maximized;
- 3) To determine the feasibility of a single treatment process employing ozone.

### 2.4. Materials and methods

#### 2.4.1. Ozonation system design

Treatability tests were conducted using a bench-scale semi-batch reactor. A schematic diagram of the installation is presented in Figure 2.1. The reactor was a fine-bubble diffuser column 510 cm long and 10 cm diameter. The height of the water in the column before ozonation was 450 cm, corresponding to a 35-liter volume. The gas flow rate was constant, initially expanding the water height to 485 cm. The remaining space (25 cm) accommodated foam formation. The reactor had a connector at the top for evacuation of foam and gases, and a valve at the bottom for water drainage when the experiments ended. A collection port for sampling was inserted into the reactor wall, at one-third distance from the bottom.

Ozone was produced from pure oxygen (stored in standard bottles) using a corona discharge ozone generator (Pacific Ozone Technology, Brentwood, California, Model

G21), and was injected at the bottom of the reactor using two silica stone diffusers (Aquatic Ecosystem Inc., Apopka, Florida). A bubble diffuser was used, because it has been reported that a high concentration of solute causes small bubbles to be formed by this technique (Hofmeir et al., 1995). The diffusers were rectangular stones, with dimensions of 20 x 4 x 4 cm. The ozone concentration in the gas phase was measured with an ozone monitor (PCI Ozone & Control Systems, Inc., Caldwell, New Jersey, Model HC-400). It displayed the volumetric or weight-based concentration of ozone in the mixed gases, and stored in memory the measurements performed. Valves were used to direct the gas stream from the diffusers to the ozone monitor for inflow ozone concentration measurements. By reversing valve positions, the gas stream went to the diffusers within the reactor. At the top of the reactor, the residual gases were collected along with foam and were directed to a foam collector for separation. The foam collector was a cone-shaped condensing tank, with a volume of 50 L. The foam/gas mixture was introduced at the top, as it was evacuated from the ozonation reactor. Another connection at the top allowed the separated gases to be evacuated, while the condensed foam remained in the foam collector. A valve at the bottom allowed drainage of the condensed foam. To measure the ozone concentration in the residual gas stream (an approximately 1 Lpm sample), stream was directed by a split connector through the ozone monitor. Exhaust from the condensing tank and from the monitor was vented to the atmosphere. The ozone monitor was also provided with a “zero sample” of pure oxygen from a separate oxygen bottle. All parts that came into contact with ozone were made from ozone-resistant materials, such as a special polymer for hoses, and PVC for reactor, connectors, and foam collector.

#### 2.4.2. Operation and experimental procedures

The wastewater samples were collected as grab samples from the BRA effluent by pumping a volume of 150 L to a tank located near the experimental installation. In the tank, the samples were allowed to settle for 45 minutes. After sedimentation, a volume of 35 liters of supernatant from the tank was introduced to the reactor, representing the testing batch volume. In parallel, as the sedimentation progressed, the ozone generator and the ozone monitor were started and warmed up for about 20 minutes. Before

beginning the test, key parameters that characterized the experimental procedure were optimized. First, gas flow rate was fixed by adjusting a gas flow meter valve incorporated in the ozone generator. Then, the voltage input to the generator was also adjusted. The influent ozone concentration was read from the ozone monitor. During these preliminary operations, the gas exiting the generator was wasted. After establishing the influent ozone concentration, the ozone monitor was set to read the ozone concentration from the effluent gas by switching a pair of valves. At the same time, the gas exiting the ozone generator was routed to enter the diffusers by switching another pair of valves, the timer was started, and the test was begun. All batch tests lasted 30-minutes.

The experimental design involved combinations of three ozone doses and six treatment times (Table 2.1). Ozone concentrations of 8, 16 and 23 g/m<sup>3</sup> were tested, under a constant gas flow rate (0.01 m<sup>3</sup>/min). Ozone alone was used in this study, because its direct reaction with organics is discriminative and relatively efficient, although it occurs at lower rate than for the OH<sup>°</sup> radical. Reaction-enhancement alternatives, such as addition of H<sub>2</sub>O<sub>2</sub>, were not considered from economic standpoints, because large doses are required in order to enhance water biodegradability and organics removal (Tosik and Wiktorowski, 2001). Additionally, H<sub>2</sub>O<sub>2</sub> promotes formation of OH<sup>°</sup>, a nonselective radical, which could promote ozone auto-destruction. The ozone feed rate was achieved by changing the gas-phase ozone concentration applied. The ozone feed rates were expressed in mass units (g), calculated as a product between the volumetric concentration of ozone (g/m<sup>3</sup>) and the volume of gas injected (m<sup>3</sup>) during a specific interval of time. The overall (cumulative) feeds of ozone achieved at the end of each test were 2.4, 4.8, and 6.9 g, also referred as dosages *D*<sub>2.4 g</sub>, *D*<sub>4.8 g</sub>, and *D*<sub>6.9 g</sub>, respectively. Six experimental runs were conducted, grouped in two separate experimental trials. In the first trial all dosages were tested on wastewaters of different qualities. In the second trial, all dosages were tested on the same wastewater. Additionally, a control test was conducted, using only oxygen. The control test was designed to assess the effect of oxygen on wastewater quality, when ozone was not applied.

#### 2.4.3. Analytical methods and parameters determination

System characteristics and water quality parameters were measured at six different reaction times, at 3, 6, 9, 15, 20, and 30 minutes, and at time zero. (Table 2.1). Some parameters, characterizing the reaction system or describing the experimental procedure, were determined by simple physical measurements or by direct readings from monitoring instruments. These parameters were: reactor volume, condensed foam volume, gas flow rates, ozone concentration in feed gas and ozone concentration in off gas. For water quality parameters, data were generated by laboratory or instrumental analyses of samples of water collected from the reactor, or from the condensed foam.

Samples of water collected for analyses were tested immediately for residual dissolved ozone concentration (Indigo method, using a Hach DR 2400 spectrophotometer, Hach Company, Loveland, Colorado), except for samples from time zero, which had not come into contact with ozone. Both water and foam samples were treated for dissolved ozone stripping by sustained air injection for about 5 minutes. Tests for turbidity (NTU), total ammonia nitrogen (TAN), nitrite nitrogen ( $\text{NO}_2^-$ -N), and nitrate nitrogen ( $\text{NO}_3^-$ -N) were conducted on site by using the Hach DR 2400 spectrophotometer. To carry these determinations, sample aliquots (20 – 30 ml) were filtered through a 0.45  $\mu\text{m}$  Fisher brand membrane. pH and temperature determinations were made by using a pH/mV/ $^\circ\text{C}$  meter (Acorn Meter Kit Model pH 6, Oakton, Vernon Hills, Illinois). Alkalinity and hardness analyses were conducted using Hach Permachem<sup>®</sup> Methods. Following these tests, the remaining water samples were split into two parts; one was refrigerated immediately with ice, and the other was acidified below pH 2 using  $\text{H}_2\text{SO}_4$ , and then refrigerated. Both categories of samples were transported for further analyses at the Department of Environmental Engineering at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. The non-acidified samples were used for determining organic material biodegradability as carbonaceous biological oxygen demand ( $\text{cBOD}_5$ ) (Standard Methods, procedure 5220C, APHA et al., 1998), total solids (TS), total suspended solids (TSS), fixed total solids (FTS), volatile total solids (VTS), fixed suspended solids (FSS), and volatile suspended solids (VSS), according the Standard Methods (APHA et al., 1998), procedures 2540B, 2540D and 2540E, respectively. The acidified samples were analyzed for total organic matter as chemical oxygen demand (COD), for dissolved organics mineralized during chemical oxidation as

dissolved organic carbon (DOC), and for total Kjeldahl nitrogen (TKN). COD was determined by using the closed reflux procedure (Standard Method 5220C, APHA et al., 1992). DOC measurements were obtained using a total organic carbon analyzer (Dorhmann Model DC 80, Santa Clara, California). TKN was determined using macro-Kjeldahl Standard Method 4500-N<sub>org</sub> B (APHA et al., 1998).

Foam samples were analyzed separately for COD, cBOD<sub>5</sub>, TKN, TS, FTS, VTS, TSS, FSS, and VSS using the same methods. For the control test foam was not analyzed, because despite of a small amount generated by oxygen injection, none exited the reactor.

Parameters regarding ozone, pollutant removal, or combinations were calculated as:

Ozone dose or feed rate,  $F(O_3)$ , ( $\text{mg L}^{-1} \text{s}^{-1}$ )

$$F(O_3) = \frac{Q_G \times C_{G0}}{V_L} \quad (2.1)$$

Ozone consumption rate,  $r(O_3) = r_L$ , ( $\text{mg L}^{-1} \text{s}^{-1}$ )

$$r(O_3) = \frac{1}{n} \sum_{i=1}^n \overline{r(O_3)}(\Delta t_i); \quad \Delta t_i = \text{const.} \quad (2.2)$$

$$\overline{r(O_3)}\Delta t_i = \frac{Q_G((C_{G0} - \overline{C_{Ge}}(\Delta t_i)))}{V_L} - \frac{C_L(\Delta t_i)}{\Delta t_i} \quad (2.3)$$

$$\overline{C_{Ge}}(\Delta t_i) = \frac{C_{Ge}(\Delta t_i)}{2} \quad (2.4)$$

$$\overline{C_L}(\Delta t_i) = \frac{(C_{L0}(\Delta t_i) + C_{Le}(\Delta t_i))}{2} \quad (2.5)$$

Ozone absorption rate,  $r_A(O_3)$ , ( $\text{mg L}^{-1} \text{s}^{-1}$ )

$$r_A(O_3) = \frac{1}{n} \sum_{i=1}^n \overline{r_A(O_3)}(\Delta t_i); \quad \Delta t_i = \text{const.} \quad (2.6)$$

$$\overline{r_A(O_3)}\Delta t_i = \frac{Q_G(C_{G0} - \overline{C_{Ge}}(\Delta t_i))}{V_L} \quad (2.7)$$

Pollutant removal rate,  $r(M)$ , ( $\text{mg L}^{-1} \text{s}^{-1}$ )

$$r(M) = \frac{c(M)_0 - c(M)_e}{t_e - t_0} \quad (2.8)$$

Specific ozone dose or input,  $I^*$ , ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )

$$I^* = \frac{F(O_3)}{c(M)_0} \times t_R \quad (2.9)$$

Specific ozone adsorption,  $A^*$ , ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )

$$A^* = \sum_{i=1}^n \frac{Q_G(C_{G0} - \overline{C_{Ge}(\Delta t_i)}) \times \Delta t_i}{V_L \times c(M)_0} \quad (2.10)$$

Ozone transfer efficiency,  $\eta(O_3)$ , (%)

$$\eta(O_3) = \frac{A^*}{I^*} \quad (2.11)$$

Degree of pollutant removal,  $\eta(M)$ , (%)

$$\eta(M) = \frac{c(M)_0 - c(M)_e}{c(M)_0} \quad (2.12)$$

Ozone yield coefficient,  $Y(O_3/M)$ , ( $\text{g O}_3 \text{ g}^{-1} \Delta \text{M}$ )

$$Y(O_3/M) = \frac{\overline{r(O_3)}}{r(M)} = \frac{\overline{r(O_3)} \times t_R}{c(M)_0 - c(M)_e} \quad (2.13)$$

where:

$Q_G$  = gas flow rate ( $\text{L s}^{-1}$ )

$C_{G0}$  = influent gas concentration ( $\text{mg L}^{-1}$ )

$C_{Ge}$  = effluent gas concentration ( $\text{mg L}^{-1}$ )

$V_L$  = liquid volume ( $\text{m}^3$ )

$G_L$  = liquid concentration (in reactor) ( $\text{mg L}^{-1}$ )

$C_{L0}$  = influent liquid concentration ( $\text{mg L}^{-1}$ )

$C_{Le}$  = effluent liquid concentration ( $\text{mg L}^{-1}$ )

$c(M)_0$  = influent pollutant concentration ( $\text{mg L}^{-1}$ )

$c(M)_e$  = effluent pollutant concentration ( $\text{mg L}^{-1}$ )

$\Delta t_i = t_e - t_0$

$t_0$  = initial time (s)

$t_e$  = final time (s)

$t_R$  = reaction time (s) =  $n$

#### 2.4.4. Kinetic determinations

Oxidation processes consist in general of direct and indirect reactions, which can occur simultaneously. Therefore, it can be assumed that oxidation of a compound  $M$  can

be described in a model as the sum of the two reaction pathways (Gottschalk et al., 2000). For example, in the case of ozone reaction with organic compounds, ozone and hydroxyl radical are the main oxidants of the pollutant. Despite these theoretical considerations, the reaction often develops in the fast, pseudo-first order kinetic regime:

$$-\frac{dc(M)}{dt} = k' c(M) \quad (2.14)$$

where:  $k'$  = pseudo-first order reaction rate coefficient.

The simplification affects  $k'$  definition ignoring the indirect component, which otherwise would be a function of both the direct and the indirect reactions:

$$k' = k_D c(O_3) + k_R c(OH^\circ) \quad (2.15)$$

direct      indirect

This is because during ozonation of a wastewater that is highly pollutant-laden, mass transfer is often limited, and the direct reaction becomes predominant. Consequently, the indirect reaction could be considered negligible (Gottschalk et al., 2000). Additionally, Beltran et al. (2001) justified the notion that the free radical reaction is not included in the kinetics model due to the unknown nature of compounds present in wastewater that can initiate, promote, or inhibit ozone decomposition. Wastewater from aquaculture presents a case where, in addition to a rich content of organic compounds, significant amounts of bicarbonate ions are usually present, scavenging the  $OH^\circ$  radical. This aspect should make the simplified model even more appropriate, reflecting the predominant occurrence of only the direct reaction.

#### 2.4.5. Statistical analysis

Linear regressions were used to determine the reaction rate coefficient for the respective parameters. To compare mean water quality parameter values, two-way ANOVAs were performed using the initial parameter values at time zero as covariates. The null hypothesis was that ozone dose or contact time had no effect on a water quality parameter. The null hypothesis was rejected when a  $p$ -value less than 0.05 was determined. Pearson's correlation coefficients were determined for comparisons among several parameters. Statistical analysis was conducted using SPSS (SPSS Science Inc., Chicago, IL) and SAS, version 8.2 (SAS Institute Inc., Cary, NC).

## 2.5. Results and discussion

### 2.5.1. Ozone reaction and COD

Results of this study quantified the responses of BRA wastewater effluent to ozone, showing responses of several key parameters to ozonation. Table 2.2 shows the initial values of those parameters in samples used for tests. Responses of COD to three ozone dosages along a 30-minute period of time were determined (Table 2.3).

The cumulative mass and percent of ozone reacted at the end of each test are presented in Table 2.4. Between 63.2 and 84.8% of the ozone fed reacted, depending on the dosage and initial pollutant concentrations. Most of the non-reacted ozone exited with the residual gas. Between 0.28 and 2.46 mg O<sub>3</sub>/L of water was observed as dissolved residual, usually after 15 or 20 minutes of reaction. Figures 2.2a and 2.2b show cumulative ozone dosages injected and reacted (grams) over time for the first and for the second set of trials, respectively. The amount of ozone reacted decreased with increasing dosage and with reaction time. Despite the lower percentage reacted, the total amount of ozone reacted was higher at larger dosages. This was not surprising; according to Henry's law, the solubility of a gas in water is nearly directly proportional to its partial pressure in the gas phase. Therefore, more ozone in the gas feed led to a higher concentration at the gas-liquid interface, triggering more transfer and more reaction (Masschelein, 1985; Mazzei et al., 1995). Additionally, comparing the amounts of ozone reacted at the same dosage among different trials, it becomes clear that more ozone reacted when the initial load of pollutant was higher.

The ozone dose or feed rate parameter [ $F(O_3)$ ] determined by equation 2.1, expresses the availability of the ozone dosage (fed constantly) to the volume of water in the reactor.  $F(O_3)$  had initial values of 0.11, 0.08, and 0.04 mg O<sub>3</sub> L<sup>-1</sup>s<sup>-1</sup> for  $D_{6.9g}$ ,  $D_{4.8g}$ , and  $D_{2.4g}$ , respectively, in both trials. As the tests proceeded,  $F(O_3)$  increased by a total of 14.5 – 18.5%, because the batch volumes decreased by these proportions by eliminating foam. This volume reduction was considered when determining parameters that were a function of batch volume or of  $F(O_3)$ . Table 2.5 shows the cumulative volumes of condensed foam

during the tests, also expressed as percent of the initial water volume. The total volume of foam increased with the ozone dosage, although differences were relatively small. The volume of foam generated decreased during tests, by a factor between 2.2 to 9.0 comparing the volumes collected between minutes 0 – 3 and 20 – 30, respectively. This reduction could be due to depletion of foam-generating pollutants in the water or to the decrease of the water level in the reactor, which created conditions for less foam to be created or evacuated.

Figures 2.3a and 2.3b show the dynamics of  $F(O_3)$ , along with rates of ozone absorption [ $r_A(O_3)$ ] (equation 2.7) and ozone consumption [ $r(O_3)$ ] (equation 2.3) as a function of time during the two experimental trials. Two observations were evident from these dynamics. The initial reaction was quite rapid, but as the reaction continued it slowed drastically. Both  $r_A(O_3)$  and  $r(O_3)$  started to decline after the 6 - 9 minutes measurement interval. During the last interval (20 to 30 minutes),  $r_A(O_3)$  ranged from 45.3 to 78.4% and  $r(O_3)$  from 42.6 to 77.9% of  $F(O_3)$  on a percentage wise basis. Both parameters were higher at lower dosages. Park et al. (2001) and Beltran et al. (2001b) suggested that ozone reactions with wastewater are fast at the beginning of the ozonation (i.e., high ozone absorption and consumption rates) because of the high pollutant concentration. Then reaction rates slow as a result of the formation of compounds more refractory toward ozone. Gottschalk et al. (2000) suggested that when ozonating wastewater in the semi-batch mode, the reaction regime can change over time because the concentrations of pollutants change, affecting reaction efficiency. Therefore, the slower decrease of  $r_A(O_3)$  and  $r(O_3)$  with respect to  $F(O_3)$  at lower dosages is probably the consequence of slower removal of pollutants with time. Comparing the results for the lowest dosage utilized ( $D_{2.4g}$ ) in Figures 2.3a and 2.3b, respectively, it can be observed that under conditions of higher initial pollutant loading (Figure 2.3b), absorption and the consumption rates are significantly higher than at lower dosages. These results are in agreement with those of Park et al. (2001), who found a relationship between TOC and the ozone consumption, although it was not linear, likely because other constituents contributed to the ozone demanding. Based on these observations, I conclude that  $r_A(O_3)$  and  $r(O_3)$  decrease over time because a dosage of ozone, fed constantly in this case, becomes available to a continuously decreasing amount of pollutant, which in turn

becomes more resistant to ozone. Additionally, higher pollutant loading of wastewater determines greater ozone absorption and consumption rates.

Table 2.6 presents the ozonation parameter rates as weighted averages, which were determined from equations 2.6 and 2.2, respectively. Referring the values of these parameters to those of  $F(O_3)$  on a percentage basis,  $r_A(O_3)$  accounted for 70.6 – 73.3%, and  $r(O_3)$  for 68.7 – 72.1% of  $F(O_3)$  at dosage  $D_{6.9g}$ . Similarly,  $r_A(O_3)$  was between 76.6 – 87.1%, and  $r(O_3)$  between 76.3 – 86.9% from  $F(O_3)$  at dosage  $D_{2.4g}$ . However, the percentages were much higher than these weighted averages for the interval 20 – 30 minutes, but lower at the beginning of ozonation. These suggest that a shorter period of reaction would result in better absorption and consumption. Furthermore, this aspect becomes more important at high dosages of ozone, as shown in Figures 2.3a and 2.3b. Overall, results of this study showed that, if ozone is injected at a constant dosage, better ozone utilization could be achieved at the expense of incomplete or poor pollutant removal, while a high degree of pollutant removal could be achieved at the expense of wasting large amounts of ozone. Because an acceptable trade-off between these two outcomes is difficult to achieve in a batch-type reactor at constant ozone feed, especially when a high degree of removal is desired, an alternative solution should be considered. Gottschalk et al. (2000) assessed that, because the ozone reacts more slowly as pollutant concentration decreases triggering the decrease of ozone consumption (oxidation) rate  $r(O_3)$ , a more economically feasible ozone efficiency  $\eta(O_3)$  could be achieved by lowering the ozone feed rate  $F(O_3)$  as pollutant removal progresses. Further, in a continuous-flow mode of ozonation, which is frequently employed for many full-scale applications for treating wastewater, ozone efficiency could be increased by increasing the number of stages. During every pass through an ozonation stage, the compounds present would be partially oxidized, and in the next stage the ozone dosage would be reduced accordingly.

In this study, COD concentration was one parameter used to assess the removal of ozone-reacting pollutants from the wastewater.  $COD_0$ , defined as the initial COD concentration for an interval of time between two measurements, was used for the normalization of  $F(O_3)$  and  $r_A(O_3)$ , generating the specific ozone dose or input ( $I^*$ ) (equation 2.9) and the specific ozone absorption ( $A^*$ ) (equation 2.10). Table 2.6 shows  $I^*$

and  $A^*$  as having different weighted average values for the cases of total COD removed and COD oxidized (e.g., COD oxidized was only a part of the total COD removed). Although  $I^*$  and  $A^*$  were comparable for the interval 0 – 3 minutes, they then diverged, with  $I^*$  increasing faster than  $A^*$ . However, they were both several-fold larger in the interval 20 – 30 minutes. This dynamic was the consequence of the dependency of  $I^*$  on  $F(O_3)$  (which increased with time) and dependency of  $A^*$  on  $r_A(O_3)$  (which decayed with time), as shown in Figures 2.3a and 2.3b. These parameters were used in determination of ozone transfer efficiency [ $\eta(O_3)$ ] in equation 2.11. Because  $\eta(O_3)$  is expressed as a percentage, it was the same regardless of the set of  $A^*$  and  $I^*$  values used to calculate it (i.e.,  $A^*$  and  $I^*$  as a function of total COD removal, or as a function of COD oxidized only). Figure 2.4 shows the evolution of  $\eta(O_3)$  for all tests conducted in this study, indicating a decay with time regardless of dosage. For example, in the second trial at dosage  $D_{6.9g}$ ,  $\eta(O_3)$  was 98.4% during the first three minutes, and decreased to 45.2% during the 20 – 30 minute interval. Instead, on a cumulative percentage basis,  $\eta(O_3)$  varied between 71.4 – 73.9 and 77.2 - 87.4% at  $D_{6.9g}$  and  $D_{2.4g}$ , respectively (Table 2.6), therefore increasing as dosage decreased. The decline of  $\eta(O_3)$  was a consequence of faster  $I^*$  increase (e.g.,  $I^*$  increased roughly twice that  $A^*$  in the 20 - 30 minute interval at dosage  $D_{6.9g}$ ). These observations lead to the conclusion that keeping a low  $A^*$ , close to  $I^*$  values, will assure a better ozone transfer efficiency  $\eta(O_3)$ . Additionally, based on  $\eta(O_3)$  values determined in this study, ozonation longer than nine minutes is not recommended.

Figure 2.5 presents the evolution of COD concentration with reaction time. COD removal was fast during the first nine minutes, and then diminished as time progressed to the 30-minute trial. COD removal curves (Figure 2.5) were inversely related to those of  $\eta(O_3)$  over time (Figure 2.4). Reduction of COD concentration was steeper when higher initial COD concentrations were combined with larger ozone dosages. Initial and final COD concentrations, COD reacted (oxidized), COD removed as foam, and the total COD removal after 30 minutes of ozonation are shown in Table 2.3, on mass and percentage basis. The percentages are based on batch mass content, and therefore consider the volume losses due to foam removal. The total COD removal was between 61.0 and 77.4%, increasing with the ozone dosage (Trial 2) and also depending on the initial COD

content of the sample (Trial 1). Among ozone dosages, after 9 minutes, COD removal at dosage  $D_{6.9g}$  became significantly higher than at the other two dosages (Table 2.7). The percentage of total COD oxidized varied similarly, representing between 17.8 and 31.3%. Of total COD removed, only between 25.5 and 40.4% was oxidized.

Figures 2.6a and 2.6b present the cumulative percentages of total COD removed and COD oxidized [ $\eta(\text{COD})$ ] (equation 2.12) for the first and second experimental trials, respectively. Table 2.6 presents these data on a cumulative percentage basis, after 30 minutes of ozonation. For example, in each trial the degree of COD oxidized,  $\eta(\text{COD})$ , was 30.4 – 35.3% of COD at dosage  $D_{6.9g}$ . Total COD removed was 68.9 – 72.6%, when COD removed with foam was included. At dosage  $D_{2.4g}$  during the first trial, 19.2% of COD was oxidized and the total COD removal was 67.6%. In this particular case, the high total  $\eta(\text{COD})$  was in part due to the initial COD concentration being lower than those in other tests; despite this,  $\eta(\text{COD})$  oxidized became significantly lower at  $D_{2.4g}$  than at  $D_{6.9g}$  and  $D_{4.8g}$  after only the sixth minute. However, there was no statistically significant difference between dosages with regard to total  $\eta(\text{COD})$  at each time point until the 20 – 30 minute interval (Table 2.7). This suggests that a high degree of COD removal can still be obtained at lower ozone dosages, if foam is removed from the reactor. The differences observed between percentages expressing the cumulative mass COD removal (Table 2.3) and those for  $\eta(\text{COD})$  (Figures 2.6a and 2.6b) are due to the fact that in the first case the losses in batch volume by foam elimination were considered, but in the second case only the changes in COD concentration were accounted for. The more rapidly-increasing removal of total COD than for its mineralization (Figures 2.6a and 2.6b) showed that combining chemical and physical removal mechanisms in a single process poses a great advantage for removing organic pollutants. The proportions of COD oxidized  $\eta(\text{COD})$  observed in this study for ozone dosages applied, between 19.2 and 35.5% COD reacted, were in agreement with those of numerous ozonation studies on domestic or industrial wastewaters (Beltran et al., 1999a; Beltran et al., 1999b; Rivas et al., 2000; Beltran et al., 2001; Gunukula and Tittlebaum, 2001; Pena et al., 2003). This agreement is noted, despite the fact that most of the results from these studies were obtained under different conditions with regard to time of reaction, ozone dosage, gas flow regime, pollutant loading, special chemical characteristics, kinetics, or other factors,

making direct comparison difficult. Nevertheless, noting total  $\eta(\text{COD})$  between 54.4 and 72.6% after 30 minutes, system performance could be considered more superior for dosages and wastewater COD initial loadings similar to those tested in this study. According to Kirk et al. (1975), these values of  $\eta(\text{COD})$  are at the maximum limit of feasibility for ozone application to wastewater. After 50 to 70% reduction of organic pollutant as COD from domestic wastewater, the economic practicality of further reduction becomes questionable, especially with regard to the amount of ozone that will not transfer and react.

The high performance for COD removal during ozonation is also described by the values obtained for COD removal rates [ $r(\text{COD})$ ] (equation 2.8), and ozone yield coefficients [ $Y(\text{O}_3/\text{COD})$ ] (equation 2.13). The weighted average forms for these parameters are presented in Table 2.6. When determined for total COD removal,  $r(\text{COD})$  was between 0.124 and 0.138 mg COD L<sup>-1</sup>s<sup>-1</sup> for  $D_{6.9g}$  and between 0.080 and 0.093 mg COD L<sup>-1</sup>s<sup>-1</sup> for  $D_{2.4g}$ . For COD oxidized only,  $r(\text{COD})$  was between 0.060 and 0.061 mg COD L<sup>-1</sup>s<sup>-1</sup> for  $D_{6.9g}$ , and between 0.023 and 0.035 mg COD L<sup>-1</sup>s<sup>-1</sup> for  $D_{2.4g}$ . Figure 2.7 shows  $r(\text{COD})$  decreasing with time. For example, in the first trial  $r(\text{COD})$  oxidized was, on average, 0.167 mg L<sup>-1</sup>s<sup>-1</sup> for the first three minutes at dosage  $D_{6.9g}$ , then diminished to 0.009 mg COD L<sup>-1</sup>s<sup>-1</sup> during the last 10-minute interval. The higher rates at the beginning of the tests were due to high COD concentration, but they decreased as the wastewater became depleted of COD.

The ozone yield coefficient  $Y(\text{O}_3/\text{COD})$  parameter was determined to quantify ozone consumption as a function of COD removal. Table 2.6 shows that over a 30-minute period of ozonation weighted averages of  $Y(\text{O}_3/\text{COD})$  varied with dosage. Table 2.6 also presents  $Y(\text{O}_3/\text{COD})$  values generated on both total COD removal and COD oxidized,  $Y(\text{O}_3/\text{COD})$  having smaller values when calculated as a function of total COD removal. Figures 2.8a and 2.8b presents  $Y(\text{O}_3/\text{COD})$  determined for both COD removed and oxidized for Trial 1 and Trial 2, respectively. In all tests,  $Y(\text{O}_3/\text{COD})$  for COD oxidized alone had low values for the first nine minutes of reaction (e.g., 1 g O<sub>3</sub> g<sup>-1</sup> COD or less), and started to increase much steeply after that to more than 6 g O<sub>3</sub>/g COD in the last 10 minutes (Figure 2.8b). Among ozone dosages, there were slightly statistically significant differences between  $D_{6.9g}$  and the other two dosages at different time points for

$Y(O_3/COD)$  for COD oxidized, but no differences for  $Y(O_3/COD)$  for total COD removed (Table 2.7). When determined as a function of the total COD removal rate,  $Y(O_3/COD)$  varied between 0.3 and 2.1 g  $O_3/g$  COD during the 30-minute period (Figure 2.8a). In comparison, Rosen (1975) found an ozone yield between 0.7 and 3.0 g  $O_3/g$  COD in a study of tertiary treatment aimed at a final COD below 15 mg/L.

Despite the variation of  $Y(O_3/COD)$  on the ozone oxidation of the organic materials, it is generally expected that only one atom of oxygen from the  $O_3$  molecule is highly reactive. On this basis, 3 g of  $O_3$  should oxidize 1g of COD, and 2 g of molecular oxygen would be yielded as a byproduct. Evans (1975) suggested that these departures are due to three factors that are likely to influence the reaction. First, the ozone consumption rate is greater at the beginning of ozonation, since the pollutant then has the highest concentration. Second, the g  $O_3/g$  COD removed increases rapidly as the treatment progresses, which reflects the increased resistance to ozone oxidation of the remaining organics. Third, the consumption of less than the expected 3 g  $O_3/g$  COD removed at the beginning of the ozonation suggests that auto-oxidation figures into the reaction mechanism. Beltran et al. (1999a) suggested two ways in which different dosages of ozone react with organic matter in water. First, for low ozone dosages, the most readily oxidizable compounds are the only organic removed through fast direct reactions with ozone, while at high dosages ozone decomposes to yield free radicals that are known to react non-specifically and to be scavenged by bicarbonate ions. Complementarily, when the ozone application begins under conditions of high pollutant concentration, the easily-oxidizable matter present in the wastewater reacted immediately and selectively with ozone, in its molecular form. As the oxidation advanced and most of this type of material disappeared (being oxidized or exiting the reactor as foam), ozone started to form radicals and attack and oxidize the refractory compounds, generating a chemical chain reaction (Gunukula and Tittlebaum, 2001).

Overall, treatment of BRA effluent benefited greatly from its strong surfactant characteristics when subjected to ozonation, because foam removal increased significantly the proportion of COD removal and reduced the ozone yield coefficient.

### 2.5.2. Organics biodegradability and degree of oxidation

In this study  $\text{cBOD}_5$  was measured to quantify biodegradable organic pollutants. The initial  $\text{cBOD}_5$  was between 100 and 60 mg/L, which represented 27 – 29% of the COD concentration. Statistical analysis showed a significant difference in  $\text{cBOD}_5$  removal among dosages  $D_{6.9g}$  and  $D_{2.4g}$  after 15 minutes of ozonation (Table 2.7). After ozonation,  $\text{cBOD}_5$  decreased to 25 – 44 mg/L. Variations in wastewater biodegradability were measured by the  $\text{cBOD}_5/\text{COD}$  ratio, which increased with time and with ozone dosage in all tests, from each trial (Figures 2.9a and 2.9b). For example, ratios between 0.28 and 0.29 found before ozonation increased to 0.36 – 0.44 in the final samplings, representing an increase of 24.5 to 34.9%. Hence, analysis of  $\text{cBOD}_5$  supports ozone's capacity for transforming the matter present in wastewater into compounds more amenable to decomposition by microorganisms, especially when considering that results showed no change in  $\text{cBOD}_5$  by oxygenation alone during the control test. Biodegradability improvement induced by ozonation also has been reported in numerous studies involving various wastewaters (Medley and Stover, 1983; Millamena, 1992; Beltran et al., 1999b; Yu and Yu, 2000; Tosik and Wiktorowski, 2001). The improvement in biodegradability has been attributed to formation of smaller, oxygenated species more suitable to microbial attack, and possibly to the reduction of compounds with bactericidal properties (e.g., phenolic-type substances) (Rivas et al., 2000). Broadly speaking, the results from this study showed that the higher the ozone consumption, the higher the final biodegradability of the effluent.

DOC concentration represented 7.9 to 10.3% of COD in the initial wastewater, and 27.9 to 46.4% after 30 minutes. DOC concentration increased by 50 to 100% after the first 3 or 6 minutes, and then varied little during the remaining testing time. DOC concentration increased proportionally with ozone dosage, and depended on the initial organic loading of the wastewater (e.g., DOC was higher when COD and  $\text{cBOD}_5$  were higher). DOC concentrations showed statistically significant differences between dosages  $D_{6.9g}$  and  $D_{2.4g}$  up to the 20-minute time-point for ozonation (Table 2.7).

Figure 2.10 shows COD/DOC ratio variation with time for the three ozone dosages applied during both trials. The ratio decreased by a maximum of more than six-fold (e.g., from 10.23 to 1.58 at dosage  $D_{2.4g}$  in the second trial), indicating that the organics had a significantly higher degree of oxidation after ozonation. To explain this, considering the

definitions of COD and DOC, oxidation can result in a reduction of COD, but not necessarily a reduction in DOC. Evans (1975) explained that no cleavage of organic compounds is needed for a COD reduction. Only on destruction of the organic residue, when oxidation leads to formation of carbon dioxide, is there a reduction in DOC. Similarly, Pena et al. (2003) suggested that ozone may transform the organic matter into more oxidized organic compounds, without the occurrence of complete mineralization. Accordingly, my results indicated that intense solubilization of organics took place during reaction with ozone, along with partial mineralization.

### 2.5.3. Solids

The concentration of solids (both total and suspended) decreased as ozonation proceeded. Tables 2.8a and 2.8b present summaries of solids removal for each testing trial. Initial TS concentrations ranged between 1212 and 1441 mg/L, of which the volatile fraction (VTS) represented 24-28 %. After 30 minutes of treatment with ozone, TS were reduced by 20-27%. Higher percent removals resulted from application of higher ozone dosages, and also depended on the initial TS. As expected, the part of TS most affected by ozone was the volatile fraction, as VTS was reduced by 53-64%. At the same time, FTS accounted for only 7-16% of TS reduction.

TSS had initial values between 142 and 229 mg/L. The volatile fraction (VSS) accounted for 79-82% of TSS. After ozonation, TSS decreased by 67-77%, depending on the ozone dosage applied and on the initial TSS concentration (Figure 2.11). VSS was the most affected component, reduced by 7-85%, at the end representing only 54-63% of the remaining TSS. Only 17-45% of the fixed fraction (FSS) was removed.

TDS was reduced by 12-17% during ozonation. VDS, which accounted for 16-18% of initial TDS, was reduced by 33-52%. 81 to 86% of the dissolved substrate was nonvolatile (FDS), and was reduced by only 6-14% by ozone treatment.

Whether by direct oxidation of organic and inorganic compounds from particles or by physical removal with foam, removal of solids was faster during the first 9-15 minutes, and then diminished considerably afterwards. These intervals also coincided with the time-points at which no statistically significant difference was found between ozone dosages with regard to TSS removal (Table 2.7). For example, TSS removal (Figure

2.11) shows two stages, a situation that also was observed for parameters describing ozone reaction and COD removal. The pattern similarities among TS and COD removal were not surprising, considering that the volatile fraction of solids was the most affected by ozone, and that fraction accounted for a part of COD. Additionally, the staging dynamics were created because highly volatile TSS were heavily removed as foam at the beginning of the tests, and much less were available to react with ozone or to be physically removed later in the experiments. Consequently, among the respective tests, the greatest TSS removal was observed for cases where the initial concentrations were high, and when higher ozone dosages were applied. A specific removal pattern was found for test  $D_{2.4g}$  in the first trial, where the low initial TSS and the low ozone dosage applied generated a low slope and an almost linear removal curve through the entire test (Figure 2.11). Lack of staging in this test suggests that for ozonation under these conditions solid removal could continue efficiently beyond the 30-minute period. These results are in agreement with Rueter and Johnson's (1995) findings, which suggested that ozone has a coagulant/flocculent effect, enhancing particulate removal from hatchery waters. In studies involving ozone treatment of aquaculture waters, Brazil (1997) and Summerfelt et al. (1997) also demonstrated the floatation and clarification effects. Grasso and Weber (1988) postulated the existence of not fewer than five different mechanisms that contribute to the ozone-induced particle destabilization. However, no studies describing physically removal of solids and organic matter by collecting the foam were found, with which to compare the results from this study.

#### 2.5.4. Turbidity

Turbidity describes the degree of process water clarity, quantifying the interference with passage of light by soluble colored organic compounds and suspended solids (Tango and Gagnon, 2003). Among the water quality parameters measured in this study in conjunction with the effect of ozonation, turbidity showed the greatest efficiency. Starting from initial values between 52.0-70.2 NTU, ozonation removed between 78-86% of turbidity. Greater removal was achieved when a lower initial turbidity combined with a higher dosage of ozone, as shown in Table 2.6. However, there was no significant difference in turbidity between ozone dosages until after the 9-minute time point, after

which the turbidity removal became significantly different between dosages  $D_{6.9g}$  and  $D_{2.4g}$  (Table 2.7). Turbidity removal was very active at the beginning of the tests, half of its reduction occurring in the first nine minutes of treatment. Unlike other parameters (e.g., TSS or COD), turbidity decline significantly and after this time, suggesting that if ozone would continue beyond 30 minutes, efficient removal could still be possible. The turbidity data showed that despite a large difference between dosages (i.e., about three-fold between  $D_{6.9g}$  and  $D_{2.4g}$ ), all tests produced highly clarified water after 30 minutes. The explanation could be that even though the ozone does not completely oxidize turbidity-producing compounds to carbon dioxide, even the partial breakdown of such organic molecules reduces turbidity. This interpretation is supported by significant differences between COD removals at different ozone dosages (especially in the case of COD oxidized), contrasting with final turbidity values that were much closer with each other. The mechanisms of turbidity and color removal are diverse, their complexity increasing with that of the water pollutant mix. For example, Tosik and Wiktorowski (2001) suggested that decolorization of dye-containing wastewaters could be due to ozone's selective oxidation characteristics, reacting rapidly with C=N and N=N bonds, and without it being necessary to completely oxidize the compounds to obtain color reduction. Additionally, Pena et al. (2003) found that ozone has a cleaving effect on conjugated C=C bonds from the dissolved organics responsible for the brown color of wastewater. The attack of ozone occurs in general on C=C bonds because they have the lowest bond-localization energy (Razumovskii and Zaikov, 1984). Selective mechanisms involving incomplete oxidation and breaking of double bonds with effect on light scattering could explain why the removal of organic matter measured by specific parameters (e.g., COD or DOC) are more dependent on ozone dosage than the turbidity. Finally, the micro-flocculation promoted by ozone followed by removal of these solids with foam also could contribute to the water clarifying process (Krumins et al., 2001b).

#### 2.5.5. pH

In all tests the initial pH was between 7.40 and 7.12, and decreased during ozonation by 0.08-0.30 units. The largest decrease was in the case of the highest initial pH, and was smaller as the initial pH was closer to neutral. This is agreement with Rueter

and Johnson (1995), who reported an average of 0.36 pH decrease for ozonated aquaculture waters with an initial pH between 6.80 and 8.02. Therefore, it is expected that such small variations of pH would not have much effect on pollutant removal (Beltran, 1997).

#### 2.5.6. Nitrogenous compounds

In the initial wastewater TKN ranged between 19.9-31.9 mg/L, but after 30 minutes of ozonation it decreased to 1.6-7.2 mg/L. This change represented 72.0-93.8% TKN removal, which was greater than the 54.4-72.6% removal of COD. The efficient TKN removal occurred despite TAN increase during ozonation. Very efficient removal of protein compounds with foam probably overwhelmed the generation of free ammonia by advanced oxidation of protein and peptide macromolecules, resulting in an overall depletion of TKN. This interpretation is supported by the observation that TAN, which had initial concentrations between 2.80-3.68 mg/L, increased during ozonation by only 13.6-45.5% (as a function of organics concentration and ozone dosage), an increase that contributed little to TKN. However, the degree of accumulation of TAN was statistically different among ozone dosages after 9 minutes. TAN concentration became significantly larger for  $D_{6.9g}$  relative to that for other ozone dosages (Table 2.7). However, this TAN increase was different between treatments after the 9 minute time point, when  $D_{6.9g}$  was statistically different. Despite TAN increase during ozonation in this study, Krumins et al. (2001a) reported that TAN decreased in a RAS receiving treatment with ozone; this was probably a consequence of organic removal, which allowed better development of nitrifiers on biofilters.

$\text{NO}_2^-$ -N, had initial concentrations between 0.83-1.20 mg/L, and was totally oxidized to  $\text{NO}_3^-$ -N after the first 3-9 minutes of contact with ozone.  $\text{NO}_3^-$ -N concentration was 6.7 to 16.7% higher after 30 minutes of ozonation. The stoichiometric unbalanced concentration between TAN and  $\text{NO}_3^-$ -N after ozonation could be due to oxidation of organic nitrogen, which also contributed to TKN reduction (Wang and Pai, 2001). Neither of these scenarios could be supported or refuted directly by TAN production overwhelming its eventual consumption. However, considering  $\text{NO}_3^-$ -N accumulation associated with pH decrease, it could be inferred that  $\text{NO}_2^-$ -N and some TAN and organic

nitrogen were converted to  $\text{NO}_3^-$ -N, which has a stronger acid character. Similar interpretations were suggested by Lin and Wu (1996).

#### 2.5.7. Alkalinity

The initial alkalinity was between 106-122 mg/L as  $\text{CaCO}_3$ , and decreased by 15-30% during ozonation. The largest removal was found with the largest dosages of ozone. Alkalinity removal was probably a consequence of the scavenging effect of carbonate and bicarbonate ions on ozone. However, detectable reduction in alkalinity was not observed until after the first 15 minutes of ozonation. Even then, there was no significant difference among ozone dosages with regard to alkalinity removal, presumably due to the small number of samples (Table 2.7). This should be possible because at a neutral pH ozone decomposes, and then organic compounds compete for  $\text{OH}^\circ$  radicals formed with carbonate/bicarbonate ions and other traps present in wastewater (Glaze and Kang, 1987). However, Wong and Pai (2001) suggested that the inhibition effects of these scavenging ions on ozone are less apparent than is generally expected, because of the heterogeneous organic composition of the wastewater. Additionally, Gottschalk et al. (2000) suggested that at high concentrations of organic pollutants (as is usually the case with wastewater), the direct reaction of ozone predominates and  $\text{OH}^\circ$  radicals have a low chance to form, unless the removal of pollutants is near completion.

#### 2.5.8. Hardness

The initial hardness of the wastewater was between 136 and 146 mg/L. Hardness decreased by a maximum of 25% at the highest ozone dosage. The loss of hardness was probably due to the characteristic bonding of divalent ions with destabilized organic molecules by acting as flocculation agents, and eventually being removed in such structures with foam. Grasso and Weber (1988) demonstrated that ozonation improved water flocculation by creating negative charges on the surface of colloids, and divalent cations in solution mediated their bonding.

#### 2.5.9. Control test

When oxygen was applied instead of ozone during the control test, a slight foam formation was observed at the top of the water column, but none was removed from the reactor. Upon contact with oxygen, neither in samples collected initially, nor in those collected at different intervals of time, were changes detected among the water quality parameters measured initially (Table 2.2). This lack of reaction suggested that ozone is responsible not only for pollutant oxidation and changes of water quality parameters, but also for the formation of abundant foam.

#### 2.5.10. Kinetic determinations

Kinetic studies are useful for modeling chemical and engineering processes, designing an effective reactor, and comparing kinetic parameters from the oxidation of single compounds (Beltran et al., 1997). Though a wastewater like that researched in this study is chemically complex, a model is necessary for predicting the proportion of pollutant removal that could be achieved in scaled-up treatment units.

For all tests I determined the reaction rate pseudo-first order kinetic constant ( $k'$ ) for turbidity removal, COD oxidation, total COD, and cBOD<sub>5</sub> removal in interaction with ozone. These rate constants were determined for conditions of different ozone dosages and parameter concentrations. Plots of  $\ln[M]/[M_0]$  versus reaction time were generated for these parameters (according to equation 2.14), and slopes of the lines so generated provided  $k'$  values. The profiles of variation of ozone content in the exiting gas and those of COD, cBOD<sub>5</sub> and turbidity reduction with time were typical of a semi-batch wastewater ozonation experiment. However, a distinguishing particularity in this case was the existence of two reaction/removal stages observed for all parameters, except turbidity. That is both ozone absorption and pollutant removal appeared to be faster in the first 9-15 minutes, and diminished considerably afterwards. Consequently,  $k'$  was determined separately for two intervals of time. For turbidity, which had a removal rate following one exponential curve during the entire reaction time (slopes of  $\ln[NTU]/[NTU_0]$  versus reaction time between -3.00 and -3.86, and  $r^2$  between 0.98 and 0.99), I determined a single  $k'$  for the entire period of ozonation for each dosage (Table 2.9). Similar outcomes were reported by Beltran et al. (2001) and Park et al. (2001) from ozonation experiments involving domestic wastewater and natural water, respectively.

Turbidity removal proceeded at a fast rate, reflected by the high proportion of removal (Table 2.9). Comparing the performance among dosages, showed that turbidity removal had a larger  $k'$  when a smaller ozone dosage reacted with an initially less pollutant-laden wastewater (e.g., comparing  $D_{4.8g}$  versus  $D_{2.4g}$  in the first trial), although the ratio between ozone dosages and the ratio between pollutant concentrations in the wastewater at a specific time were not proportional.

For COD oxidation (Table 2.10),  $k'$  was 4.7-9.3 times larger in the first 9 minutes than afterwards, and represented 25-83% of the turbidity removal rates. For total COD removal, the difference in  $k'$  was smaller among the two stages (e.g., 1.3-3.4 times as large), and comparable to turbidity removal rates (Table 2.11). The non-proportional removal rate variation for the two components of COD (total and oxidized) were probably due to the ozone reaction with COD dropping very much after 15 minutes, while the physical removal (i.e., by foam formation and evacuation) continuing at higher rates, resulting in a smaller reduction of  $k'$  for the total COD removal.

For  $cBOD_5$  removal,  $k'$  values were generally slightly smaller than those for total COD removal (Table 2.12). The removal of  $cBOD_5$  at high rates was somehow surprising, considering that there was also production of  $cBOD_5$  following ozone interaction with COD. To explain the high removal rates of  $cBOD_5$ , we might assume that it mainly was exerted by small, highly destabilized molecules. These molecules had ideal surfactant characteristics, and were removed efficiently by foam fractionation.

#### 2.5.11. Statistical results

To get an overall sense of the relationships between the several water quality parameters, a correlation analysis was performed. Pearson's correlation coefficients are presented in Table 2.13. The highly significant positive correlations between TSS, COD,  $cBOD_5$ , turbidity, alkalinity and TKN parameters, show that ozonation affects all these parameters in a similar way. TAN was negatively correlated with the above parameters; while those parameters decreased with time, TAN increased. DOC was not correlated with any other parameter, which indicates that to obtain a reduction of DOC, treatment methods other than ozonation should be employed.

To compare the effects of ozone dosage and reaction time on the values of water quality parameters, two-way ANOVAs with ozone dosage and reaction time as factors were performed. To adjust for different batches of water, the initial value of each parameter at time zero was introduced into the model as a covariate. The results showed significant dosage\*time interaction for all parameters. For instance, COD decreased over time much faster at dosage  $D_{6.9g}$ , than at  $D_{4.8g}$  or  $D_{2.4g}$ . Simultaneous 95% confidence intervals for values of the parameters at each dosage and time were obtained using Tukey's adjustment. These confidence intervals allowed comparisons of parameter values at different dosage and time settings. An overall significance level of 0.05 was used. As expected, the values of parameters at  $D_{4.8g}$  fell between the values of parameters at  $D_{6.9g}$  and  $D_{2.4g}$ . A comparison between dosages  $D_{6.9g} - D_{2.4g}$  at different times is given in Table 2.7 .

## 2.6. Summary and recommendations

This study examined the effects of ozone on BRA wastewater effluent, and the influence of ozonation conditions on the treatability of this wastewater. Important parameters characterizing ozonation and different pollutants removal were monitored through a 30-minute period under three ozone dosages (6.9, 4.8 and 2.4 g, respectively). The following results and conclusions were reached:

1. Between 63.2-84.8% of the fed ozone reacted in this study. Percent reacted decreased with dosage, and with time. In a quantitative sense, more ozone reacted at higher dosages. Higher pollutant loading also triggered more ozone reaction.
2.  $r_A(O_3)$  and  $r(O_3)$  increased as dosage decreased, and decreased drastically with time. Consequently, shorter periods of ozonation yielded greater  $r_A(O_3)$  and  $r(O_3)$ . Higher pollutant loadings of wastewater triggered greater  $r_A(O_3)$  and  $r(O_3)$ , due to better transfer. Their decrease with time was due to a constant ozone feed being provided to a continuously decreasing amount of pollutant, which in its turn became more resistant to ozone attack. A shorter period of ozonation should also result in a greater  $r_A(O_3)$  and  $r(O_3)$ .

3.  $\eta(\text{O}_3)$  was as high as 98.4% in the first 3 minutes of ozonation, and decreased to 45.2% in the last 10 minutes at the highest ozone dosage. Having a large (i.e., efficient)  $\eta(\text{O}_3)$  is constrained by keeping a low  $A^*$ , but it should be close to  $I^*$ . To achieve this,  $F(\text{O}_3)$  should be lowered as the pollutant concentration decreases in the batch reactor. This recommendation is supported by the values found for  $r(\text{COD})$ , which decreased by up to 18-fold in 30 minutes of ozonation.
4. COD removal was much faster in the first 9 minutes, and declined thereafter. It was found to be in inverse relationship with  $\eta(\text{O}_3)$ . Larger ozone dosages resulted in better COD removal, but also in more ozone wasted. The total mass COD removal was between 61.0-77.4%, of which 25.5-40.4% was oxidized, with the balance removed in the foam.  $\eta(\text{COD})$  determinations did not account for batch volume losses, and consequently, slightly lower values than those for mass total COD removal were generated.  $\eta(\text{COD})$  oxidized was between 19.2-35.5%, and total  $\eta(\text{COD})$  removed between 54.4-72.6%.
5.  $Y(\text{O}_3/\text{COD})$  was greatly improved by foam removal from the reactor. Between 1 – 6 g  $\text{O}_3$  was consumed per gram COD for the oxidized fraction of COD. Between 0.3-2 g  $\text{O}_3$  was consumed per gram COD for total COD removal at the end of the tests.
6. Water biodegradability index (cBOD<sub>5</sub>/COD) increased with the ozone consumption, ranging between 24.5 and 34.9% higher as tests ended.
7. Decreasing up to six-fold in 30 minutes, the COD/DOC ratio indicated that ozonation produced a high degree of oxidation of organic materials, achieving some degree of mineralization.
8. The volatile fraction of suspended solids was most affected by ozonation, contributing to a TSS removal of 67-77%. Two-stage removal kinetics was observed in five of six tests. The particulate matter was actively removed with foam. Volatile solids were greatly mineralized, resulting in an increase of total/volatile solids ratio in foam. Instead, TDS were not greatly reduced because of their large fixed fraction.
9. Turbidity showed the greatest reduction among all parameters, with a removal between 78-86%. Staging with time was not observed for turbidity, suggesting

- that a longer ozonation could still result in further efficient removal. Ozone's chemical attack on organics and microflocculation of colloidal particles were both believed to have contributed to effective turbidity removal.
10. TKN was very efficiently removed, between 72.0-93.8%. Foam-mediated removal played an important role in TKN reduction, because of the surfactant qualities of the polarized nitrogen-containing organics (e.g., protein and peptide macromolecules) resulting from interaction with ozone. Additionally, some mineralization of TAN and organic N to  $\text{NO}_3^-$ -N could have contributed to lowering TKN, despite an increase of TAN concentration by 13.6-45.5%.
  11. Alkalinity decreased by 15 to 30%. This occurred mostly toward the end of the tests, suggesting that the scavenging effect on the  $\text{OH}^\circ$  radical was not significant when the wastewater was laden with organic pollutant.
  12. Hardness decreased by as much as 25%, suggesting that divalent ions from the wastewater acted as flocculent agents by bonding with colloidal and dissolved macromolecules.
  13. The control test demonstrated that ozone is responsible for the pollutant oxidation and for other water quality changing during ozonation. In addition, ozone is responsible for the abundant foam generation.

Results from this study demonstrated that ozone application becomes limiting when a high quality effluent is desired, in terms of the amount of ozone injected versus the amount transferred and reacting with the wastewater. In the semi-batch reactor mode of operation utilized in this study, ozone transfer efficiency  $\eta(\text{O}_3)$  could be improved with time if the feed rate is lowered as pollutant removal proceeds. Improved ozone transfer efficiency could be obtained for a continuous-flow system by employing multi-stage ozonation in consecutive reactors, each successive reactor being fed with a lower ozone dosage. Pollutant removal could also be improved by pretreatment of the wastewater through efficient sedimentation and/or flocculation. This improvement would be realized because particulate organic matter has substantial ozone consumption capacity, and if present in large amounts, could divert a significant amount of ozone from reacting with dissolved compounds. The foam removal from the reactor observed in this study proved

to be a means by which significant amounts of pollutants could be removed. Foam removal improved most of the ozonation and pollutant removal parameters. For example, COD removal efficiency increased by 200-280% when the oxidation was combined with foam fractionation. The foam stream proved more biodegradable than the effluent, and hence could be used as a source for producing volatile fatty acids for use in fueling the denitrification process.

Based on the range of ozone dosages used and on findings from this study, it is recommended that ozonation of BRA effluent be approximately 9 minutes, and not exceed 15 minutes. This ozonation time should accomplish the desired effect when injecting 23 g O<sub>3</sub>/m<sup>3</sup>. However, this recommendation is based on the highest concentration tested in this study, and therefore, it is possible that better results could be obtained if higher dosages were applied. A high amount of ozone is required for this type of wastewater in order to decrease the organic content to a degree that will allow effective nitrification during subsequent aerobic treatment. To accommodate the TAN generation and improvement of water biodegradability, ozonation should be followed by biological oxidation rather than ozonation being employed as a single treatment process.

The results from this study were used as design data of a two-stage biological/chemical effluent treatment and recovery pilot system (see following chapter). The results from the pilot station were used to establish basic criteria for establishing the most cost-effective application of ozone with BRA wastewater effluent. Pilot system results will assist in optimizing and integrating a full-scale system used to recover effluent otherwise directed for treatment at the municipal treatment facility.

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Table 2.1. Study matrix presenting the ozone dosages supplied at different times during tests. Ozone concentration in influent gas:  $D_{16.9g} = 23 \text{ g/m}^3$ ,  $D_{4.8g} = 16 \text{ g/m}^3$ , and  $D_{2.4g} = 8 \text{ g/m}^3$ .

| Reaction time,<br>(min) | Ozone dosage, (g/ $\Sigma$ time) |            |            |
|-------------------------|----------------------------------|------------|------------|
|                         | $D_{6.9g}$                       | $D_{4.8g}$ | $D_{2.4g}$ |
| 0                       | 0.00                             | 0.00       | 0.00       |
| 3                       | 0.69                             | 0.48       | 0.24       |
| 6                       | 1.38                             | 0.96       | 0.48       |
| 9                       | 2.07                             | 1.44       | 0.72       |
| 15                      | 3.45                             | 2.40       | 1.20       |
| 20                      | 4.60                             | 3.20       | 1.60       |
| 30                      | 6.90                             | 4.80       | 2.40       |

Table 2.2. Initial values of parameters in samples used for tests. In the first trial, samples from different initial batches of wastewater were used. In the second trial, samples from the same initial batch of wastewater were used.

| Parameter                       | Units | Trial/Dosage   |                |                |                                    |
|---------------------------------|-------|----------------|----------------|----------------|------------------------------------|
|                                 |       | $T_1/D_{6.9g}$ | $T_1/D_{4.8g}$ | $T_2/D_{2.4g}$ | $T_2/D_{6.9g, D_{4.8g}, D_{2.4g}}$ |
| COD                             | mg/L  | 360            | 334            | 213            | 307                                |
| cBOD <sub>5</sub>               | mg/L  | 100            | 91             | 60             | 88                                 |
| DOC                             | mg/L  | 38             | 34             | 27             | 30                                 |
| TKN                             | mg/L  | 31.9           | 26.8           | 19.9           | 25.7                               |
| Turbidity                       | NTU   | 70.2           | 66.1           | 52.0           | 62.4                               |
| pH                              | -     | 7.34           | 7.30           | 7.12           | 7.40                               |
| TAN                             | mg/L  | 3.12           | 2.80           | 3.68           | 3.16                               |
| NO <sub>2</sub> <sup>-</sup> -N | mg/L  | 1.04           | 0.83           | 1.20           | 0.90                               |
| NO <sub>3</sub> <sup>-</sup> -N | mg/L  | 48.5           | 39.8           | 22.0           | 54.2                               |
| Alkalinity                      | mg/L  | 114            | 122            | 106            | 118                                |
| Hardness                        | mg/L  | 146            | 142            | 136            | 140                                |
| TS                              | mg/L  | 1441           | 1398           | 1212           | 1400                               |
| VTS                             | mg/L  | 375            | 388            | 295            | 386                                |
| FTS                             | mg/L  | 1066           | 1010           | 917            | 1014                               |
| TSS                             | mg/L  | 229            | 210            | 142            | 218                                |
| VSS                             | mg/L  | 181            | 173            | 112            | 176                                |
| FSS                             | mg/L  | 48             | 37             | 30             | 42                                 |
| TDS                             | mg/L  | 1212           | 1188           | 1070           | 1182                               |
| VDS                             | mg/L  | 194            | 215            | 183            | 210                                |
| FDS                             | mg/L  | 1018           | 937            | 887            | 972                                |

Table 2.3. COD concentration (initial and final), and mass and percent cumulative COD oxidized and removed under ozone doses during the respective tests, following 30 minutes of reaction.

| Trial # | Dosage                   | COD initial<br>g (mg/L) | COD final<br>g (mg/L) | COD reacted<br>g (%) | COD in foam<br>g (%) | Total COD removed<br>g (%) |
|---------|--------------------------|-------------------------|-----------------------|----------------------|----------------------|----------------------------|
| 1*      | <i>D<sub>6.9 g</sub></i> | 12.60 (360)             | 3.19 (112)            | 3.36 (26.7)          | 6.05 (48.0)          | 9.41 (74.7)                |
|         | <i>D<sub>4.8 g</sub></i> | 11.69 (334)             | 3.85 (133)            | 3.07 (26.2)          | 4.77 (40.8)          | 7.84 (67.0)                |
|         | <i>D<sub>2.4 g</sub></i> | 7.46 (213)              | 2.04 (69)             | 1.33 (17.8)          | 4.08 (54.7)          | 5.41 (72.6)                |
| 2**     | <i>D<sub>6.9 g</sub></i> | 10.75 (307)             | 2.43 (84)             | 3.37 (31.3)          | 4.95 (46.1)          | 8.32 (77.4)                |
|         | <i>D<sub>4.8 g</sub></i> | 10.75 (307)             | 3.19 (109)            | 2.91 (27.1)          | 4.64 (43.2)          | 7.55 (70.3)                |
|         | <i>D<sub>2.4 g</sub></i> | 10.75 (307)             | 4.19 (140)            | 1.99 (18.5)          | 4.57 (42.5)          | 6.56 (61.0)                |

\*Samples from different initial batches of wastewater were used for tests.

\*\*Samples from the same initial batch of wastewater were used for tests.

Table 2.4. Ozone injected (concentration and maximum dosage), cumulative mass and total percent consumed and wasted. Test conditions: 35 L water volume, 10 Lpm gas flow rate, 10 psi backpressure, and 30 minutes maximum reaction time.

| Trial # | Dosage                    | O <sub>3</sub> conc.<br>(g/m <sup>3</sup> gas) | O <sub>3</sub> dosage<br>(g/ 30 min) | O <sub>3</sub> consumed |        | O <sub>3</sub> wasted |        |
|---------|---------------------------|--|--------------------------------------|-------------------------|--------|-----------------------|--------|
|         |                           |  |                                      | g                       | (%)    | g                     | (%)    |
| 1*      | <i>D</i> <sub>6.9 g</sub> | 23   | 6.9                                  | 4.57                    | (66.2) | 2.33                  | (33.8) |
|         | <i>D</i> <sub>4.8 g</sub> | 16   | 4.8                                  | 3.36                    | (70.0) | 1.44                  | (30.0) |
|         | <i>D</i> <sub>2.4 g</sub> | 8  | 2.4                                  | 1.73                    | (72.2) | 0.67                  | (28.8) |
| 2**     | <i>D</i> <sub>6.9 g</sub> | 23   | 6.9                                  | 4.36                    | (63.2) | 2.54                  | (36.8) |
|         | <i>D</i> <sub>4.8 g</sub> | 16   | 4.8                                  | 3.37                    | (70.2) | 1.43                  | (28.8) |
|         | <i>D</i> <sub>2.4 g</sub> | 8  | 2.4                                  | 2.04                    | (84.8) | 0.36                  | (15.2) |

\*Samples from different initial batches of wastewater were used for tests.

\*\*Samples from the same initial batch of wastewater were used for tests.

Table 2.5. Cumulative volume of foam ( $V$ ) (percent of the initial volume) collected at various times for each ozone dosage.

| Time (min) | Volume of foam (L) (%) - Trial 1 |              |              | Volume of foam (L) (%) - Trial 2 |              |              |
|------------|----------------------------------|--------------|--------------|----------------------------------|--------------|--------------|
|            | $D_{6.9g}$                       | $D_{4.8g}$   | $D_{2.4g}$   | $D_{6.9g}$                       | $D_{4.8g}$   | $D_{2.4g}$   |
| 0          | 0.00 (0.00)                      | 0.00 (0.00)  | 0.00 (0.00)  | 0.00 (0.00)                      | 0.00 (0.00)  | 0.00 (0.00)  |
| 3          | 2.35 (6.71)                      | 2.15 (6.14)  | 1.55 (4.43)  | 2.00 (5.71)                      | 1.82 (5.20)  | 1.64 (4.46)  |
| 6          | 3.85 (11.00)                     | 3.71 (10.60) | 2.60 (7.43)  | 3.48 (9.94)                      | 3.32 (9.49)  | 2.74 (7.83)  |
| 9          | 5.05 (14.43)                     | 4.71 (13.46) | 3.40 (9.71)  | 4.54 (12.97)                     | 4.29 (12.26) | 3.68 (10.51) |
| 15         | 5.82 (16.63)                     | 5.36 (15.31) | 4.10 (11.71) | 5.36 (15.31)                     | 4.91 (14.03) | 4.68 (13.37) |
| 20         | 6.22 (17.77)                     | 5.78 (16.51) | 4.65 (13.29) | 5.78 (16.51)                     | 5.33 (15.23) | 4.98 (14.23) |
| 30         | 6.48 (18.51)                     | 6.05 (17.29) | 5.37 (15.34) | 6.11 (17.46)                     | 5.71 (16.31) | 5.08 (14.51) |

Table 2.6. Parameters values characterizing ozonation and COD removal (weighted averages and cumulative percents) at different ozone doses.

| Parameter         | Units              | Trial 1    |            |            | Trial 2    |            |            |
|-------------------|--------------------|------------|------------|------------|------------|------------|------------|
|                   |                    | $D_{6.9g}$ | $D_{4.8g}$ | $D_{2.4g}$ | $D_{6.9g}$ | $D_{4.8g}$ | $D_{2.4g}$ |
| $F(O_3)$          | $mg L^{-1}s^{-1}$  | 0.130      | 0.089      | 0.043      | 0.128      | 0.088      | 0.043      |
| $r_A(O_3)$        | $mg L^{-1}s^{-1}$  | 0.095      | 0.069      | 0.033      | 0.091      | 0.067      | 0.038      |
| $r(O_3)$          | $mg L^{-1}s^{-1}$  | 0.094      | 0.068      | 0.033      | 0.088      | 0.066      | 0.038      |
| $I^{*1a}$         | $g O_3 g^{-1} COD$ | 0.314      | 0.195      | 0.145      | 0.374      | 0.212      | 0.095      |
| $I^{*2a}$         | $g O_3 g^{-1} COD$ | 0.184      | 0.134      | 0.091      | 0.225      | 0.144      | 0.064      |
| $A^{*1a}$         | $g O_3 g^{-1} COD$ | 0.191      | 0.130      | 0.098      | 0.213      | 0.140      | 0.079      |
| $A^{*2a}$         | $g O_3 g^{-1} COD$ | 0.117      | 0.091      | 0.064      | 0.135      | 0.098      | 0.053      |
| $\eta(O_3)^a$     | %                  | 73.9       | 77.5       | 77.2       | 71.4       | 76.8       | 87.4       |
| $r(COD)^{1a}$     | $mg L^{-1}s^{-1}$  | 0.138      | 0.112      | 0.080      | 0.124      | 0.110      | 0.093      |
| $r(COD)^{2a}$     | $mg L^{-1}s^{-1}$  | 0.061      | 0.050      | 0.023      | 0.060      | 0.052      | 0.035      |
| $\eta(COD)^{1b}$  | %                  | 68.9       | 60.2       | 67.6       | 72.6       | 64.5       | 54.4       |
| $\eta(COD)^{2b}$  | %                  | 30.4       | 29.4       | 19.2       | 35.3       | 30.4       | 20.6       |
| $Y(O_3/COD)^{1a}$ | $g O_3 g^{-1} COD$ | 1.11       | 0.98       | 0.47       | 0.97       | 0.78       | 0.91       |
| $Y(O_3/COD)^{2a}$ | $g O_3 g^{-1} COD$ | 3.61       | 2.79       | 2.30       | 3.27       | 2.28       | 1.86       |

<sup>1</sup> Total COD removed (oxidized + foam).

<sup>2</sup> COD oxidized by ozone.

<sup>a</sup> Calculated as weighted averages between different times.

<sup>b</sup> Cumulative values over 30 minutes.

Table 2.7. Pairwise comparisons of parameter means at different ozone dosages and reaction times, performed with Tukey's adjustment for multiple comparisons.

| Time (min) | Dosage                    | Parameter           |                    |                       |                                      |   |                    |                           |                    |                    |                    |                  |                    |
|------------|---------------------------|---------------------|--------------------|-----------------------|--------------------------------------|---|--------------------|---------------------------|--------------------|--------------------|--------------------|------------------|--------------------|
|            |                           | $\eta(O_3)$<br>%    | $\eta(COD_t)$<br>% | $\eta(COD)_{ox}$<br>% | $Y(O_3/COD_t)$<br>$g O_3 g^{-1} COD$ | $Y(O_3/COD)_{ox}$<br>$g O_3 g^{-1} COD$ | COD<br>mg/L        | cBOD <sub>5</sub><br>mg/L | Turb.<br>NTU       | Alk.<br>mg/L       | TSS<br>mg/L        | TAN<br>mg/L      | DOC<br>mg/L        |
| 3          | <i>D</i> <sub>6.9 g</sub> | 98.46 <sup>a*</sup> | 19.5 <sup>a</sup>  | 8.8 <sup>a</sup>      | 0.44 <sup>a</sup>                    | 0.72 <sup>b</sup>                       | 266.7 <sup>a</sup> | 82.5 <sup>a</sup>         | 51.2 <sup>a</sup>  | 111.9 <sup>a</sup> | 174.4 <sup>a</sup> | 3.6 <sup>a</sup> | 53.9 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 98.84 <sup>a</sup>  | 17.1 <sup>a</sup>  | 7.3 <sup>a</sup>      | 0.37 <sup>a</sup>                    | 0.62 <sup>a</sup>                       | 270.6 <sup>a</sup> | 81.2 <sup>a</sup>         | 50.6 <sup>a</sup>  | 118.6 <sup>a</sup> | 176.1 <sup>a</sup> | 3.3 <sup>a</sup> | 48.2 <sup>ab</sup> |
|            | <i>D</i> <sub>2.4 g</sub> | 99.00 <sup>a</sup>  | 15.3 <sup>a</sup>  | 4.5 <sup>a</sup>      | 0.25 <sup>a</sup>                    | 0.63 <sup>a</sup>                       | 261.7 <sup>a</sup> | 81.5 <sup>a</sup>         | 49.3 <sup>a</sup>  | 114.0 <sup>a</sup> | 174.9 <sup>a</sup> | 3.5 <sup>a</sup> | 38.5 <sup>b</sup>  |
| 6          | <i>D</i> <sub>6.9 g</sub> | 97.97 <sup>a</sup>  | 36.0 <sup>a</sup>  | 16.8 <sup>a</sup>     | 0.45 <sup>a</sup>                    | 0.81 <sup>a</sup>                       | 218.7 <sup>a</sup> | 75.0 <sup>a</sup>         | 39.5 <sup>a</sup>  | 109.9 <sup>a</sup> | 140.9 <sup>a</sup> | 4.0 <sup>a</sup> | 55.9 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 98.14 <sup>a</sup>  | 32.6 <sup>a</sup>  | 14.3 <sup>a</sup>     | 0.36 <sup>a</sup>                    | 0.67 <sup>a</sup>                       | 229.1 <sup>a</sup> | 75.7 <sup>a</sup>         | 38.8 <sup>a</sup>  | 115.6 <sup>a</sup> | 144.1 <sup>a</sup> | 3.5 <sup>a</sup> | 54.2 <sup>ab</sup> |
|            | <i>D</i> <sub>2.4 g</sub> | 97.81 <sup>a</sup>  | 28.4 <sup>a</sup>  | 8.7 <sup>b</sup>      | 0.25 <sup>a</sup>                    | 0.69 <sup>a</sup>                       | 232.7 <sup>a</sup> | 75.9 <sup>a</sup>         | 41.9 <sup>a</sup>  | 112.0 <sup>a</sup> | 149.9 <sup>a</sup> | 3.8 <sup>a</sup> | 40.5 <sup>b</sup>  |
| 9          | <i>D</i> <sub>6.9 g</sub> | 92.17 <sup>a</sup>  | 51.5 <sup>b</sup>  | 23.7 <sup>a</sup>     | 0.42 <sup>a</sup>                    | 0.92 <sup>a</sup>                       | 167.7 <sup>a</sup> | 63.0 <sup>a</sup>         | 30.3 <sup>a</sup>  | 106.9 <sup>a</sup> | 105.9 <sup>a</sup> | 4.6 <sup>b</sup> | 56.9 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 92.47 <sup>a</sup>  | 44.9 <sup>a</sup>  | 19.7 <sup>a</sup>     | 0.39 <sup>a</sup>                    | 0.84 <sup>a</sup>                       | 191.2 <sup>a</sup> | 66.7 <sup>a</sup>         | 32.0 <sup>a</sup>  | 112.6 <sup>a</sup> | 114.2 <sup>a</sup> | 3.7 <sup>a</sup> | 53.2 <sup>a</sup>  |
|            | <i>D</i> <sub>2.4 g</sub> | 93.03 <sup>a</sup>  | 39.4 <sup>a</sup>  | 11.7 <sup>b</sup>     | 0.28 <sup>a</sup>                    | 0.92 <sup>a</sup>                       | 206.7 <sup>a</sup> | 69.3 <sup>a</sup>         | 34.2 <sup>a</sup>  | 110.5 <sup>a</sup> | 122.4 <sup>a</sup> | 3.9 <sup>a</sup> | 42.5 <sup>a</sup>  |
| 15         | <i>D</i> <sub>6.9 g</sub> | 81.90 <sup>a</sup>  | 63.4 <sup>b</sup>  | 29.0 <sup>a</sup>     | 0.91 <sup>a</sup>                    | 2.18 <sup>a</sup>                       | 125.7 <sup>b</sup> | 49.5 <sup>a</sup>         | 21.0 <sup>a</sup>  | 100.9 <sup>a</sup> | 81.9 <sup>a</sup>  | 5.0 <sup>b</sup> | 56.9 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 84.17 <sup>a</sup>  | 54.8 <sup>a</sup>  | 24.9 <sup>a</sup>     | 0.82 <sup>a</sup>                    | 1.64 <sup>a</sup>                       | 158.6 <sup>a</sup> | 56.7 <sup>a</sup>         | 23.2 <sup>ab</sup> | 107.1 <sup>a</sup> | 93.1 <sup>a</sup>  | 4.0 <sup>a</sup> | 53.2 <sup>ab</sup> |
|            | <i>D</i> <sub>2.4 g</sub> | 85.75 <sup>a</sup>  | 52.3 <sup>a</sup>  | 16.1 <sup>b</sup>     | 0.44 <sup>a</sup>                    | 1.40 <sup>a</sup>                       | 172.2 <sup>a</sup> | 61.3 <sup>a</sup>         | 26.3 <sup>b</sup>  | 106.0 <sup>a</sup> | 107.4 <sup>a</sup> | 4.1 <sup>a</sup> | 40.5 <sup>b</sup>  |
| 20         | <i>D</i> <sub>6.9 g</sub> | 69.71 <sup>a</sup>  | 70.2 <sup>b</sup>  | 31.1 <sup>a</sup>     | 1.04 <sup>a</sup>                    | 3.76 <sup>b</sup>                       | 100.2 <sup>b</sup> | 41.0 <sup>a</sup>         | 15.1 <sup>a</sup>  | 93.4 <sup>a</sup>  | 63.4 <sup>a</sup>  | 4.8 <sup>b</sup> | 52.9 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 75.89 <sup>a</sup>  | 62.5 <sup>a</sup>  | 28.0 <sup>a</sup>     | 0.78 <sup>a</sup>                    | 2.18 <sup>a</sup>                       | 132.1 <sup>a</sup> | 49.2 <sup>ab</sup>        | 17.6 <sup>ab</sup> | 101.6 <sup>a</sup> | 75.7 <sup>b</sup>  | 3.8 <sup>a</sup> | 52.7 <sup>a</sup>  |
|            | <i>D</i> <sub>2.4 g</sub> | 78.56 <sup>a</sup>  | 59.1 <sup>a</sup>  | 18.0 <sup>b</sup>     | 0.61 <sup>a</sup>                    | 2.19 <sup>a</sup>                       | 154.7 <sup>a</sup> | 56.3 <sup>b</sup>         | 21.3 <sup>b</sup>  | 102 <sup>a</sup>   | 93.4 <sup>c</sup>  | 3.9 <sup>a</sup> | 41.5 <sup>a</sup>  |
| 30         | <i>D</i> <sub>6.9 g</sub> | 47.38 <sup>a</sup>  | 76.0 <sup>b</sup>  | 32.8 <sup>a</sup>     | 1.66 <sup>a</sup>                    | 6.40 <sup>a</sup>                       | 78.2 <sup>a</sup>  | 33.5 <sup>a</sup>         | 7.5 <sup>a</sup>   | 85.9 <sup>a</sup>  | 45.9 <sup>a</sup>  | 5.3 <sup>b</sup> | 49.4 <sup>a</sup>  |
|            | <i>D</i> <sub>4.8 g</sub> | 56.17 <sup>a</sup>  | 68.7 <sup>a</sup>  | 29.9 <sup>a</sup>     | 1.41 <sup>a</sup>                    | 4.88 <sup>b</sup>                       | 110.1 <sup>b</sup> | 43.2 <sup>ab</sup>        | 11.3 <sup>ab</sup> | 95.6 <sup>a</sup>  | 61.1 <sup>b</sup>  | 4.2 <sup>a</sup> | 44.2 <sup>a</sup>  |
|            | <i>D</i> <sub>2.4 g</sub> | 69.16 <sup>b</sup>  | 66.8 <sup>a</sup>  | 19.9 <sup>b</sup>     | 1.32 <sup>a</sup>                    | 3.63 <sup>c</sup>                       | 135.2 <sup>c</sup> | 51.8 <sup>b</sup>         | 14.7 <sup>b</sup>  | 97.0 <sup>a</sup>  | 80.4 <sup>c</sup>  | 4.1 <sup>a</sup> | 36.5 <sup>a</sup>  |

\*Means in the same cell with the same superscript are not statistically different ( $p > 0.05$ ).

Table 2.8a. Summary of solids removal parameters for tests using different initial wastewaters.

| Parameter    | <i>D</i> <sub>6.9g</sub> |       |          | <i>D</i> <sub>4.8g</sub> |       |           | <i>D</i> <sub>2.4g</sub> |       |           |
|--------------|--------------------------|-------|----------|--------------------------|-------|-----------|--------------------------|-------|-----------|
|              | Initial                  | Final | %removal | Initial                  | Final | % removal | Initial                  | Final | % removal |
| TS (mg/L)    | 1441                     | 1070  | 26       | 1398                     | 1100  | 21        | 1212                     | 965   | 20        |
| FTS (mg/L)   | 1066                     | 901   | 16       | 1010                     | 920   | 9         | 917                      | 853   | 7         |
| VTS (mg/L)   | 375                      | 169   | 55       | 388                      | 180   | 54        | 295                      | 112   | 62        |
| VTS as % TS  | 26                       | 16    | -        | 28                       | 16    | -         | 24                       | 12    | -         |
| TSS (mg/L)   | 229                      | 65    | 72       | 210                      | 70    | 67        | 142                      | 43    | 70        |
| FSS (mg/L)   | 48                       | 27    | 44       | 42                       | 26    | 38        | 30                       | 18    | 40        |
| VSS (mg/L)   | 181                      | 39    | 79       | 173                      | 44    | 75        | 112                      | 25    | 78        |
| VSS as % TSS | 79                       | 60    | -        | 82                       | 63    | -         | 79                       | 58    | -         |
| TDS (mg/L)   | 1212                     | 1005  | 17       | 1188                     | 1030  | 13        | 1079                     | 922   | 14        |
| FDS (mg/L)   | 1018                     | 874   | 14       | 968                      | 894   | 8         | 887                      | 835   | 6         |
| VDS (mg/L)   | 194                      | 130   | 33       | 215                      | 136   | 37        | 183                      | 87    | 53        |
| VDS as % TDS | 16                       | 13    | -        | 18                       | 13    | -         | 17                       | 9     | -         |

Table 2.8b. Summary of solids removal parameters for tests using identical initial wastewaters.

| Parameter    | Initial | <i>D</i> <sub>6.9 g</sub> |           | <i>D</i> <sub>4.8 g</sub> |           | <i>D</i> <sub>2.4 g</sub> |           |
|--------------|---------|---------------------------|-----------|---------------------------|-----------|---------------------------|-----------|
|              |         | Final                     | % removal | Final                     | % removal | Final                     | % removal |
| TS (mg/L)    | 1400    | 1028                      | 27        | 1079                      | 23        | 1120                      | 20        |
| FTS (mg/L)   | 1014    | 890                       | 12        | 919                       | 9         | 940                       | 7         |
| VTS (mg/L)   | 386     | 138                       | 64        | 160                       | 59        | 180                       | 53        |
| VTS as % TS  | 28      | 13                        | -         | 15                        | -         | 16                        | -         |
| TSS (mg/L)   | 218     | 50                        | 77        | 65                        | 70        | 82                        | 62        |
| FSS (mg/L)   | 42      | 23                        | 45        | 26                        | 38        | 35                        | 17        |
| VSS (mg/L)   | 176     | 27                        | 85        | 39                        | 78        | 53                        | 70        |
| VSS as % TSS | 81      | 54                        | -         | 60                        | -         | 65                        | -         |
| TDS (mg/L)   | 1182    | 978                       | 17        | 1024                      | 14        | 1038                      | 12        |
| FDS (mg/L)   | 972     | 867                       | 11        | 893                       | 8         | 905                       | 7         |
| VDS (mg/L)   | 210     | 111                       | 47        | 121                       | 42        | 133                       | 38        |
| VDS as % TDS | 18      | 11                        | -         | 12                        | -         | 13                        | -         |

Table 2.9. Influence of ozone dosage on turbidity removal pseudo-first order reaction rate constants ( $k'$ ), slope values ( $r^2$ ), turbidity (NTU) at time zero and 30 minutes, and percent turbidity removal at the end of the reaction period.

| O <sub>3</sub> dosage<br>(g/0.5 h) | $k'$<br>(h <sup>-1</sup> ) | $r^2$ | NTU <sub>0</sub> <sup>1</sup> | NTU <sub>30</sub> | % removal |
|------------------------------------|----------------------------|-------|-------------------------------|-------------------|-----------|
| 6.9                                | 3.86                       | 0.99  | 70.2                          | 10.4              | 85        |
| 4.8                                | 3.29                       | 0.98  | 66.1                          | 12.9              | 81        |
| 2.4                                | 3.57                       | 0.99  | 52.0                          | 8.5               | 84        |
| 6.9*                               | 3.79                       | 0.99  | 62.4                          | 9.0               | 86        |
| 4.8*                               | 3.22                       | 0.98  | 62.4                          | 12.2              | 86        |
| 2.4*                               | 3.00                       | 0.99  | 62.4                          | 13.9              | 78        |

\* Indicates tests using samples from the same initial batch of wastewater;

<sup>1</sup>The subscript indicates the time of reaction when was recorded the value (min).

Table 2.10. Influence of ozone dosage on COD oxidation pseudo-first order reaction rate constants ( $k'$ ), slope values ( $r^2$ ), and COD % oxidation at the end of the reaction period.

| O <sub>3</sub> dosage<br>(g/0.5 h) | Test intervals 1 to 4 (0 to 9 min) |       |               | Test intervals 5 to 7 (15 to 30 min) |       |               |
|------------------------------------|------------------------------------|-------|---------------|--------------------------------------|-------|---------------|
|                                    | $k'$<br>(h <sup>-1</sup> )         | $r^2$ | % COD removal | $k'$<br>(h <sup>-1</sup> )           | $r^2$ | % COD removal |
| 6.9                                | 1.67                               | 0.99  | 20            | 0.18                                 | 0.94  | 27            |
| 4.8                                | 1.60                               | 0.99  | 18            | 0.21                                 | 0.91  | 24            |
| 2.4                                | 0.88                               | 0.99  | 7             | 0.18                                 | 0.96  | 11            |
| 6.9*                               | 1.94                               | 1.00  | 20            | 0.24                                 | 0.92  | 27            |
| 4.8*                               | 1.40                               | 0.99  | 15            | 0.30                                 | 0.89  | 23            |
| 2.4*                               | 0.79                               | 0.99  | 9             | 0.17                                 | 0.97  | 16            |

\* Indicates tests using samples from the same initial batch of wastewater.

Table 2.11. Influence of ozone dosage on total COD removal (oxidized plus foam) pseudo-first order reaction rate constants ( $k'$ ), slope values ( $r^2$ ), and percent COD removal at the end of the reaction period.

| O <sub>3</sub> dosage<br>(g/0.5 h) | Test intervals 1 to 4 (0 to 9 min) |       |               | Test intervals 5 to 7 (15 to 30 min) |       |               |
|------------------------------------|------------------------------------|-------|---------------|--------------------------------------|-------|---------------|
|                                    | $k'$<br>(h <sup>-1</sup> )         | $r^2$ | % COD removal | $k'$<br>(h <sup>-1</sup> )           | $r^2$ | % COD removal |
| 6.9                                | 3.84                               | 0.98  | 52.           | 1.31                                 | 0.94  | 75            |
| 4.8                                | 3.16                               | 0.99  | 46.           | 1.02                                 | 0.94  | 67            |
| 2.4                                | 2.80                               | 0.99  | 41            | 2.13                                 | 0.99  | 73            |
| 6.9*                               | 2.67                               | 0.96  | 51            | 1.78                                 | 0.97  | 77            |
| 4.8*                               | 2.47                               | 0.99  | 44            | 1.60                                 | 0.966 | 70            |
| 2.4*                               | 2.11                               | 0.97  | 38            | 0.62                                 | 0.91  | 61            |

\* Indicates tests using samples from the same initial batch of wastewater.

Table 2.12. Influence of ozone dosage on cBOD<sub>5</sub> removal (oxidized plus foam) pseudo-first order reaction rate constants ( $k'$ ), slope values ( $r^2$ ), and percent cBOD<sub>5</sub> removal at the end of the reaction period.

| O <sub>3</sub> dosage<br>(g/0.5 h) | Test intervals 1 to 4 (0 to 9 min) |       |               | Test intervals 5 to 7 (15 to 30 min) |       |               |
|------------------------------------|------------------------------------|-------|---------------|--------------------------------------|-------|---------------|
|                                    | $k'$<br>(h <sup>-1</sup> )         | $r^2$ | % COD removal | $k'$<br>(h <sup>-1</sup> )           | $r^2$ | % COD removal |
| 6.9                                | 3.18                               | 0.97  | 38            | 1.14                                 | 0.93  | 64            |
| 4.8                                | 2.59                               | 0.99  | 33            | 0.93                                 | 0.92  | 56            |
| 2.4                                | 2.59                               | 0.99  | 32            | 1.59                                 | 0.97  | 62            |
| 6.9*                               | 2.65                               | 0.99  | 33            | 1.63                                 | 0.97  | 65            |
| 4.8*                               | 2.40                               | 0.98  | 30            | 1.19                                 | 0.95  | 56            |
| 2.4*                               | 2.08                               | 0.98  | 27            | 0.50                                 | 0.91  | 47            |

\* Indicates tests using samples from the same initial batch of wastewater.

Table 2.13. Pearson's correlation matrix for water quality parameters. The top value in each cell represents the correlation coefficient, the second value represents the  $p$ -value, and the third value represents the sample size.

| Parameter         |                     | COD        | DOC      | cBOD <sub>5</sub> | Turbidity  | TAN        | Alkalinity | TKN       | TSS |
|-------------------|---------------------|------------|----------|-------------------|------------|------------|------------|-----------|-----|
| COD               | Pearson Correlation | 1          |          |                   |            |            |            |           |     |
|                   | $p$ -value          | .          |          |                   |            |            |            |           |     |
|                   | $n$                 | 36         |          |                   |            |            |            |           |     |
| DOC               | Pearson Correlation | 0.264      | 1        |                   |            |            |            |           |     |
|                   | $p$ -value          | 0.120      | .        |                   |            |            |            |           |     |
|                   | $n$                 | 36         | 36       |                   |            |            |            |           |     |
| cBOD <sub>5</sub> | Pearson Correlation | 0.969(**)  | 0.401(*) | 1                 |            |            |            |           |     |
|                   | $p$ -value          | 0.000      | 0.015    | .                 |            |            |            |           |     |
|                   | $n$                 | 36         | 36       | 36                |            |            |            |           |     |
| Turbidity         | Pearson Correlation | 0.967(**)  | 0.193    | 0.903(**)         | 1          |            |            |           |     |
|                   | $p$ -value          | 0.000      | 0.260    | 0.000             | .          |            |            |           |     |
|                   | $n$                 | 36         | 36       | 36                | 36         |            |            |           |     |
| TAN               | Pearson Correlation | -0.724(**) | 0.144    | -0.603(**)        | -0.742(**) | 1          |            |           |     |
|                   | $p$ -value          | 0.000      | 0.402    | 0.000             | 0.000      | .          |            |           |     |
|                   | $n$                 | 36         | 36       | 36                | 36         | 36         |            |           |     |
| Alkalinity        | Pearson Correlation | 0.822(**)  | 0.290    | 0.829(**)         | 0.812(**)  | -0.761(**) | 1          |           |     |
|                   | $p$ -value          | 0.000      | 0.086    | 0.000             | 0.000      | 0.000      | .          |           |     |
|                   | $n$                 | 36         | 36       | 36                | 36         | 36         | 36         |           |     |
| TKN               | Pearson Correlation | 0.975(**)  | 0.112    | 0.923(**)         | 0.974(**)  | -0.754(**) | 0.805(**)  | 1         |     |
|                   | $p$ -value          | 0.000      | 0.516    | .000              | .000       | 0.000      | 0.000      | .         |     |
|                   | $n$                 | 36         | 36       | 36                | 36         | 36         | 36         | 36        |     |
| TSS               | Pearson Correlation | 0.985(**)  | 0.225    | 0.952(**)         | 0.973(**)  | -0.710(**) | 0.819(**)  | 0.981(**) | 1   |
|                   | $p$ -value          | 0.000      | 0.187    | 0.000             | 0.000      | 0.000      | 0.000      | 0.000     | .   |
|                   | $n$                 | 36         | 36       | 36                | 36         | 36         | 36         | 36        | 36  |

\*Indicates  $0.05 > p > 0.01$ , \*\*Indicates  $p < 0.01$

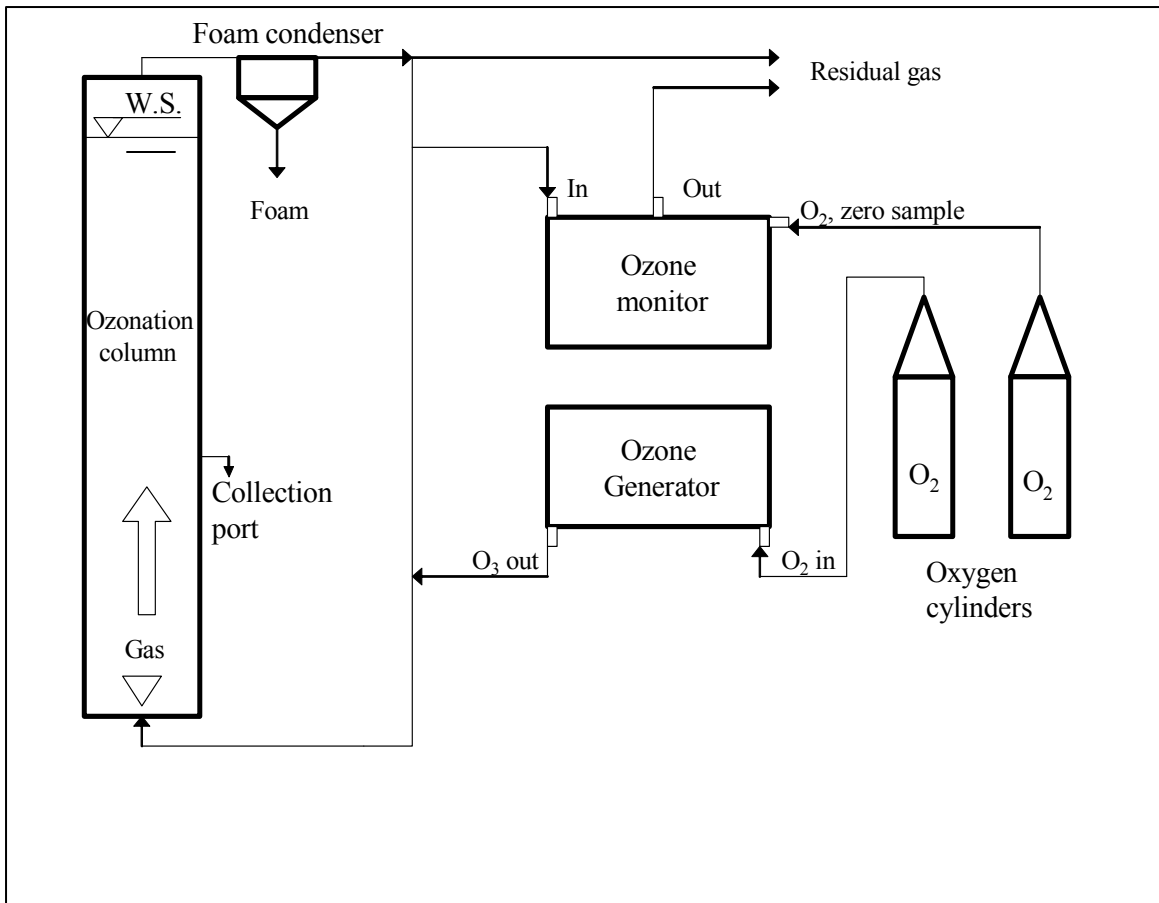


Figure 2.1. Schematic diagram of gas flow in the experimental installation used for the treatability study with ozone. W.S. = free water surface.

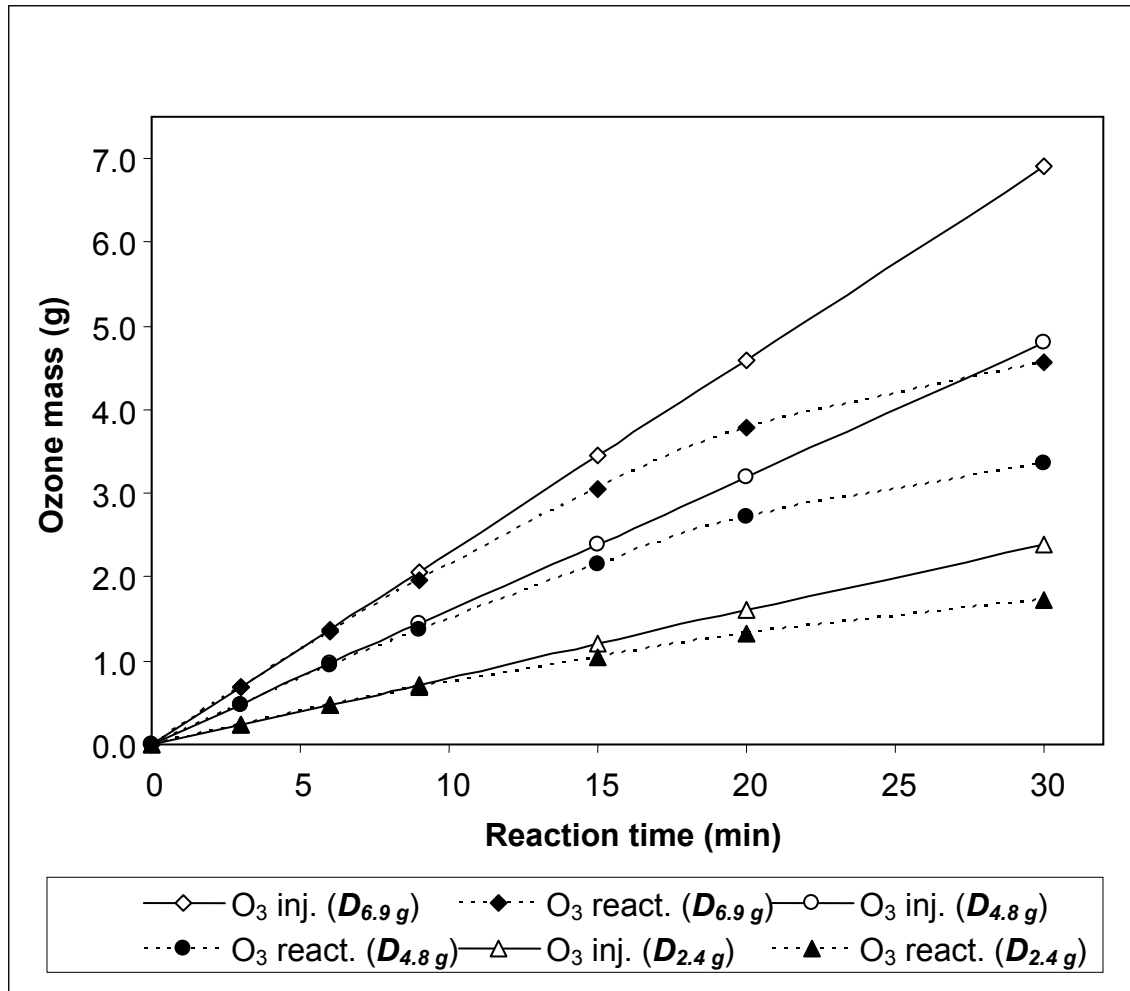


Figure 2.2a. Mass of ozone injected and reacted for three ozone dosages applied in trials using different wastewater.

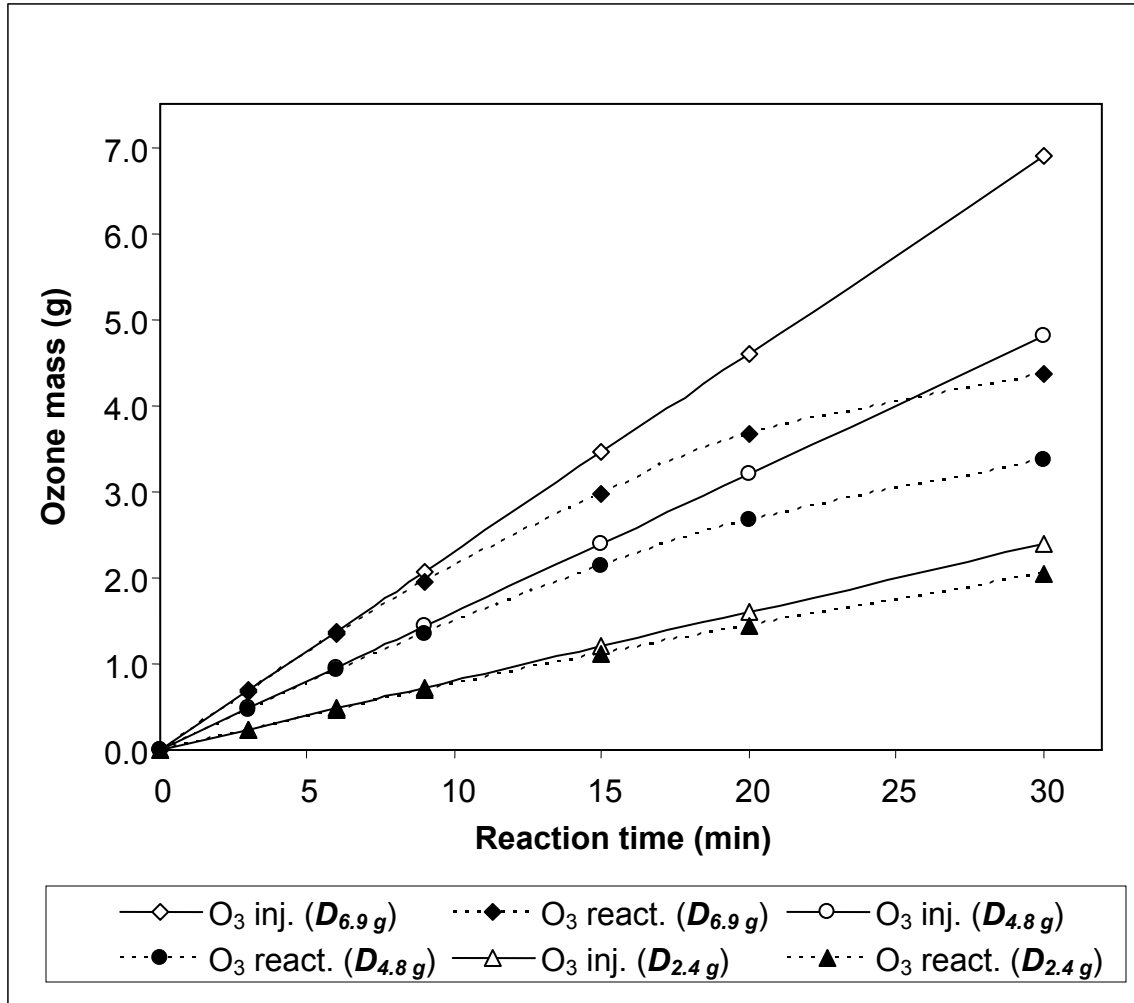


Figure 2.2b. Mass of ozone injected and reacted for three ozone dosages applied in trials using identical wastewater.

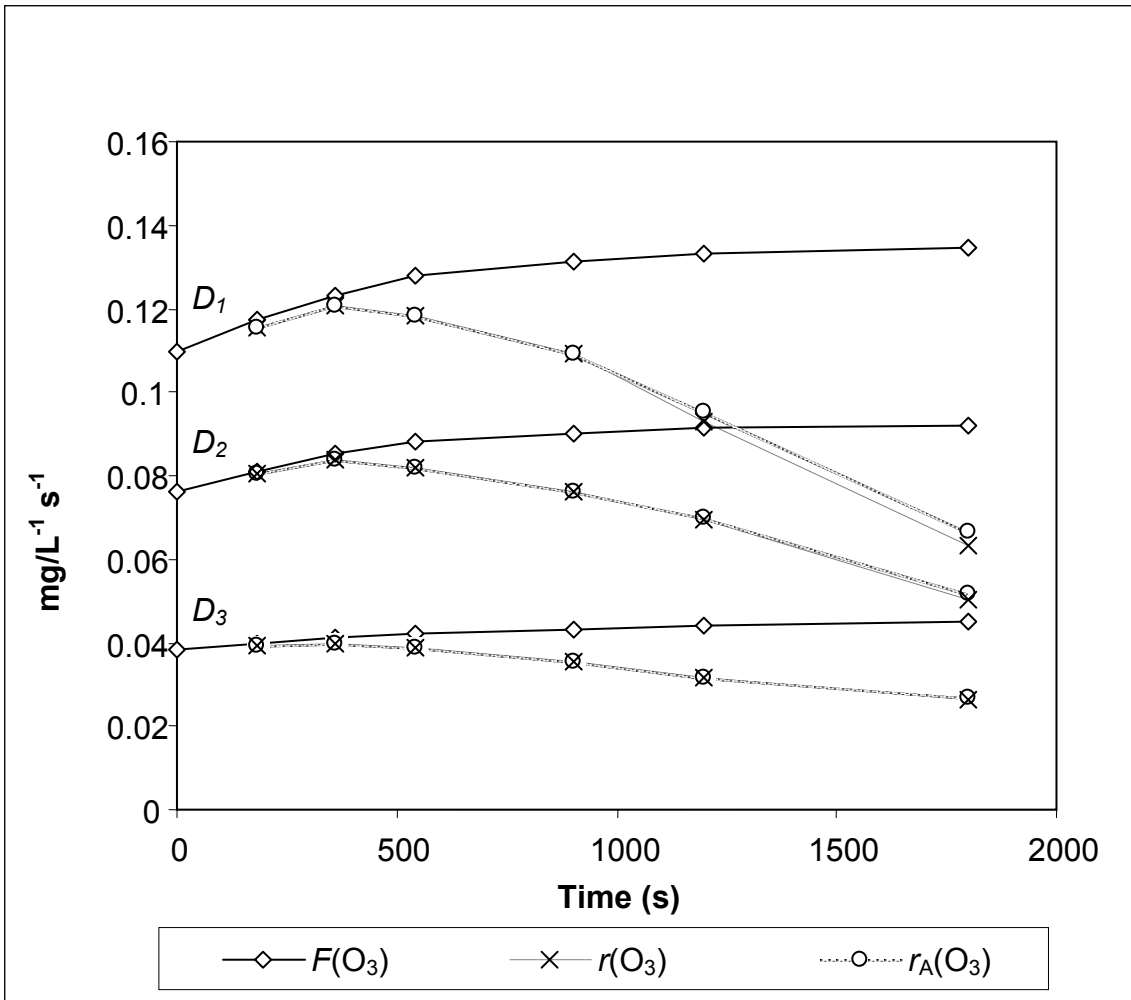


Figure 2.3a. Rates of ozone feed  $[F(O_3)]$ , consumption  $[r(O_3)]$ , and absorption  $[r_A(O_3)]$  for the three ozone dosages applied in trials using different wastewaters.

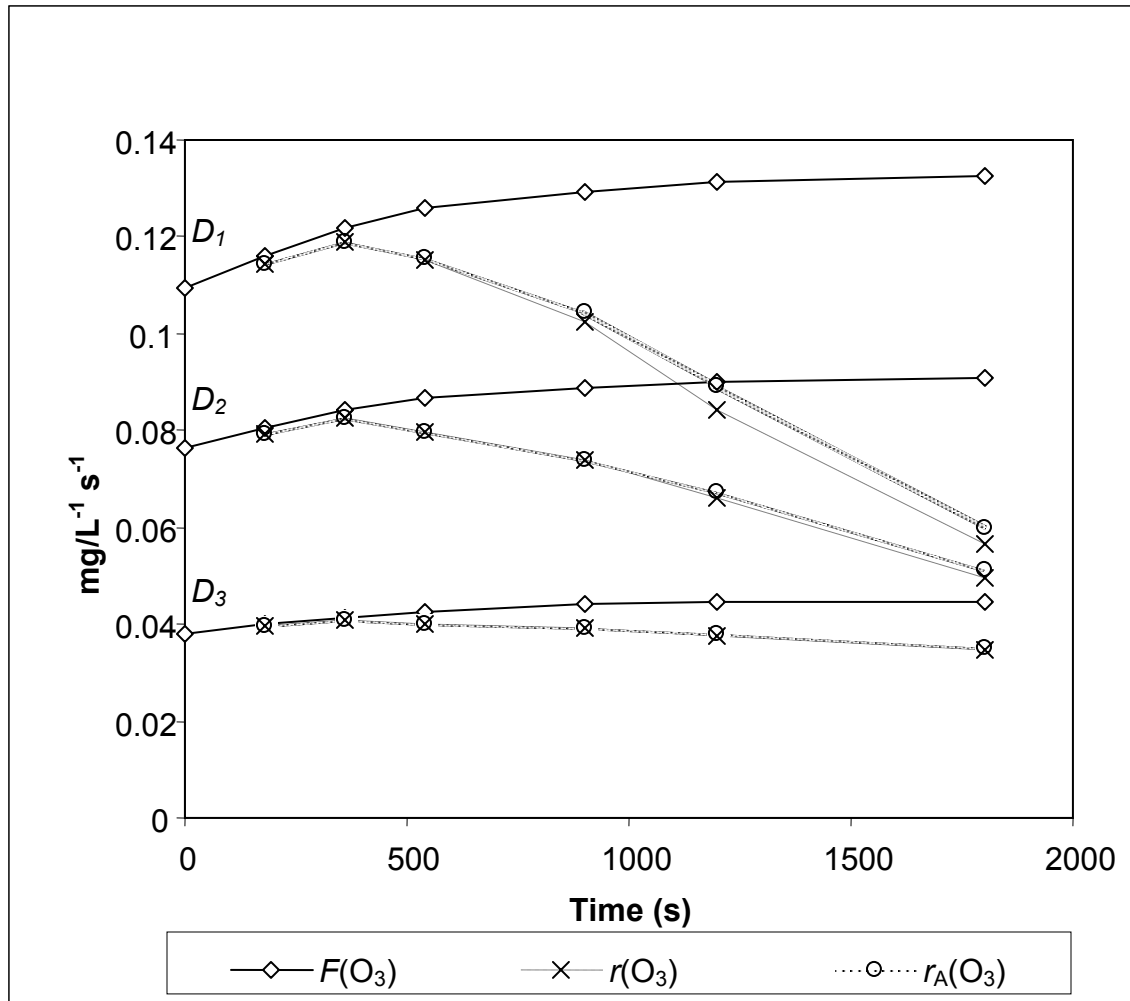


Figure 2.3b. Rates of ozone feed  $[F(O_3)]$ , consumption  $[r(O_3)]$ , and absorption  $[r_A(O_3)]$  for the three ozone dosages applied in trials using identical wastewaters.

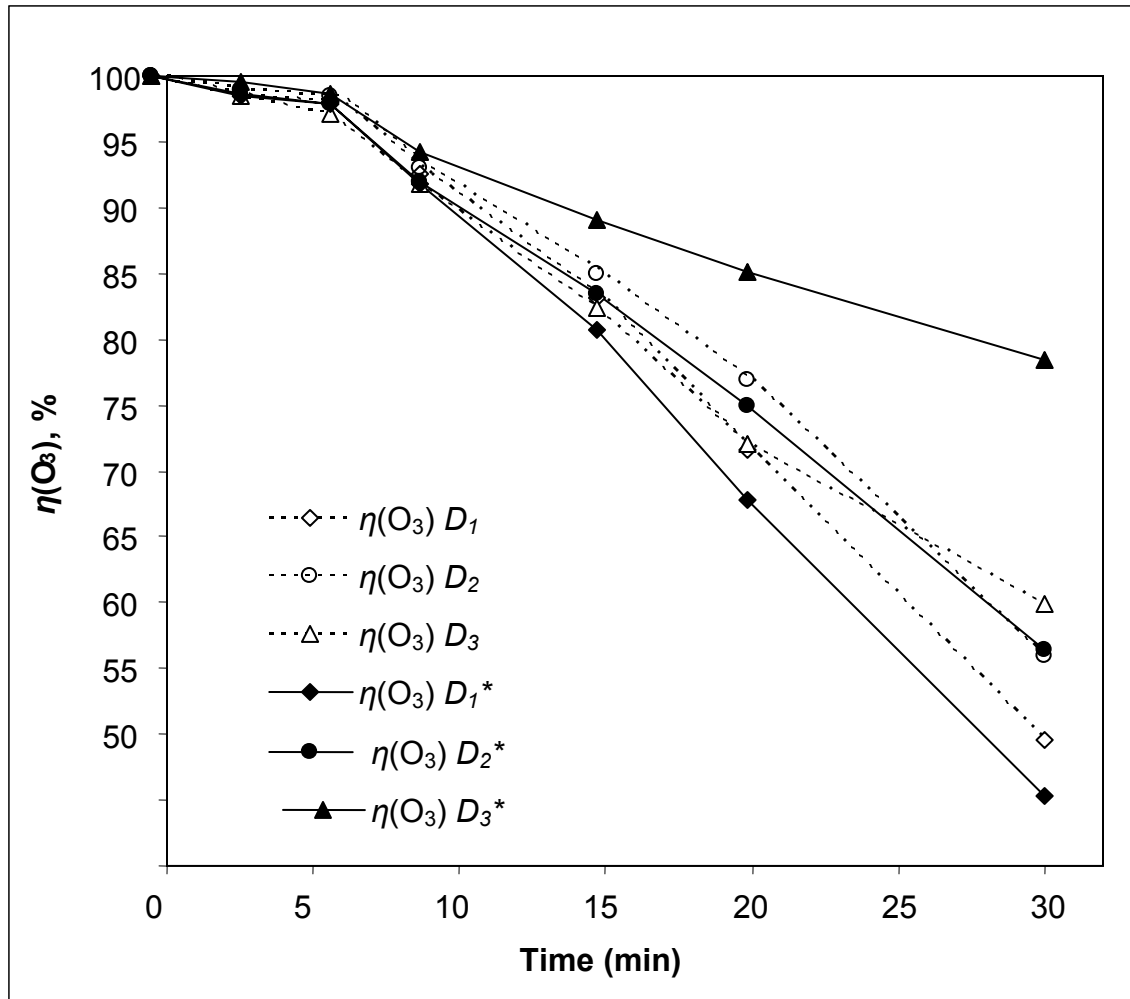


Figure 2.4. Ozone transfer efficiency [ $\eta(O_3)$ ] variation over time for three ozone dosages applied in trials using either different or identical wastewater.  
 \* Indicates tests using samples from the same initial wastewater.

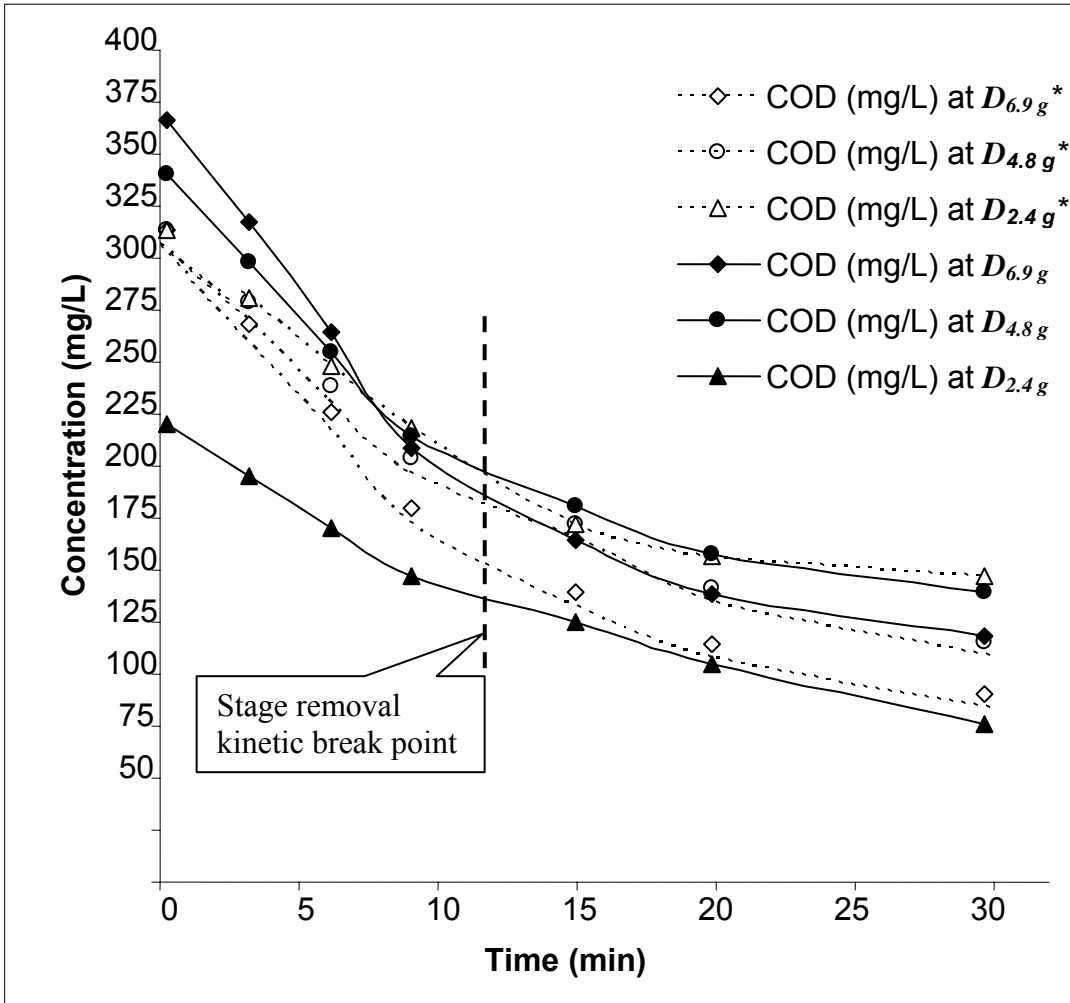


Figure 2.5. COD concentration over time for three ozone dosages applied in trials using either different or identical wastewaters.

\* Indicates tests using samples from the same initial wastewater.

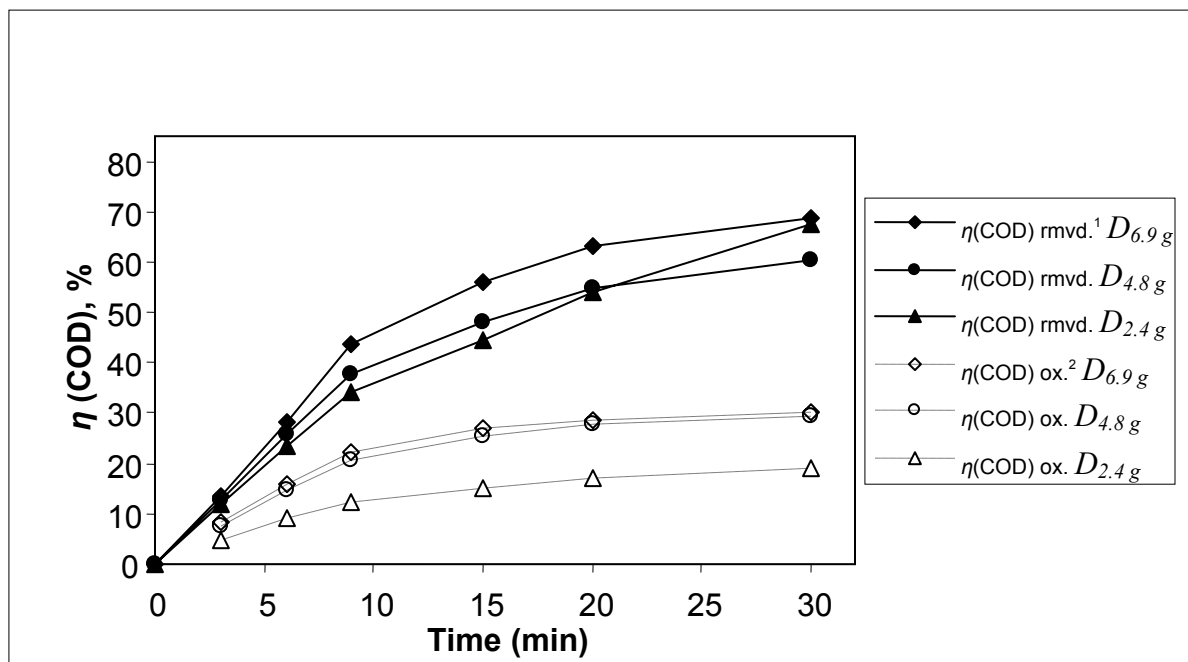


Figure 2.6a. COD removal efficiency [ $\eta(\text{COD})$ ] (<sup>1</sup>total removed and <sup>2</sup>oxidized) for three ozone dosages applied in trials using different wastewaters.

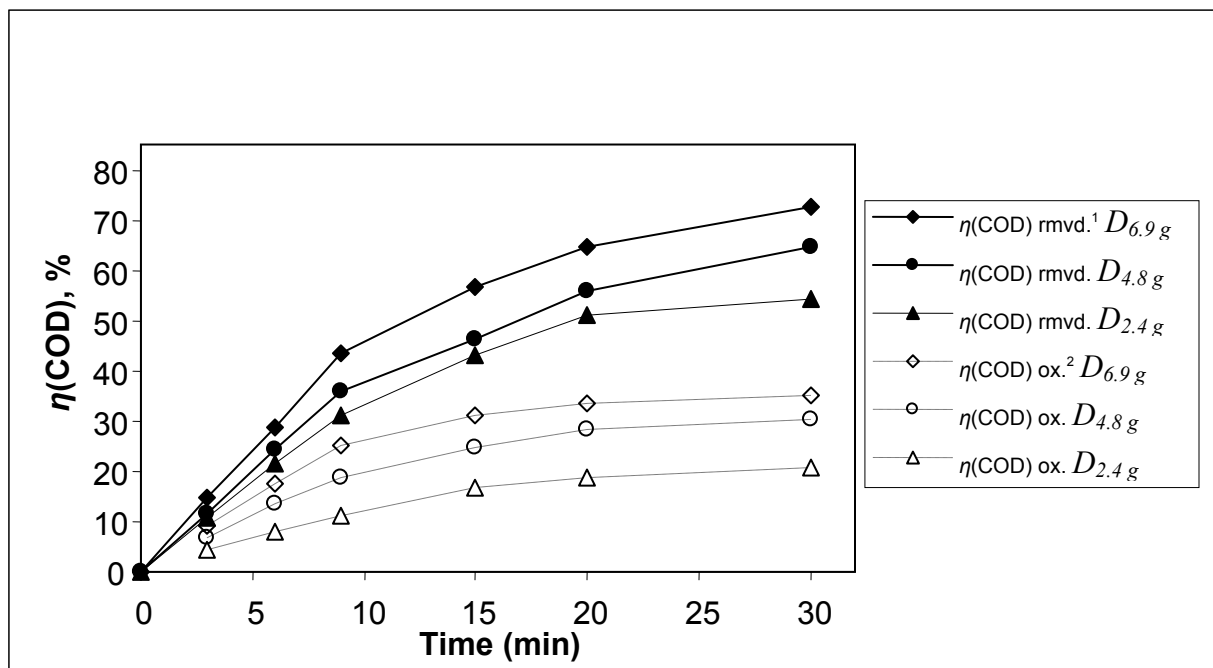


Figure 2.6b. COD removal efficiency [ $\eta(\text{COD})$ ] (<sup>1</sup>total removed and <sup>2</sup>oxidized) for three ozone dosages applied in trials using identical wastewater.

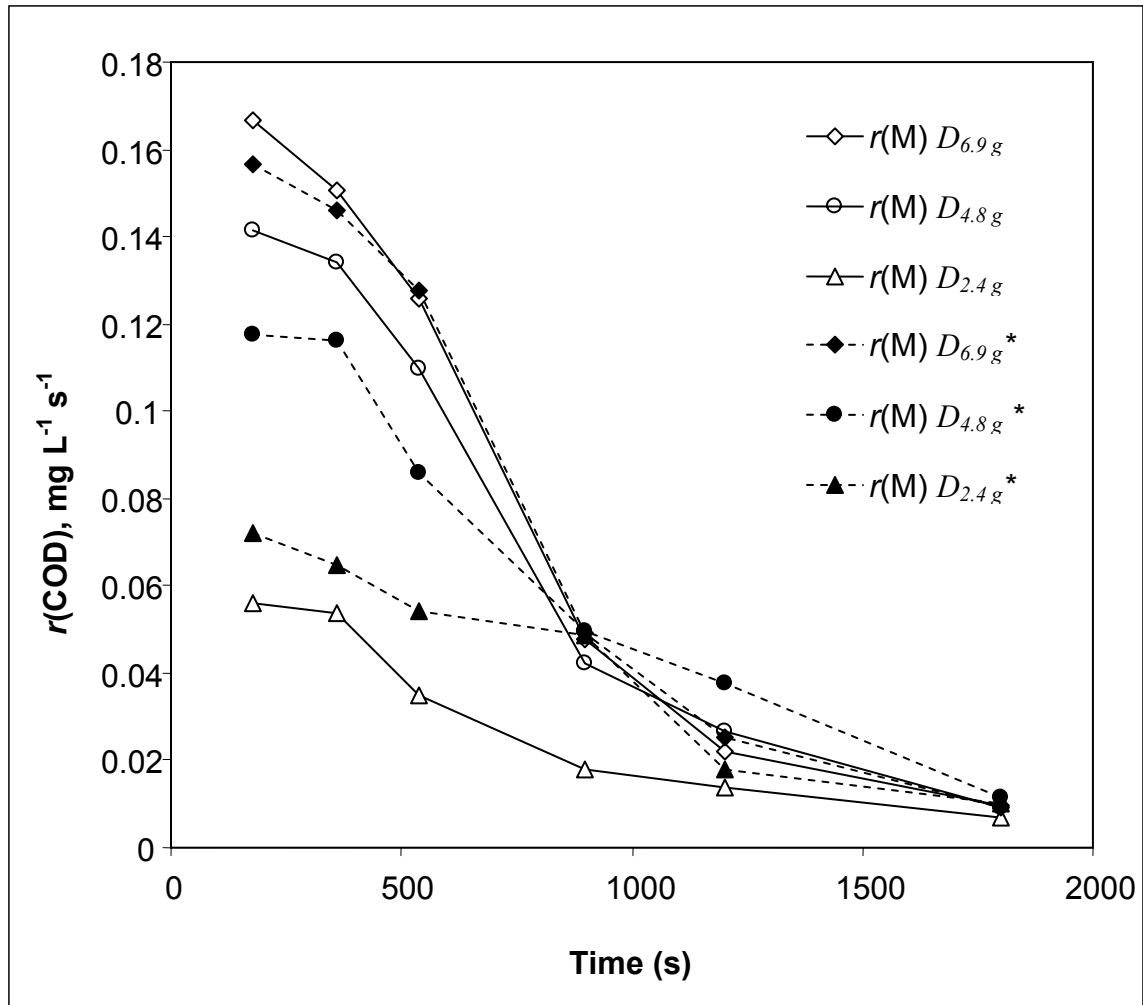


Figure 2.7. COD removal rate [ $r(\text{COD})$ ] for three ozone dosages applied, determined as COD removed by oxidation.

\* Indicates tests using samples from the same wastewater.

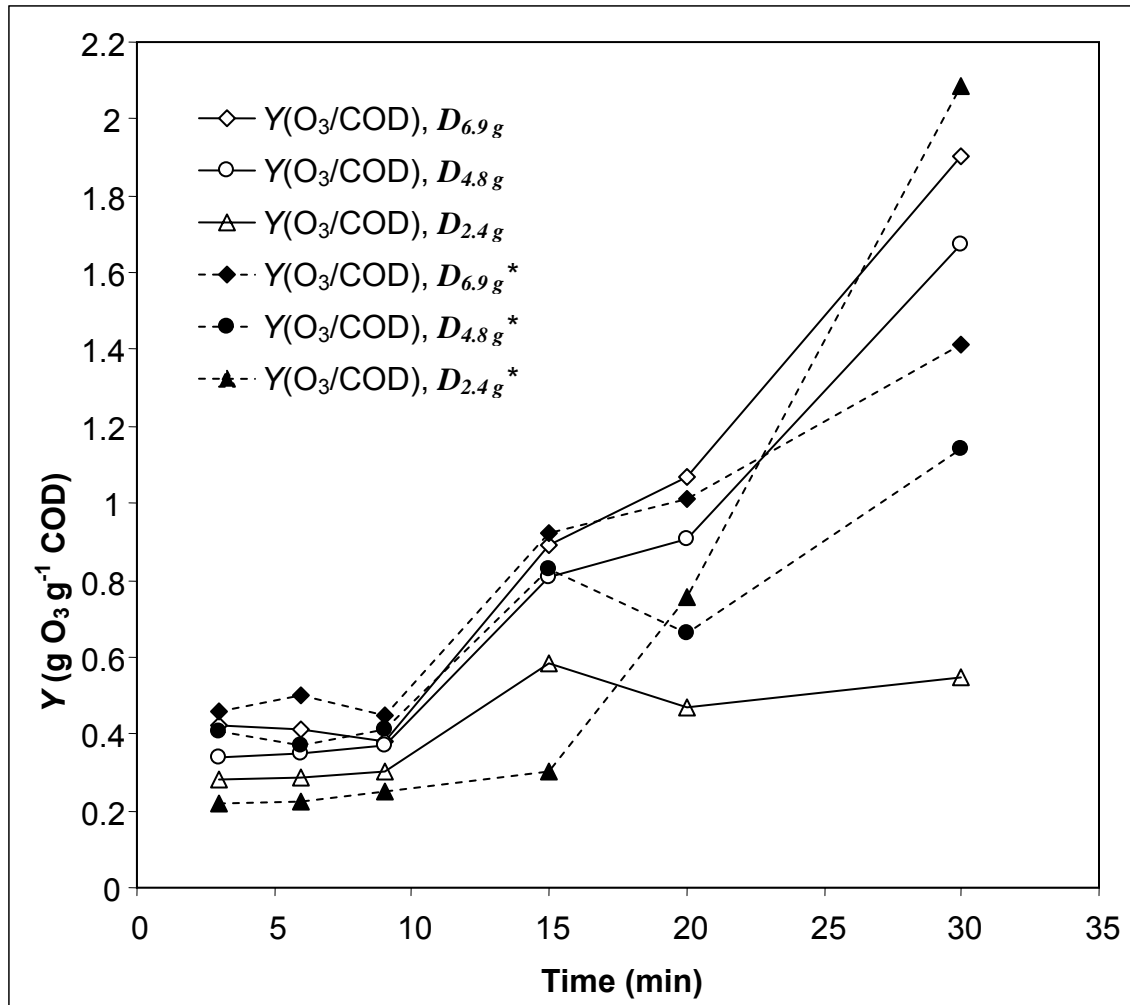


Figure 2.8a. Ozone consumption coefficient [ $Y(O_3/COD)$ ] over time for total COD removal (foam + oxidation) for three ozone dosages applied in trials using either different or identical wastewaters.

\* Indicates tests using samples from the same wastewater.

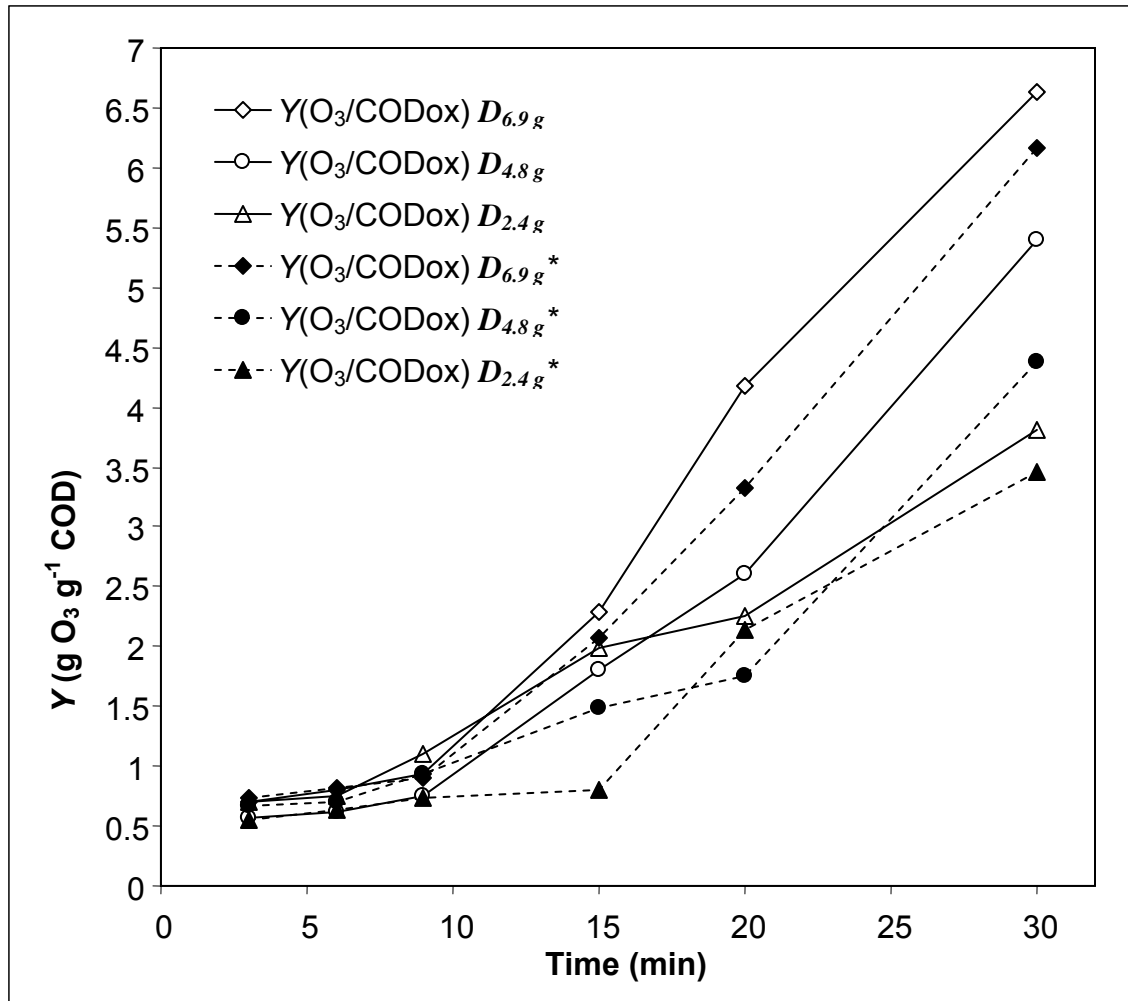


Figure 2.8b. Ozone consumption coefficient [ $Y(\text{O}_3/\text{COD})$ ] over time for COD removal by oxidation for three ozone dosages applied in trials using either different or identical wastewaters.

\* Indicates tests using samples from the same wastewater.

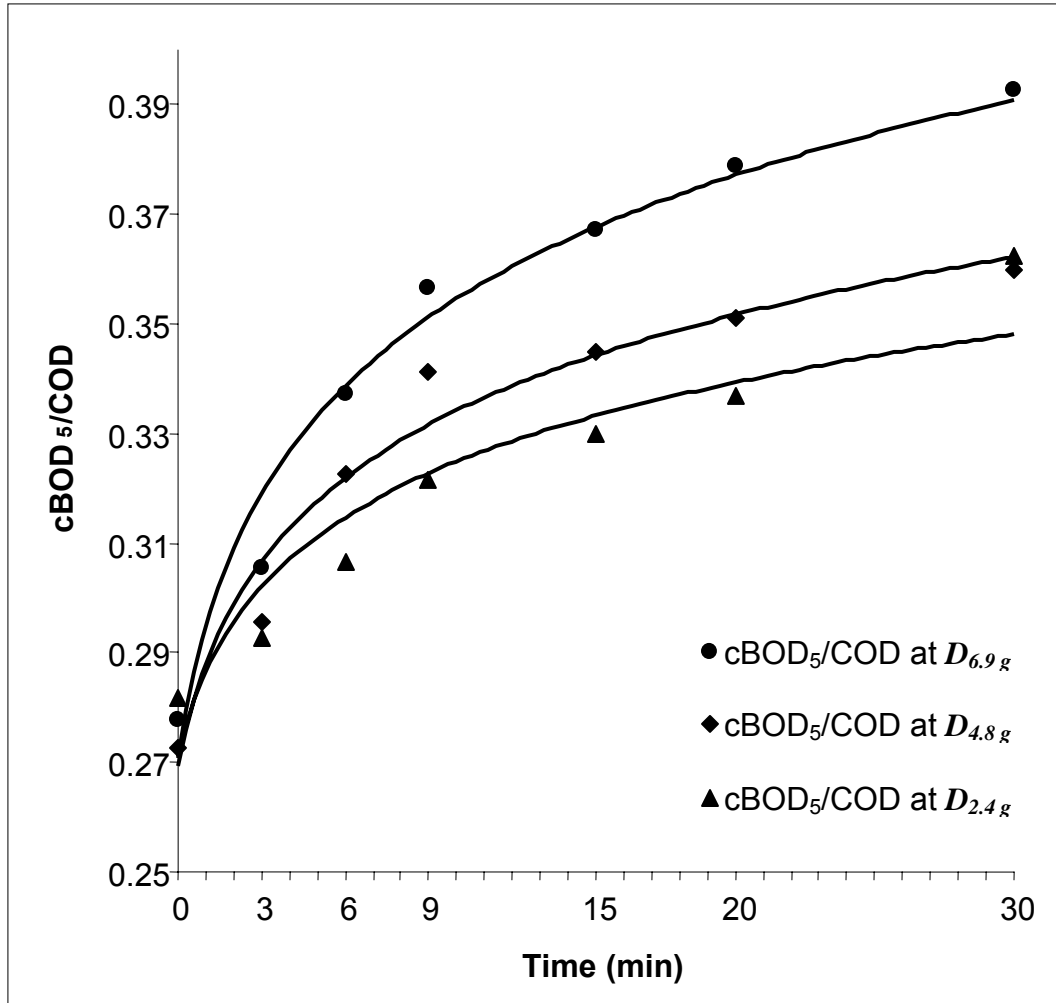


Figure 2.9a. cBOD<sub>5</sub>/COD ratio over time for three ozone dosages applied for trials using different wastewaters. The curves represent the exponential regression line between values measured at different reaction times.

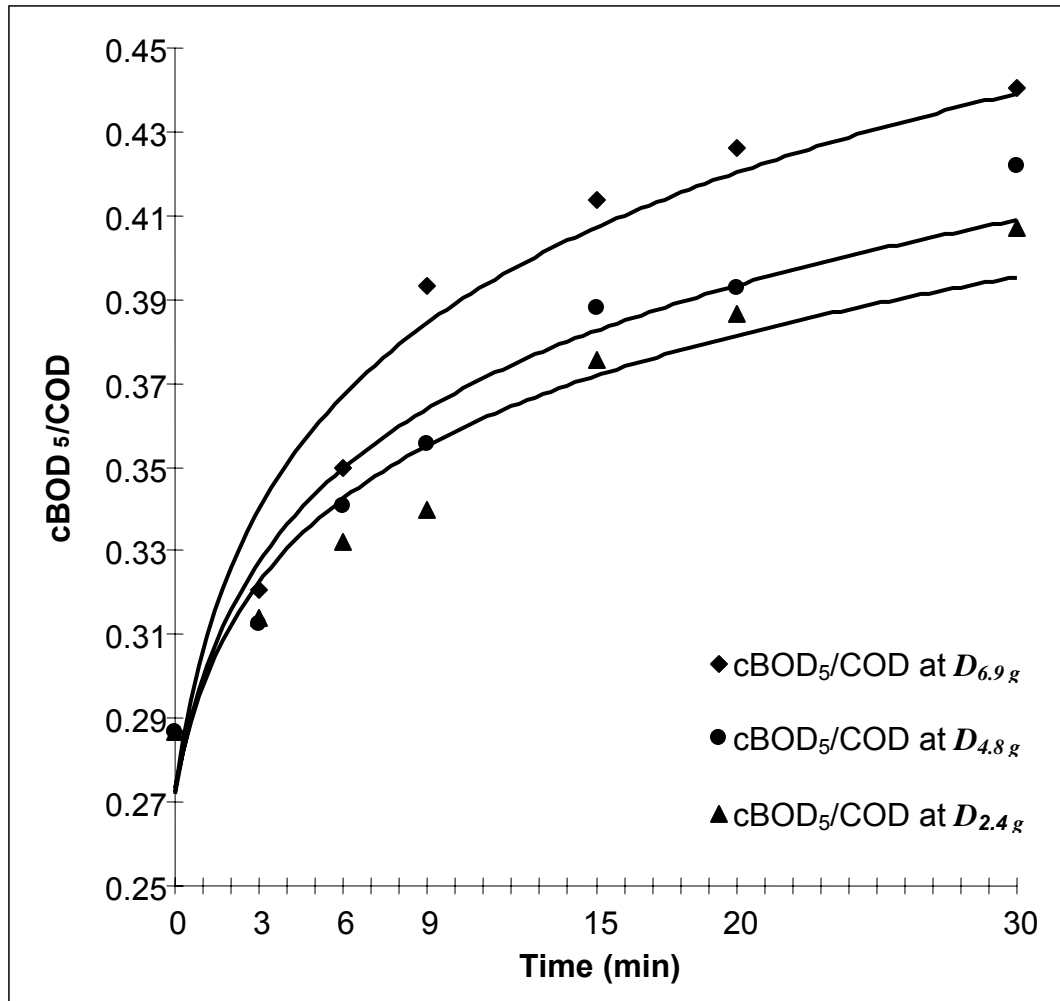


Figure 2.9b. cBOD<sub>5</sub>/COD ration over time for three ozone dosages applied for trials using identical wastewater. The curves represent the exponential regression line between values measured at different reaction times.

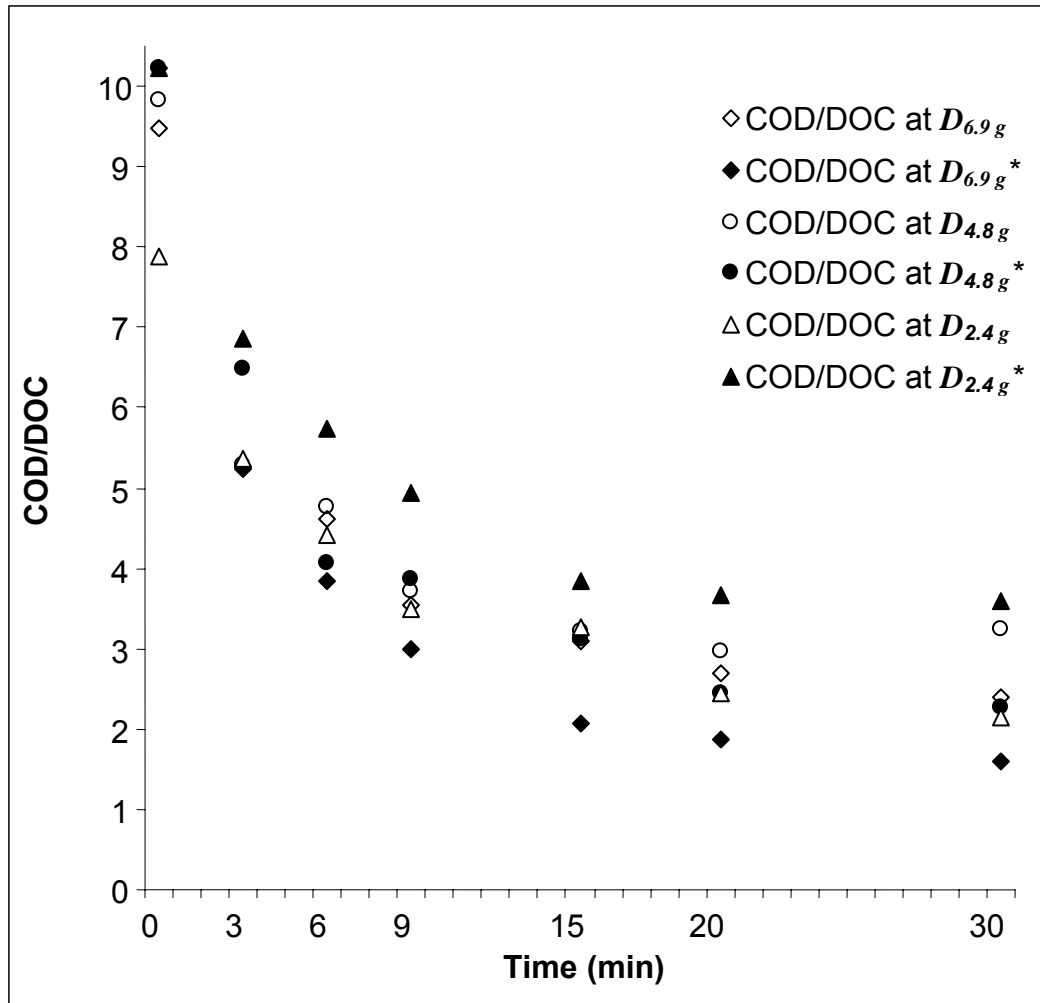


Figure 2.10. Change of COD/DOC ratio over time for three ozone dosages applied in trials using different or identical wastewaters.

\* Indicate tests using samples from the same wastewater.

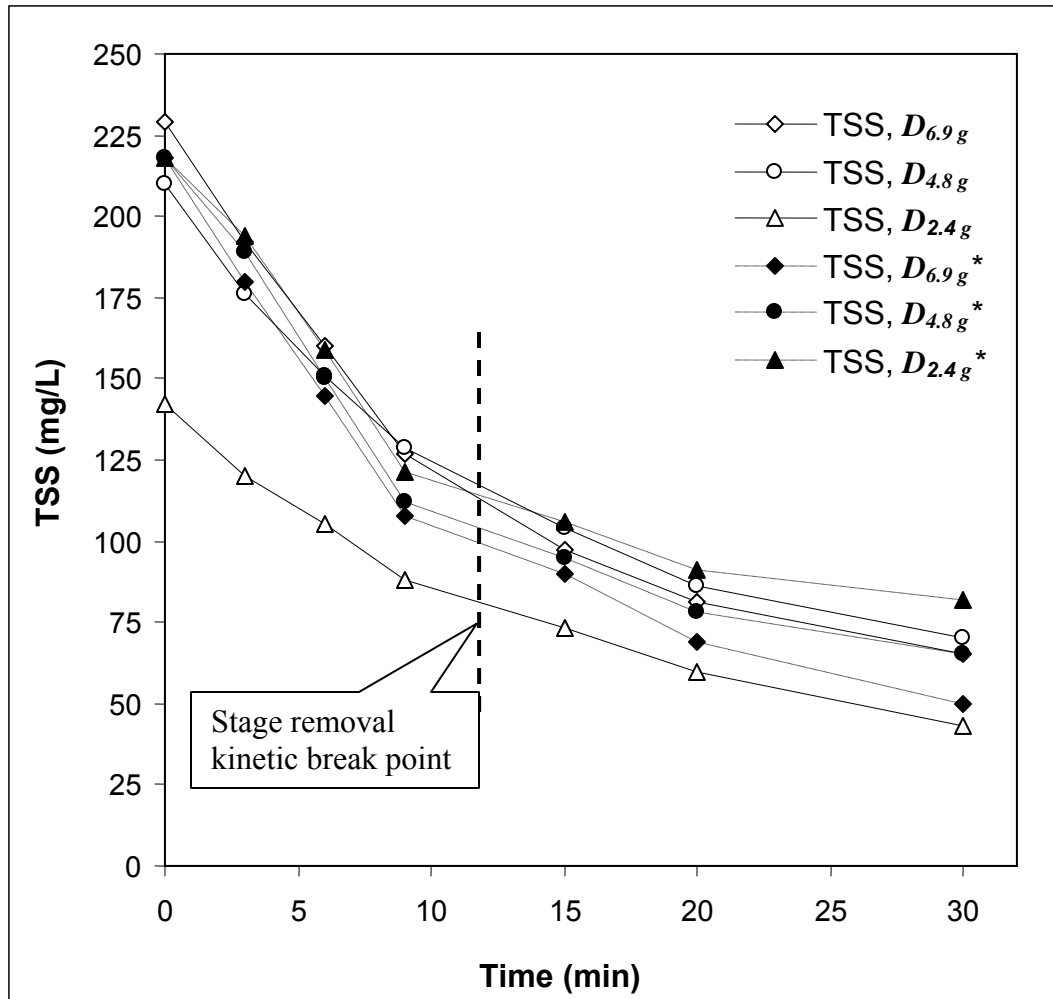


Figure 2.11. Changes in TSS concentration over time for three ozone dosages applied in trials using either different or identical wastewaters.  
 \* Indicate using samples from the same wastewater.

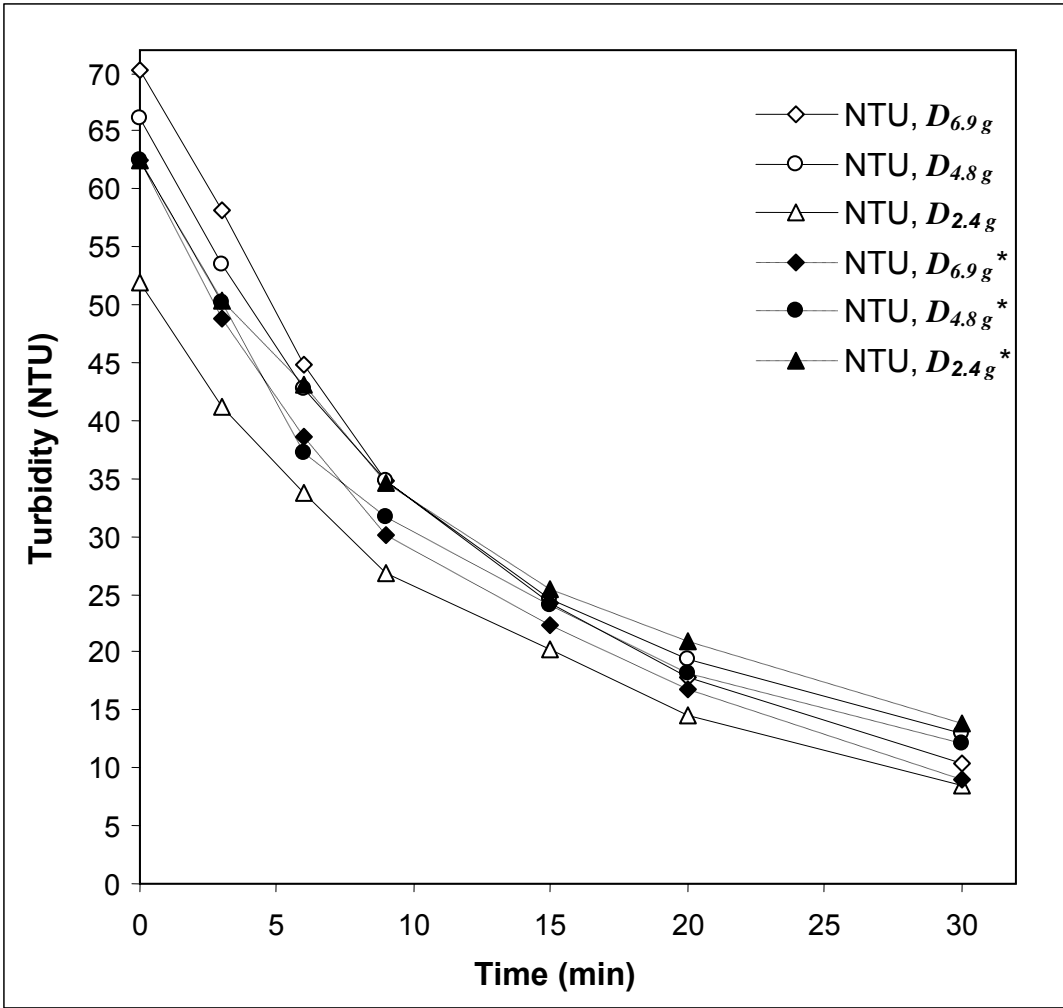


Figure 2.12. Changes in turbidity over time.  
 \* Indicate tests using samples from the same wastewater.

### Chapter 3. EFFICACY OF A PILOT-SCALE WASTEWATER TREATMENT PLANT FOR BLUE RIDGE AQUACULTURE

#### ABSTRACT

A pilot-scale wastewater treatment station was built and operated at Blue Ridge Aquaculture (BRA) in order to initiate, characterize, and optimize the operation of a treatment strategy for effluent recovery and reuse. Solid, dissolved organic, and nitrogenous compounds were the pollutants targeted in this study. The sequential treatment process employed physical (sedimentation basin), anaerobic biological (denitrification reactor), chemical (ozonation reactor), and aerobic biological (trickling filter) treatment processes, followed by (jar test) chemical flocculation. The study consisted of four different experimental stages, differentiated by alternative use of 6 or 4 Lpm flow and recycling rates, ozone doses between 36.6 – 82.5 mg O<sub>3</sub>/L water, and 6- or 9-minute ozonation time.

Over 70% of solids were removed by sedimentation in all experimental stages. At the end of treatment, up to 99% of TSS was removed due to the combined action of ozonation and chemical flocculation. COD removal (59.2-62.7%) was not significantly different among the stages in the sedimentation basin ( $p>0.05$ ), but was positively correlated with ozone dose (slope = 0.452,  $r^2 = 0.99$ ), yielding total COD removal  $\eta(\text{CODt})$  of 19.8-40.7%. Of these amounts, 60.4-66.5% of COD was removed with foam, while the balance was mineralized. Ozone reactivity was 83.7% at a dose of 82.5 mg O<sub>3</sub>/L water. The ozone yield coefficient  $Y(\text{O}_3/\text{CODox})$  for COD oxidized was 1.92-2.23 g O<sub>3</sub> g<sup>-1</sup> COD, and 0.70-0.78 g O<sub>3</sub> g<sup>-1</sup> COD when total COD removed was considered. Overall, 87.9-92.4% of COD was removed by the treatment train, to an average of 44 mg/L in the experimental stage using the highest ozone dose. In that same stage, cBOD<sub>5</sub> was reduced by 88% (to 9 mg/L), while the water's organic biodegradability increased by over 20%. DOC did not change significantly through the treatment train, and experienced a large fluctuation throughout the system due to methanol addition. From 95.2-96.0% of TKN was removed, mostly as organics were removed. TAN was produced during ozonation, but was partially removed in the trickling filter, decreasing by 34.5-39.9% after treatment. From an average influent of 42.8-43.2 mg/L in the influent, 94.0-95.4%

of  $\text{NO}_3^-$ -N was removed by the end of the treatment train (to 2.0-2.7 mg/L). The biological yield for denitrification  $Y(\text{g biomass VSS/g NO}_3^- \text{-N})$  was 0.69, and the maximum nitrogen removal was  $23.4 \text{ kg NO}_3^- \text{-N m}^3 \text{ day}^{-1}$ . The work with the pilot station showed that the treatment strategy employed could support effective recovery and recycling of BRA effluent. However, salts and refractory organics may accumulate in the system.

### 3.1. Introduction

A major advance from traditional pond-based aquaculture toward industrial production of aquatic organisms was achieved by development of tank-based systems that allowed increased rearing densities while reducing land usage and water consumption (Losordo et al., 1998; Ackefors, 1999; Waller, 2001). Another advantage of land-based tank systems is that water quality can be controlled and adjusted to the requirements of the species (e.g., temperature, salinity, dissolved oxygen, alkalinity, pH, etc.). To achieve the desired environmental control in rearing systems, different unit processes were developed and combined with the rearing tanks, generically known as recirculating aquaculture systems (RAS). Basically, in a RAS, the water is alternatively polluted by the crop and then cleaned by the filtration system (Wheaton, 2002). Consequently, many aspects of management of an aquaculture system are affected by water quality (Cripps, 1993). Poor water quality may cause a variety of problems, including physiological stress, which negatively affects growth rate, feed conversion efficiency, and sensitivity to diseases (Poxton and Allouse, 1982). For these reasons, water quality control in RAS is of paramount importance, and is attained through water treatment and water exchange, in which the rate of water exchange needed depends on the efficiency of water treatment components (Waller, 2001). As long as the filtration systems adequately remove enough pollution to restore water quality to acceptable levels, the water can be recycled indefinitely, with some makeup water to replace the amount that evaporates or leaks from the system (Wheaton, 2002). However, at present, most commercially operated RAS contain only a subset of the components that could allow complete reuse of the water, (e.g., ammonia removal, oxygenation/carbon dioxide

stripping, and dissolved and suspended solids removal devices), confining themselves to a loop of recirculation that achieves only a primary treatment of the water. A RAS equipped with a primary water treatment system will still demand a high water exchange rate, which often exceeds 10% of system volume per day (Owsley, 1993; Westerman et al., 1996; Waller, 2001). To further reduce water requirements or to totally eliminate the need for water exchange, the water discharged from the primary loop is purified in a secondary treatment loop. If brought to desired quality after the secondary treatment, the water is fed back into the primary recirculation system or is discharged, imposing significantly less impact to the downstream environment while meeting water quality standards.

The primary treatment of aquaculture water is a well-studied area, as researchers have focused on maintaining water quality in the rearing units and optimizing performance of RAS (Spotte, 1979; Bovendeur et al., 1987; Lucchetti and Gray, 1988; Losordo, 1991; Wheaton et al., 1991b; Losordo and Timmons, 1994; Van Gorder, 1994; Westerman et al., 1996; Heinsbroek and Kamstra, 1990; Losordo et al., 1998). Different authors have recommended various strategies for aquaculture water treatment and recirculation. Common strategies include use of devices for removing suspended solids, biological filtration, degassing and oxygenation, and temperature control. In more sophisticated systems, foam fractionation, ion exchange, ultraviolet radiation, or ozonation are employed, achieving a higher degree of water quality, but raising production costs. Hence, the efficacy of these technologies is the focus of current research.

When a secondary loop is employed to treat effluent from a RAS, two major types of methods provide technical solutions for water recovery, and are often combined into hybrid technologies. The first category includes techniques analogous to post-treatment of effluents from land-based farms, using settling ponds or lagoons for organics removal (i.e., sedimentation followed by digestion), often by encouraging aquatic macrophyte or phytoplankton development for the purpose of reducing nutrients. Naegel (1977) and Rennert (1992, cited by Waller, 2001) have demonstrated the feasibility of such systems. Also in this category of methods is utilization of wetlands, which are highly productive ecosystems, and which have the potential to incorporate excess nutrients without adverse effects on overall system function. Finally, systems integrating aquaculture with

hydroponic plant production productively remove nutrients released to water by fish, converting them to terrestrial plant biomass (Racocy et al., 1993; Seawright, 1993; Van Gorder 2002). Because these technologies require large surface areas to be functional, a second category of methods tries to eliminate this disadvantage using techniques analogous to secondary or tertiary treatment of domestic and industrial wastewater discharges. Rosenthal and Black (1993) proposed a so-called "advanced quasi-closed multiple cycle system" that besides fish tank unit, biofilter, sediment trap, and reaeration or oxygenation, includes a suspended solids removal, denitrification, ultraviolet disinfection, and ozonation units. Such a system will allow recycling of nearly 100% of the aquaculture system's water, and makes it possible to install such systems on a small footprint. No such complex water treatment trains have been yet operated in connection with a commercial fish production system. However, several authors have reported successes from laboratory and pilot-scale closed RAS (Thoman et al., 2001; Behrends et al., 2002; Gelfand et al., 2002) and even from commercial closed RAS facilities, when hybrids of the two technologies were employed to treat the effluent (Van Gorder, 2002). Therefore, there are many waste management scenarios that can be considered for treatment of RAS discharges. The most appropriate form of treatment depends on system characteristics, economics, and the ultimate destination of the effluents. The technology of second loop treatment is still in its infancy and a range of developments is needed to successfully introduce these components into commercial aquaculture operations (Waller, 2001).

### **3.2. Literature review**

#### **3.2.1. Effluent decontamination, recovery and reuse in RAS**

Dramatic reduction in concentrations of suspended solids, dissolved organics, and other pollutants such as nutrients are required for an effluent stream to be recycled through a closed RAS.

For a commercial hybrid striped bass (*Morone saxatilis*) production facility with a total volume of 1,760 m<sup>3</sup> of water and discharging 140 m<sup>3</sup>/day, Van Gorder (2002) showed that a secondary closed-loop system treated the waste stream to sufficient quality

to be reused. The entire volume lost from recirculation was that of the cleaning water used to spray the microscreen clarifiers, which were the only devices used for the primary treatment of the effluent. The secondary loop employed cone-bottom centrifugal separators. The settleable solids removed were directed to an aerobic stabilization tank, and after stabilization, the sludge was transferred to two reed beds (*Phragmites sp.*) for final dewatering. The effluent from the reed beds (which was very low in organics) was combined with the overflow from the centrifugal separators (totaling more than 150 mg/L nitrates), polished through a microscreen clarifier, and then directed to a greenhouse, where plants in aquaponics systems production removed the nitrates and polished the dissolved organics and other contaminants. Before returning, the recovered water was disinfected with ozone, at the same time achieving reoxygenation. Although there was a high degree of water decontamination, Van Gorder reported a series of negative consequences from using this system, such as loss of fish appetite, decrease of fish condition, decreased efficacy of biofilters, and calcium depletion of the water.

Seawright (1993) demonstrated the technical feasibility of integrating hydroponic cultivation of terrestrial plants with Nile tilapia (*Oreochromis niloticus*) production in a completely closed, recirculating system. However, he indicated that the nutrient content of water generated in recirculating culture systems in which fish were fed commercial diets was inadequate for meeting and maintaining the nutritional and physiological requirements of plants over prolonged periods of time. This observation was in agreement with those of Racocy et al. (1993), who reviewed integration of vegetable hydroponics with fish culture, and concluded that the ability to control the nutrient composition of fish culture water is limited. Nutrients in fish culture water do not reach levels normally utilized in commercial vegetable hydroponics without nutrient supplementation, and integrated systems have not been designed and managed to meet the production requirements of both fish and vegetables.

Gelfand et al. (2002) evaluated the functionality of a zero-discharge, pilot-scale marine recirculating system growing red tilapia (*Oreochromis niloticus x O. aureus*) and then gilthead seabream (*Spratus aurata*) without discharging either water or particulate matter. Their system of water treatment incorporated an aerobic (trickling filter) and an anaerobic loop (sedimentation/digestion tank and fluidized bed reactor), respectively incorporating

treatment steps for biological oxidation of ammonia to nitrate and nitrate, reduction to nitrogen gas, and biological digestion of the organic matter. A third loop of water treatment was an air-driven foam fractionator, fed directly from the fish tanks, removing dissolved and suspended solids with foam that was directed to the digestion tank, and returning water to the fish tank. The system was run for 392 days, reaching a maximum biomass loading of 50 kg fish /m<sup>3</sup>. Ammonia, nitrite, and nitrate did not exceed 1.0 mg /L TAN, 0.5 mg/L NO<sub>2</sub>-N and 50 mg/L NO<sub>3</sub>-N, respectively. The alkalinity lost during nitrification was recovered partially by denitrification. The digestion in the sedimentation tank provided in full the volatile fatty acids necessary to sustain the denitrification, and the sludge remaining in the sedimentation basin at the end of the experimental period accounted for 9.2% of the total feed addition to the system. They found the system technically feasible, fish at harvest of good quality and free of microbial contamination, and reported no operational drawback or significant water quality deterioration.

Behrends et al. (2002) operated an experimental pilot-scale integrated waste management system to treat high-strength wastewater from a recirculating tilapia aquaculture system. They employed a novel technology referred to as “subsurface-flow reciprocating wetlands” (ReCip<sup>TM</sup>), a technology used previously only for treating municipal, industrial, and agricultural wastewater. They described improvements in the design and operation of paired subsurface-flow constructed wetlands, such that contiguous cells are filled and drained on a recurrent basis. For their study, the system configuration was designed to treat water-borne nutrients, manure solids, reduced gases, and odor compounds associated with high-strength wastewater from a small recirculating system. The RAS consisted of 24 tanks holding a total of 17,100 L of water. The first loop of the water treatment system included a 60-micron drum screen filter, a fluidized sand biofilter for nitrification, and a degassing/reoxygenation reactor. The organic sludge resulting from the microscreening (10 – 14% solids on a volume basis) entered the second loop of the wastewater treatment system, consisting of a primary clarifier, two-stage (mesophilic) anaerobic digester, two perched intermittent sand-bed filters, a two-stage reciprocating subsurface – flow wetland, and an algal polishing pond. Water exiting the algal polishing unit was either discharged to the sewer or was returned as make-up water to the aquaculture system. The system proved to be very efficient, removing more

than 90% of BOD<sub>5</sub> and COD (15 mg/L BOD<sub>5</sub> in the effluent), 94% of nitrogen (TKN, TAN and NO<sub>3</sub> were 2.4, 0.8, and 1.0 mg/L in the effluent, respectively), and 74.3% of phosphorous. They concluded that the integrated waste management system provided significant reductions of waste solids, organic matter, nutrients and odors associated with high-strength wastewater.

### 3.2.2. Techniques for wastewater treatment with applications in aquaculture

#### 3.2.2.1. Suspended solids removal

Particles in RAS are mainly organic molecules originating from uneaten feed, fish feces, sloughed slime, fragmented tissue, biofloc, and other inorganic and organic materials (Libey, 1993), as the new water added to the systems is virtually particle-free (Herbst, 1994). Suspended solids tend to accumulate in the system with negative impact on the fish; hence, their management is a key factor determining the success of recirculating systems. Removal of suspended solids is usually addressed by settling, centrifugal concentration, or mechanical filtration processes (Tetzlaff, 2001).

The settling basin is the simplest type of particulate waste removal system, where particulate wastes are allowed to settle by gravity to the bottom (as a function of the retention time), due to slow water velocity. Tetzlaff (2001) defined retention time as the time it takes to exchange the water in a container at a given flow rate. The retention time needed to settle wastes depends on their settling rate, which in turn, depends on the density of the particle and the amount of water turbulence in the basin. Depending on these factors, retention times often vary from 15 minutes to 2 hours. Slow water velocities and laminar flow patterns enhance the settling process (Piper et al., 1982). Wheaton (2002) reported retention times for settling basins used in aquaculture of 15 to 60 minutes. Chen et al. (1993) determined a specific gravity of 1.19 for the solid particles removed from a RAS. They noted that 95% of the suspended particles (by number) had a diameter less than 20 microns, accounting for 40 – 70% of the total suspended solids by weight. Wong and Piedrahita (2000) characterized the settling proprieties of the solids in the discharge water from a commercial rainbow trout (*Oncorhynchus mykiss*) production

facility. Using manually stripped fecal material, they determined the performance of a sedimentation basin at various overflow rates (OFR). They recommended a minimum  $0.5 \text{ cm/s}^{-1}$  OFR for sedimentation basins treating aquaculture effluents, achieving a removal efficiency of 81% of the particulate solids. Lekang et al. (2001) tested the principle of biological lamella sedimentation for solids removal at a fish farm in Norway. They demonstrated improved solids removal efficiency by using bioblocks installed in the sedimentation basin below the water surface, because the suspended solids attach to the biofilm developed on them when water passed through the tubes. Removal rates were up to 40% TSS, 37.3% COD, 43.2% total phosphorus, and 7.3% total nitrogen. However, they recognized that the removal efficiency was lower than those attained using rotating microsieves. In a similar test of multitube clarifier, Libey (1993) reported an average suspended solids removal efficiency of 56%.

Centrifugal concentrators, known also as swirl separators, hydrocyclones and vortex concentrators, operate on the principle of spinning particulate-laden water so that the denser particulate waste is forced to the wall of a cone by centrifugal force (Tetzlaff, 2001). In a study of a hydrocyclone prefilter removing particulates from a trout production facility effluent, Scott and Allard (1983) demonstrated that the device removed 56% of the net solids pumped through the system, and resulted in more effective biological nitrification and less disruption of the bacterial bed when backwash was necessary. Scott and Allard (1984) reported that 90% of the particulate waste produced by the trout was larger than 77 microns in diameter, at which the concentrator removed 70%. For smaller particles, the concentrator was able to remove only 10%. Centrifugal concentrators are not very efficient for aquaculture applications, because of poor removal of small particles, the need to maintain flows with high headloss (Lucchetti and Gray, 1988), and waste of up to 5% of the total system flow through their effluent. However, these systems have the benefit of compact size, which make them adaptable to indoor culture facilities (Tetzlaff, 2001).

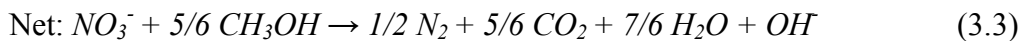
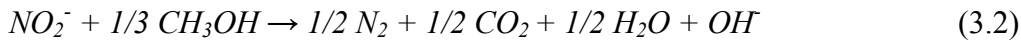
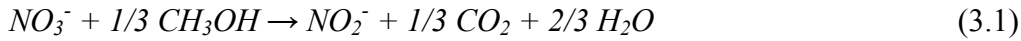
Drum screens are used frequently in aquaculture production systems for solids removal (Wheaton, 2002). Tetzlaff (2001) defined screen filtration as any mechanism where particulate matter is trapped on a thin, porous membrane. Screen filters include rotary drums (often referred to as microscreens in sewage treatment), and plates. The

water flows through the screen while the particulates resting on the screen surface are pushed by the influent and high pressure cleaning water to the end of screen (Goldberg et al., 1988; Makinen et al., 1988). The efficiency of particulate removal depends mainly on the mesh size of the screen, the particulate load, and backwash mechanism efficiency. Libey (1993) investigated the effectiveness of a drum filter (40 microns mesh size) for removing particles from a RAS containing fish at commercial density and feeding rate. He found that the drum filter removed an average of 9.1% of all particles between 5 and 300  $\mu\text{m}$  and that average removal efficiencies were above 81% for particles 70  $\mu\text{m}$  and larger with a decrease in efficiency to 56% for particles below 30  $\mu\text{m}$  in size. Heinen (1996) reported similar results from an experiment using an 80  $\mu\text{m}$  mesh size microscreen filter. He stated that particles that accumulate within a recirculating system using a microscreen filter are smaller than 20-40  $\mu\text{m}$  and constitute 50% (by weight) or more of the particles approaching the filter in the recirculated flow. Using a 65  $\mu\text{m}$  triangle filter for aquaculture effluent polishing, Cripps (1992) found a phosphorus reduction of 70 to 80%, consistent with evaluations of this type of filter by Enqvist and Larsson (1988) and Goldberg et al. (1988). Cripps (1992) demonstrated the removal of 80-100% of total solids, 27-70% of nitrogen, 67-79% of total phosphorous, and 75-82% of BOD for various operational conditions.

#### 3.2.2.2. Denitrification in fluidized bed biological reactors

Denitrification (or dissimilative nitrate reduction) is the conversion of nitrate-N to nitrogen gas by heterotrophic bacteria that utilize nitrate as the terminal electron acceptor as they oxidize organic matter in the absence of oxygen. These bacteria also utilize ammonia as a nitrogen source for growth. Denitrifiers can also grow on  $\text{CO}_2$  (autotrophs), but until now, only chemorgano-heterotrophic bacteria have been used in wastewater treatment (Wiesmann, 1994). Denitrifying bacteria belong to facultative aerobic or strictly anaerobic groups. The facultative aerobic bacteria would prefer to use elemental oxygen rather than nitrate as their energy source. Hence, denitrification occurs in anoxic zones. Although nitrate also could be removed through assimilative nitrate reduction to ammonia (e.g., when ammonia is not available as a nitrogen source), the main

mechanism for nitrate removal is through this anaerobic respiratory action. Overall, the chemical reactions for the nitrogen reduction in the presence of methanol proceed as follows:



Although this chain of reactions proposed by Jeris and Owens (1975) is a simple way to describe the process of denitrification, intermediate nitrogen species such as nitric (NO) and nitrous (N<sub>2</sub>O) oxide were identified during the transit from NO<sub>2</sub><sup>-</sup> to N<sub>2</sub> in equation 3.2.

Biological denitrification appears to be one of the most effective methods to remove nitrates from wastewater streams (Christensen and Harremoes, 1977). Denitrification may be achieved by using different types of bioreactors, classified by their physical design as suspended growth, fixed media, and mobile bed bioreactors (Grady et al., 1999). The fluidized bed biological reactor (FBBR) design belongs to the class of mobile bed bioreactors, and is one in which the biofilm grows attached to small carrier particles (e.g., sand, activated carbon, or plastic beads) that remain suspended in the fluid, i.e., fluidized by the drag forces associated with the upward flow of water (Wheaton et al., 1994; Grady et al., 1999). Moving media provide several distinct advantages: they allow better control of biofilm thickness, have superior mass transfer characteristics, are not subject to clogging, and provide very high surface areas for biofilm development while maintaining low pressure drops (Lazarov and Manem, 1994). Additional beneficial characteristics, like higher biomass concentration and higher volumetric reaction rates, lower space requirements, and lower capital costs (Coelhoso et al., 1992), make FBBR the most popular type of reactor used for denitrification of aquaculture wastewaters.

Stephenson and Murphy (1980), in an experiment describing the kinetic performance of a pilot scale FBBR for municipal wastewater denitrification, found that the fluidized

bed process provided equivalent nitrate-N and nitrite-N removal at volumetric rates approximately ten times those of suspended growth or submerged RBC processes. Temperature dependency of the removal rates was less than that for suspended growth or submerged RBC processes, but similar to that in packed columns. Jeris and Owens (1977) reported an order of magnitude higher biomass concentration and removal efficiency per volume unit for FBBR as compared to suspended growth processes, explaining that the key to the success of the reactor is the enormous surface area provided by the sand for microbial growth.

To optimize removal of nitrogen by denitrification, external carbon and energy sources normally are required to fuel the process (McCarthy, 1969). Among exogenous carbon sources, methanol is readily degradable and proves a handy solution from both kinetic and economic points of view. Jeris and Owens (1975), Stephenson and Murphy (1980), Balderston and Sieburth (1976), Semon et al. (1997), and Regan et al. (1998) supplemented wastewater passing through a FBBR with methanol, and reported complete denitrification when methanol was dosed appropriately. Akunna (1993) reported the completion of denitrification by adding acetate and glucose as exogenous carbon sources. Coelhoso et al. (1992) and Cecen and Gonenc (1992) used molasses for the same purpose. Van Rijn and Rivera (1990) fed two FBBRs with concentrated organic effluent from an experimental aquaculture pond, and reported large variations in nitrate-N removal and nitrite-N accumulation in the system. In RAS organic compounds accumulate, unless treated or removed by exchange water. Because these organics are composed mostly of long carbon chain molecules, they have proven unsuitable to sustain denitrification (Balderston and Sieburth, 1976; Arbiv and van Rijn, 1995). Surprising results on denitrification of aquaculture water were reported by Gelfand et al. (2002) from an experiment involving a pilot-scale closed RAS. They assessed that denitrification was self sustained, based on volatile fatty acids produced inside the system. The volatile fatty acids resulted from sludge fermentation in a sedimentation basin inserted on line with other devices for recirculated stream treatment. Additionally, nitrate reduction by denitrification took place not only in the anaerobic treatment stage (FBBR and solid separation basin), but also in the trickling filter. Regarding exogenous carbon material to fuel denitrification in the FBBR under various reaction conditions, Jeris and Owens

(1975) reported optimum methanol : nitrate ratios between 2.9 and 4.2 with 6.5 minutes contact time. Semon et al. (1997) and Jeris and al. (1977) both found a ration of 3 : 1 to be sufficient to remove up to 95% and 99% of the influent nitrate with 5.7 and 6.5 minutes contact time, respectively. Cecen and Gonenc (1992) showed that maximum denitrification rates could only be achieved at an influent COD/NO<sub>x</sub>-N ratio of approximately 5. They suggested that a process with a variable influent concentration should be designed with this factor in mind. Whiston et al. (1993) demonstrated that an atomic ratio of 1 : 1 carbon : nitrogen (using methanol as carbon source) was sufficient to drop system nitrate concentration from 43 mg/L to below 15 mg/L and to maintain it below this value. Menasveta et al. (2001), in a denitrification experiment employing a FBBR in a closed RAS for shrimp, showed that a carbon : nitrogen ration of only 0.21 to 0.56 will keep the nitrate concentration below 25 mg/L throughout the system. However, McCarthy (1969, cited by Jeris and Owens, 1975) estimated the amount of methanol required for cell growth and denitrification as:

$$C_m = 2.47 (NO_3^- - N) + 1.53 (NO_2^- - N) + 0.87 (DO) \quad (3.4)$$

where:

$C_m$  = required methanol concentration, mg/L;

$NO_3^- - N$  = influent nitrate nitrogen concentration, mg/L;

$NO_2^- - N$  = influent nitrite nitrogen concentration, mg/L;

$DO$  = influent dissolved oxygen concentration, mg/L.

Nitrate removal efficiency by FBBR of 6.4 kg NO<sub>3</sub>-N/m<sup>3</sup> day was found by Semon et al. (1997) over a temperature range of 11 to 25°C, but only about 30% of the bed's nitrate removal capacity was used. In the same experiment were reported reductions of soluble BOD<sub>5</sub> and soluble COD from an average influent concentration of 20 and 60 mg/L, to 8 and 30 mg/L, respectively, in the effluent. Coelho et al. (1992) found between 5.4 and 10.4 kg NO<sub>3</sub>-N/m<sup>3</sup> day nitrogen removal at 26°C with 4.73 minutes of contact time. Moore and Schroeder (1971) showed a correlation of ratios between methanol utilized and nitrate concentration in the treated effluent. They found the minimum methanol:nitrate ratio when nitrate nitrogen was in the range of 30-40 mg/L, and for a given influent concentration, an optimal residence time that would maximize nitrate utilization.

### 3.2.2.3. Ozonation

Biological treatments are inexpensive and reliable methods for eliminating pollutants from wastewater, but there are substances with which they are unable to deal. On the other hand, advanced oxidation processes have shown utility for toxic compound elimination, but total mineralization through these methods is very expensive. Consequently, a combination of both kinds of processes provides a cheaper option for total organics degradation from a wastewater (Marco et al., 1997). The idea of using combined chemical/biological processes for the treatment of wastewaters became popular around the mid-1980s, when found a viable treatment option (Gottschalk et al., 2000; Gunukula and Tittlebaum, 2001). Water and wastewater ozonation mostly is performed in directly gassed systems, where ozone-containing gas is produced by an electrical discharge ozone generator and introduced into the reactor by a gas diffuser (Gottschalk et al., 2000). Because good gas-liquid transfer rates can be obtained in bubble columns, this type of reactor is used frequently in laboratory-scale ozonation, being applied in either concurrent or countercurrent flow (Martin et al., 1994).

Rivas et al. (2000) studied the effect of ozonation on biodegradability of wastewater from olive-related industries, finding an increase in BOD<sub>5</sub>:COD ratio from 24 to 144% after ozonation, as a function of the wastewater strength. They attributed the biodegradability improvement to formation of smaller oxygenated species more vulnerable to microbial attack and to reduction of compounds with bactericidal properties. Kamiya and Hirotsuji (1998) studied a system combining both biological treatment and intermittent ozonation to reduce excess sludge production. They showed that using intermittent ozonation, 50% of the sludge generation was cut, with only 30% of the ozone dose required for continuous ozonation. They concluded that the system reduced operating costs and stabilized the performance of the biological wastewater treatment process.

Some investigations have focused on ozone mass transfer in water and wastewater, seeking to demonstrate enhancement of ozone absorption with the presence of chemical reaction. Studying ozonation of deionized water, tap water, and pulp mill effluent in a bubble contact reactor, Zhou and Smith (2001) found gas sparger and water types as the

most important factors affecting bubble size, while the impacts of ozone dose and gas flow on bubble size distribution were minor. Additionally, the specific surface area was strongly dependent on the gas flow rate, and the mass transfer coefficient increased as the gas flow rate increased. Further enhancement of mass transfer was observed for pulp mill wastewaters as compared to tap and deionized water samples, because the occurrence of chemical reactions depleted the dissolved ozone within the gas-liquid film.

#### 3.2.2.4. Aerobic treatment in trickling filters

The term “trickling filter” (TF) denotes an array of attached, grown biochemical operations in which wastewater is applied to fixed media (e.g., rocks, sheets of PVC formed in various configurations, or extruded plastic elements) in an air-filled packed tower (Grady et al., 1999). Treatment of wastewater is accomplished by consortia of microbes growing attached to the media. TF are aerobic, oxidize biodegradable organic matter, and achieve nitrification while forming microbial biomass. TF consist of five major components: the media bed, the containment structure, the wastewater dosing system, the drainage system, and the ventilation system (Water Environment Federation, 1997).

The liquid flow pattern through a trickling filter generally may be considered as plug-flow with dispersion. Because of this flow pattern, and because the microorganisms are fixed to the media, variation in the composition of the biomass often exists along through the depth of a trickling filter (Grady et al., 1999). Due to competition between heterotrophic and autotrophic bacteria for space within the biofilm, carbon oxidation typically occurs in the upper portion of trickling filters, while nitrification occurs in the lower portion. Thus, nitrifiers predominate in the biofilm only where conditions on the media are such that heterotrophic and nitrifying bacteria growth rates are at least equal. For these conditions to occur, oxygen must fully penetrate the biofilm, and dissolved organics must be at low concentration (Parker and Richards, 1986). High organic concentration in the liquid film will diminish oxygen penetration into the film and promote the growth of autotrophs. If oxygen concentration is low, conditions conducive to denitrification may appear in TF (Wanner and Gujer, 1984).

Heightened interest in the application of trickling filters in wastewater treatment emerged during the 1980s, largely because this process is less expensive to initiate and maintain than competitive processes (Feodoroff et al., 1982, cited by Parker and Richards, 1986). In wastewater treatment, TFs could be employed for secondary or tertiary treatment, after prior treatments have reduced COD to less than 60 mg/L and BOD<sub>5</sub> to less than about 20 mg/L (Kirk et al., 1975). Parker and Richards (1986) compared two TFs in a study meant to remove organics and to nitrify ammonia, confirming the relationship between nitrification and the sBOD<sub>5</sub>. They found that TFs did not produce nitrate until the sBOD<sub>5</sub> concentration was less than 20 mg/L. Grady et al. (1999) assessed that autotrophs will be excluded from the biofilm until the concentration of biodegradable organic matter drops below about 20 mg/L COD. Operating one of the experimental TF's with partial effluent recycle, they demonstrated that denitrification could also occur in TF in the combined carbon oxidation-nitrification mode. They explained that recycled effluent brought oxidized nitrogen in contact with the biofilm simultaneously with highly soluble BOD in the upper portion of the tower, and that penetration of both oxidized nitrogen and dissolved BOD<sub>5</sub> into the biofilm beyond the point of oxygen penetration would cause denitrification. This inference is in accord with Wanner and Gujer's (1984) theoretical model of competition between autotrophic and heterotrophic bacteria in a biofilm. Trying to prove that the existence of organic matter in a RAS tends to reduce the nitrification efficiency of biofilters under steady-state conditions, Zhu and Chen (2001) evaluated an experimental reactor series using sucrose as a carbon source and ratios of carbon : nitrogen of 1.0, 2.0, and 0.0 (no carbon). Their data showed that a carbon/nitrogen ratio of 1.0 or 2.0 resulted in an approximately 70% reduction of total ammonia nitrogen as compared with a solution that has a similar nitrogen level, but without carbon (C/N=0). The data showed that nitrification rate decreased with an increase in the organic concentration, but the impact became less pronounced when the carbon concentration became sufficiently high.

In fish farms, trickling filters generally are used for nitrification. These filters are relatively easy to build and operate and also serve an important function in degassing and re-oxygenating the water (Kamstra et al., 1998). Nijhof (1994b) studied the effects of plug-flow characteristics, influent ammonia concentration, and hydraulic loading rate on

filter performance. He found that plug-flow characteristics appear to be a prerequisite for the understanding of trickling filters, that ammonia oxidation capacity decreased considerably down the filter column, and that higher nitrification rates were obtained at higher hydraulic loading rates. Greiner and Timmons (1998) tested and compared a downflow floating polystyrene bead (microbead filter) to a trickling media biofilter using common influent from a 53m<sup>3</sup> fish-rearing tank stocked with red tilapia. They showed that nitrification rates increased linearly with influent TAN concentrations up to a concentration of 2.5 mg/L for both the microbead and trickling filters. The trickling filter had a specific nitrification rate 7.5 times higher than the microbead filter, although volumetric nitrification rates were 3.2 times greater for the microbeads than the trickling filter.

Kamstra et al. (1998) validated a model of ammonium removal in a pilot-scale TF (Nijhof, 1994a) for a range of full-scale TF used on commercial eel (*Anguilla sp.*) farms. They described the performance of 14 full-scale reactors based on plug-flow characteristics, which differ considerably with respect to dimensions, hydraulic and substrate loads. The hydraulic loadings applied were between 50 and 400 m/day, while the average removal rate observed was between 0.12 and 0.36 g/m<sup>2</sup>-day ammonia (maximum observed 1.1 g/m<sup>2</sup>-day). The effluent ammonium concentrations observed were in the range of 0.3 to 0.5 g/m<sup>3</sup> (maximum observed 1.8 g/m<sup>3</sup>). In most cases, the effluent nitrite concentrations were in the range of 0.2 to 0.5 g/m<sup>3</sup>. Lekang and Kleepe (2000) conducted a study regarding the efficiency of nitrifying trickling filters using different media types (dried expanded clay and plastic media), using a synthetic influent containing 1.5 mg/L TAN and hydraulic loads below 0.25 m<sup>3</sup>/m<sup>2</sup>-day. They found that 40 to 100% of TAN was removed by nitrification in different media types, and the nitrification rate was between 0.1 and 0.2 g TAN/m<sup>2</sup>-day in the different columns. Evaluating a combination of two different technologies used for fish production, Twarowska et al. (1997) determined the nitrification performance of a high-rate linear-path trickling biological filter. In six efficiency tests with a mean total ammonia nitrogen (TAN) concentration in the culture tank of 0.62 mg /L, they found that the biofilter removed approximately 65% on a single pass through the filter, with an average removal rate of 0.33 g TAN/m<sup>2</sup>-day.

### **3.3. Problem statement and design concept**

Considerable amounts of water are removed from recirculating aquaculture systems (RAS) to achieve pollutant removal. This water exchange poses significant costs to aquaculture operations regarding disposal of the effluent stream, and acquisition of plentiful, fresh replacement water. Sustainable tilapia production at Blue Ridge Aquaculture (BRA) is constrained by the availability of high quality influent water. At the same time, BRA discharges an estimated 2290 m<sup>3</sup> of wastewater per day to the municipal sewer system. In addition to carrying waste products, this effluent carries heat energy, because the systems' water temperature is artificially raised and maintained at about 28 - 30°C (i.e., the optimum for tilapia growth). Concerned about the costs and reliability of the wells and city water system, BRA wants to find an alternative solution. Developing a wastewater treatment system that recovers and reuses the water presently discharged to the City sewage system could minimize these problems.

Previous pilot-scale research at BRA (Brazil, 2001, unpublished data) employed foam fractionation, mechanical filtration, and biological treatment of wastewater for nitrate removal, and demonstrated significant improvement in quality of recovered water, although not all targeted water quality improvements were achieved. Therefore, a new, more comprehensive design had to be developed, refined and tested.

Based on the information provided by the ozone treatability study described in Chapter 2, a pilot-scale wastewater treatment station was built at BRA in order to initiate, characterize and optimize the operation of a more complex treatment strategy for effluent recovery and reuse at BRA. The need to eliminate settleable solids, colloids, dissolved organic substances and nitrogenous compounds led to selection of a sequential treatment process employing physical, biological, chemical, and again, biological steps. The physical step targeted solids separation by sedimentation and mechanical filtration. The first biological step was anaerobic, intending the reduction of nitrate; the low dissolved oxygen level of the incoming effluent supported the denitrification reaction. The chemical treatment step employed ozone, the treatability study showed that ozone partially oxidized organic substrates to carbon dioxide, and partially broke them into

smaller, more biodegradable molecules. Results highly supported inclusion of ozonation in the treatment strategy. The second biological step was an aerobic process, which capitalized on stream enrichment in oxygen from ozonation, and increased organics biodegradability. Besides further removal of organics, the process was also designed to help removing ammonia, a byproduct which accumulated during ozonation. The effluent resulting from the aerobic biological process was subject to a final polishing step of chemical flocculation and sand filtration.

Considered now in greater specificity, the treatment train included:

- a primary sedimentation basin,
- mechanical filtration using a microscreen drum filter,
- denitrification using a fluidized bed biological reactor with sand with recirculation,
- methanol addition to provide a source of carbon and energy for cellular growth,
- application of ozonation and foam fractionation in a bubble-contact ozone reactor,
- dissolved ozone quenching in an air-bubble stripping chamber,
- aerobic biological treatment using a trickling filter, and
- jar test-scale chemical flocculation, followed by sand filtration.

Figure 3.1 shows a schematic representation of the effluent treatment train built and operated at BRA. Although water recovery for RAS has been the subject of other studies, no system like that proposed for this study has been installed and operated at an industrial-scale recirculating aquaculture facility.

### **3.4. Objectives**

The development of the pilot-scale wastewater treatment plant for Blue Ridge Aquaculture involved introduction of sedimentation, innovative improvement of denitrification conditions, chemical oxidation with ozone, aerobic biological treatment and chemical flocculation into the treatment train. Evaluation of the treatment train addressed following objectives:

1. Initiate, characterize, and optimize the operation of solids removal devices (sedimentation basin and microscreen drum filter);

2. Initiate, characterize, and optimize the operation of the denitrification unit process;
3. Initiate, characterize, and optimize the operation of ozonation and foam-mediated solids and protein removal processes in a flow-through bubble reactor;
4. Initiate, characterize, and optimize the operation of combined, aerobic nutrient and carbon removal processes in a trickling filter; and
5. Initiate, characterize, and optimize the process of chemical flocculation and sand filtration.

### **3.5. Methods**

#### 3.5.1. Experimental design

At BRA, water exchange is executed discontinuously among the various production units (i.e., the nursery, greenhouses and growout systems). Water exchange practices are influenced by the availability of replacement water, labor, and need for water exchange. During morning hours (8:00 a.m. – 1:00 p.m.), the greenhouses and the nursery tanks are flushed. The amount of the wastewater coming from these systems was estimated at 12% of the entire volume of 2290 m<sup>3</sup>/day flushed at BRA during a 24-hr period. Over the rest of the day, the growout systems are flushed one by one, whenever they reach a feeding level of about 227 kg feed/system. This volume accounts for the remaining 88% of the effluent. Due to these fluctuations in the effluent flow, a capacity to store wastewater was needed in order to provide a continuous wastewater feed to the pilot station. To meet this need, two tanks were installed with a total capacity of 2.7 m<sup>3</sup> (1.0 and 1.7 m<sup>3</sup>, respectively), which assured a reserve for a maximum of four hours of independent operation of the pilot station. The use of this storage capacity altered the proportion of each type of wastewater that flowed through the treatment train, estimated at 18.4% from greenhouse and 81.6% for grow-out system wastewater, respectively. This proportion was used when calculating the non-flow weighted averages for different water quality parameters. To promote influent homogeneity, a 4” pipe connected the tanks at the bottom. Each tank was equipped with a low speed propeller (90 RPM) to prevent sedimentation, and an overflow device to release any excess incoming water. A flow

switch-controlled submersible pump located in the main effluent discharge pipe fed the first storage tank. When the storage tanks were full and the pumped stream exceeded the pilot station needs, excess wastewater was released back to the main effluent discharge system through the overflow devices.

A submersible pump located in the first storage tank fed the wastewater to the upper part of a sedimentation basin (SB), shown as “Influent” in Figure 3.1. The feed was discontinuous, with the pump operating two of every five minutes. The SB was cylindrically shaped, with a conical bottom (45° side angle), 76-cm diameter, 115 cm depth, and volume of 400L. These characteristics provided a 55 – 75 minute residence time for an average overflow rate (OFR) of 1.15 and 1.75 cm/min, respectively, as a function of the flow rate used by the treatment train. A submerged pump linked to a timer (operating 15 seconds during every 5 minutes) periodically flushed a volume of 4.5 L of sludge accumulated at the bottom, which corresponded to a continuous flow rate of 0.9 Lpm. Because of flow variations caused by intermittent feed and by sludge removal, the OFR was variable in the SB. The treatment stream (identified in this study as the working stream) exited the basin at the top through a connection located across from the influent point, and entered the microscreen filter for additional solids removal.

The microscreen filter (MSF) had 120 µm mesh. Its vessel had a volume of 160L. It was equipped with a vacuum system that removed the sludge from the screen surface, and disposed of it to the main sewer. A backwash device (controlled by a timer) sprayed the screen with fresh water for 15 seconds at 10-minute intervals to dislodge particles stuck in the screen.

From the MSF, the working stream entered a mixing basin (MB) at the top. The MB was a truncated conical PVC vessel, 65 cm tall, 34 cm average diameter, and holding a volume of 55 L of water. At the bottom, the MB also received the recycled stream from the fluidized bed biological denitrification reactor (DR). From the MB, a submersible pump passed water onward to the DR. Methanol was introduced continuously into the MB by a peristaltic pump. The flow rates varied between 81 and 54 ml/hr at wastewater working streams rates of 6 and 4 Lpm, respectively.

DR had a J-shaped cylindrical PVC vessel, with an enlarged fiberglass capsule attached to the top. The PVC cylinder was 360 cm long (300 cm on the long arm, 30 cm

along the curvature and 30 cm on the short arm), 15 cm diameter, and 64 L volume. It held an initial volume of 15 L of 0.7 mm diameter silica sand. Along the length of the reactor were four transparent PVC windows for visual inspection. The water from the mixing basin was pumped to the short arm of the reactor, exerting backpressure on the sand bed, which expanded and fluidized. A capsule at the top of the DR had a truncated conical shape at the lower part, with the base connected to the PVC body. It continued upward with a cylindrical section of 34 cm diameter, and a cone-shaped lid at the top. The capsule had 60 cm total height, and a volume of 30 L. The large diameter of the capsule reduced water velocity, limiting the loss of sand. Additionally, inside the capsule was a flat-bladed propeller, driven by a submersed, electrical motor. The propeller sheared off excess biofilm from sand particles reaching its level, thereby preventing loss of sand at the top of the reactor. A flow-splitting device at the top of the reactor's capsule (i.e., a 4" cylinder with connections and valves) allowed controlled, partial return of the flow to the mixing basin, while the rest of the flow (the working stream) went onward to the ozone reactor. An opening at the top allowed release of biologically produced nitrogen to the atmosphere. Regardless of the returned and working streams flows, the flow injected to the DR was always 10 Lpm (0.91 cm/s). The necessity of maintaining this minimum flow rate to sustain sand fluidization combined with the need to provide a sufficient residence time for ozonation in the next process unit were the rationale for using recirculation, rather than concerns about completion of denitrification in a single pass.

The ozone reactor (OR) was modified from the semi-batch reactor used for the treatability tests (Chapter 2) into a flow-through configuration. An influent connection was inserted at the top, while all other devices from the ozonation system (e.g., ozone generator, ozone monitor, foam condenser, gaseous exhaust system and upstream connections) remained unchanged. This top connection was the point where a pump delivered the working stream from the DR into the OR. The stream exited the OR at the bottom (below the diffusion stones). Water flowed downward through the column, countercurrent to upward gas bubble movement. After separation of gas and foam in the condensing tank, a hose connected the bottom of the condenser to a U-shaped 2" PVC pipe, which drained the liquefied foam, at the same time preventing escape of residual

gas to the room. The residual gas stream was collected at the top of the condenser, allowing sampling for ozone content.

The working stream flowed gravitationally to the stripping chamber (SC), placed on a high platform, at the top with the same water level as the OR. The chamber was a 55 L PVC truncated conical basin (65 cm deep and 34 cm average diameter), with influent at the bottom and effluent at the top. An air pump injected air through a submersed, 4 x 4 x 20 cm silica stone diffuser, removing residual dissolved ozone and other dissolved gases from the stream.

The stream from the SC flowed gravitationally to the trickling filter (TF), a rectangular-shaped reactor (425 cm tall x 45 x 45 cm), made from PVC. The TF was filled with Biodeck® 60° cross-flow plastic bundles. A wall vertically split the interior of the TF into two equal chambers, allowing the use of one or both as necessary. The water was distributed at the top by five, 1" diameter pipes (perforated laterally and end-plugged), and further distributed by a perforated PVC plate placed beneath them. A plastic tray located at the bottom of the TF collected the exiting stream, allowing sampling and flow rate monitoring. The effluent stream then was released to the facility's main effluent discharge canal.

### 3.5.2. Operation and analytical techniques

This study was designed to test four different combinations of working conditions, under two wastewater stream flow rates. Two ozone doses were tested for each flow rate, as shown in Table 3.1. After completing construction of the pilot station, operations began on March 1<sup>st</sup>, 2003 with the solids removal in SB and MSF, and acclimation of the DR. The working stream flow rate used for acclimation was 6 Lpm, with a recycled flow rate of 4 Lpm.

Methanol was added to the mixing basin from the beginning, at a stoichiometric ratio of 3.6 mg methanol/mg nitrate-nitrogen ( $\text{NO}_3^-$ -N), based on the average influent  $\text{NO}_3^-$ -N concentration of 50 mg/L found during preliminary tests. The selection of methanol as a source of carbon for denitrification was based on its popularity, its being known as one of the most suitable compounds for the process, and also from economic considerations. In the first month, effluent from the FBBR was released to the sewer canal. Samples were

collected twice per week at 4:00 p.m. from the SB effluent, MB, and FBBR effluent, beginning with the second week of operation. They were tested for total ammonia nitrogen (TAN),  $\text{NO}_3^-$ -N and nitrite-nitrogen ( $\text{NO}_2^-$ -N) content using a spectrophotometer (Hach DR 2400, Loveland, CO), and for temperature and pH using a pH/mV/ $^\circ\text{C}$  meter (Oakton<sup>®</sup> Acorn Meter Kit model pH 6, Vernon Hills, Illinois). Dissolved oxygen (DO) was measured using an YSI (Model 550, Yellow Springs, Ohio) instrument.

Beginning with the 30<sup>th</sup> day of operation, the OR became available, along with the complete results from the treatability study. The OR was connected to the system after reconfiguration to allow flow-through mode of operation. Consequently, the effluent stream from the DR was then treated with ozone (6 minutes hydraulic residence time), passed through the SC and then through the TF, completing the treatment train. This moment marked the start of TF acclimation. Because the working flow of 6 Lpm was the maximum intended for this study, the entire TF was exposed for acclimation. The TF was designed to remove 40 mg/L carbonaceous biological oxygen demand ( $\text{cBOD}_5$ ) and 6 mg/L TAN when fully acclimated and working under appropriate conditions. The tests assessing acclimation were extended to the TF influent and effluent, monitoring the same water quality parameters described for DR acclimation. Additionally, chemical oxygen demand (COD) was measured to assess organics removal, using the closed reflux procedure of Standard Method 5220C (APHA et al., 1998).

Acclimation was considered completed in both biofilters around May 20<sup>th</sup> (day 81 of the TF operation). However, the DR was found to remove about 95% of the  $\text{NO}_3^-$ -N from the passing stream for a two-week period before that time. For the same interval of time, the TF treated about 50% of influent TAN and 30-35% of COD. These findings indicated that both reactors had reached a steady state mode of operation. Additionally, a consistent brown biofilm was observed to coat the entire amount of sand in the DR, and bed expansion was significantly increased.

During the acclimation, as well as during the testing period, maintenance operations proved necessary to keep the pilot station working properly. Every 48 hours, the sedimentation basin was skimmed manually to remove floating solids that accumulated on the surface. Solids floatation appeared to be due to solids attaching to the walls of the tank. The layer broke sporadically, and chunks were buoyed up by gas bubbles that had

formed due to microbial activity. Solids depositions also were observed on the connecting hoses and walls throughout the treatment train, requiring weekly cleaning.

Stage 1. Testing of the first stage was conducted under the same conditions as the acclimation: 6 Lpm working flow, 4 Lpm recycling in the DR, and 22 mg ozone/L gas injected into the OR (Table 3.1). The equivalent dose was 36.6 mg O<sub>3</sub>/L water. This represented the highest working flow rate, and the lowest dose and time of exposure to ozone tested. The system was operated under these conditions for four weeks, during which three sets of tests were conducted at 10-day intervals. A listing of parameters recorded and the collection points within the system are shown in Table 3.2.

Each set of tests included three consecutive runs, extended over a 24-hour period. Samplings began at 2:00 p.m., 10:00 p.m. and 6:00 a.m. At the beginning of each sampling solids settleability tests were performed on the raw influent. The collection of sample aliquots was timed to match the residence time of water in each treatment unit, allowing sampling of the same parcel of the stream as it passed through a particular collection point (Table 3.2). Because the total volume of the treatment train was about 800 L and the working flow rate in this stage was 6 Lpm, the sampling stretched over a 2 hour and 15 minutes period (i.e., the time between the working stream entering the SB and exiting the TF). Another two tests were conducted on days 10 and 20 at 8 p.m., when only cBOD<sub>5</sub>, COD, and dissolved organic carbon (DOC) were measured. The sample locations were those indicated for cBOD<sub>5</sub> in Table 3.2.

TAN, NO<sub>2</sub><sup>-</sup>-N, and NO<sub>3</sub><sup>-</sup>-N, pH, temperature, and DO were determined by the methods already described. The analyses for nitrogenous compounds were conducted on aliquots passed through a 0.45 µm filter. A spectrophotometer was used for determining turbidity (NTU) and dissolved ozone (DO<sub>3</sub>). Alkalinity and hardness were measured using Hach (Permachem®) Reagent Methods. Raw influent settleability tests were determined following the Inhoff Cone Standard Method 2540F (APHA et al., 1998). All these analyses were performed on site. In addition, a set of sample aliquots were passed through 1.5 µm filters for total suspended solids (TSS) determination. These filters, along with a set of refrigerated aliquots and another set acidified below pH 2 with H<sub>2</sub>SO<sub>4</sub> were transported to the Civil and Environmental Engineering Laboratories at Virginia Polytechnic Institute and State University for further processing. TSS determinations

were completed there (along with volatile suspended solids [VSS] and fixed suspended solids [FSS]), following Standard Methods 2540D and 2540 E, respectively (APHA et al., 1998). The fresh samples were analyzed for total solids (TS), volatile total solids (VTS) and fixed total solids (FTS) following Standard Methods 2540 B and 2540E, respectively (APHA et al., 1998). For cBOD<sub>5</sub> determinations, fresh aliquots were collected, refrigerated on ice and transported immediately to the same facility, where they were processed according to Standard Method 5210B (APHA et al., 1998). A set of aliquots also was preserved with H<sub>2</sub>SO<sub>4</sub> and refrigerated. COD was determined by using the closed reflux procedure (Standard Method 5220C, APHA et al., 1998), DOC by using a total carbon analyzer (Dorhmann model DC 80, Santa Clara, California, Standard Method 5310A) on samples passed through 0.45 µm filters. Total Kjeldahl Nitrogen (TKN) was determined by using macro-Kjeldahl, Standard Method 4500-N<sub>org</sub> B, (APHA et al., 1998). A jar-test chemical flocculation treatment was applied to the TF effluent. FeCl<sub>3</sub> was used as the flocculating agent, because it operates over a wide pH window and fits the requirements of the TF effluent, which often had pH higher than 8. Preliminary tests involving doses of 33, 50 and 75 mg/L FeCl<sub>3</sub> showed that a dose of 50 mg FeCl<sub>3</sub>/L effluent was the appropriate dose for this effluent. This dose is higher than generally is required for drinking water treatment, but the load of solids from this water is also considerably higher and justifies the large amount of FeCl<sub>3</sub> applied. Foam samples were collected, handled and assayed for COD, BOD<sub>5</sub>, TKN, TS, VSS, FTS, TSS, VTS, VFS, and turbidity. Additionally, volumes were recorded for sludge from the sedimentation basin, microscreen filter, and foam streams.

Stage 2. At the beginning of Stage 2, a new ozone generator (Model CD1500P, ClearWater Tech, San Luis Obispo, CA) was connected in parallel with the existing one. The oxygen flow (10 Lpm) then was split equally between the two generators. By receiving a lower oxygen flow rate, each generator increased its ozone production efficiency, achieving a total concentration of 33 mg ozone/L of gas stream (a dose of 55 mg O<sub>3</sub>/L water). This stage lasted for 18 days, with 24-hour sampling runs occurring at 6-day intervals. The tests that included BOD<sub>5</sub> were performed on days 4 and 16.

Stage 3. The conditions for Stage 3 were set by reducing the working flow to 4 Lpm, and increasing the recycle flow rate in the FBBR to 6 Lpm. This modification increased

the residence time of water in each component of the treatment train, extending the sampling time to 3 hours and 20 minutes. Consequently, the water residence time in the OR increased from 6 to 9 minutes. The ozone concentration was kept at 33 mg/L gas, but extension of residence time increased the dose to 82.5 mg O<sub>3</sub>/L water. The smaller working flow rate led to closing half of the cross section of the TF, in order to keep the hydraulic loading rate above the minimum necessary for this type of filter (1.8 m/hr). Under these conditions, Stage 3 represented the best-case scenario with regard to the ozone dose applied to treatment stream (Table 2). The stage lasted for 21 days, following the same sampling protocol as in Stage 2, except for timing of sample collection as a parcel of water passed through the system. The first three days of the stage were allowed for the system to adjust to the new conditions.

Stage 4. In Stage 4, the ozone concentration was returned back to 22 mg/L gas, keeping the flow rate at 4Lpm. The oxygen flow rate was increased to 10 Lpm through the original ozone generator, and the second was detached. This stage was designed to assess application of a dose of 55 mgO<sub>3</sub>/L water (e.g., similar to Stage 2), but during a 9-minute period. The stage lasted four weeks, and sampling days were fixed at intervals similar to Stage 1. Instead, the sampling events through treatment train were timed as in Stage 3.

Data analysis. Throughout the study, the percent pollutant removal was determined by equation:

$$\eta(M) = 100 - \frac{c(M)}{c_0(M)} \times 100 \quad (3.5)$$

Parameters regarding ozone performance were calculated using steady state equations presented by Gottschalk et al. (2000):

1. Ozone dose or feed rate,  $F(O_3)$ , (mg L<sup>-1</sup>s<sup>-1</sup>)

$$F(O_3) = \frac{Q_G \times C_{G0}}{V_L} \quad (3.6)$$

2. Ozone consumption rate,  $r(O_3) = r_L$ , (mg L<sup>-1</sup>s<sup>-1</sup>)

$$r(O_3) = \frac{\frac{Q_G}{Q_L} (C_{G0} - C_{Ge}) - C_{Le}}{t_H} \quad (3.7)$$

3. Ozone absorption rate,  $r_A(O_3)$ , ( $\text{mg L}^{-1}\text{s}^{-1}$ )

$$r_A(O_3) = \frac{\frac{Q_G}{Q_L}(C_{G0} - C_{Ge})}{t_H} \quad (3.8)$$

4. Pollutant removal rate,  $r(M)$ , ( $\text{mg L}^{-1}\text{s}^{-1}$ )

$$r(M) = \frac{c(M)_0 - c(M)_e}{t_H} \quad (3.9)$$

5. Specific ozone dose or input,  $I^*$ , ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )

$$I^* = \frac{Q_G \times C_{G0}}{Q_L \times c(M)_0} \quad (3.10)$$

6. Specific ozone adsorption,  $A^*$ , ( $\text{g O}_3 \text{ g}^{-1} \text{ M}$ )

$$A^* = \frac{Q_G(C_{G0} - C_{Ge})}{Q_L \times c(M)_0} \quad (3.11)$$

7. Ozone transfer efficiency,  $\eta(O_3)$ , (%)

$$\eta(O_3) = \frac{A^*}{I^*} \times 100 \quad (3.12)$$

8. Degree of pollutant removal,  $\eta(M)$ , (%)

$$\eta(M) = \frac{c(M)_0 - c(M)_e}{c(M)_0} \times 100 \quad (3.13)$$

9. Ozone yield coefficient,  $Y(O_3/M)$ , ( $\text{g O}_3 \text{ g}^{-1} \Delta \text{M}$ )

$$Y(O_3/M) = \frac{C_{G0} - C_{Ge}}{c(M)_0 - c(M)_e} \quad (3.14)$$

where:

$Q_L$  = liquid flow rate ( $\text{L s}^{-1}$ ),

$Q_G$  = gas flow rate ( $\text{L s}^{-1}$ ),

$C_{G0}$  = influent gas concentration ( $\text{mg L}^{-1}$ ),

$C_{Ge}$  = effluent gas concentration ( $\text{mg L}^{-1}$ ),

$V_L$  = liquid volume ( $\text{m}^3$ ),

$C_{Le}$  = effluent liquid concentration ( $\text{mg L}^{-1}$ ),

$c(M)_0$  = influent pollutant concentration ( $\text{mg L}^{-1}$ ),

$c(M)_e$  = effluent pollutant concentration ( $\text{mg L}^{-1}$ ), and

$t_H$  = hydraulic retention time (s)

### 3.5.3. Denitrification kinetic determinations

The study aimed at estimating the denitrification kinetic response with respect to  $\text{NO}_x^-$ -N concentration. Oxidized nitrogen species ( $\text{NO}_x^-$ -N =  $\text{NO}_2^-$ -N +  $\text{NO}_3^-$ -N) reduction rates were calculated using a half-order kinetic model ( $\text{mg L}^{-1} \text{min}^{-1}$ ):

$$r_{\text{NO}_x\text{N}} = \frac{dC}{dt} = -kC_0^{\frac{1}{2}} \quad (3.15)$$

and the rate constant ( $k$ ,  $\text{mg}^{\frac{1}{2}} \text{L}^{-\frac{1}{2}} \text{min}^{-1}$ ) was calculated from the equation:

$$k = 2(C_0^{\frac{1}{2}} - C^{\frac{1}{2}})/t \quad (3.16)$$

where:  $r_{\text{NO}_x\text{N}}$  = removal rate ( $\text{mg L}^{-1} \text{min}^{-1}$ ),

$C$  = concentration ( $\text{mg L}^{-1}$ ),

$C_0$  = initial concentration ( $\text{mg L}^{-1}$ ), and

$t$  = hydraulic residence time (min)

The biological (anoxic) yield ( $Y$ ) (i.e., the amount of microbial biomass formed per unit of substrate used) was determined using a bench-scale batch reactor with regard to both methanol (as COD) and  $\text{NO}_3^-$ -N substrates. The biomass produced was quantified as VSS and COD. A hermetically closed 5L vessel was initially filled with water seeded with biofilm sheared from a sample of coated sand from the DR. Potassium nitrate and methanol were added in amounts accounting for initial concentrations of  $\text{NO}_3^-$ -N and methanol (as dissolved COD) of 207 and 1666 mg/L, respectively. Methanol was well in excess of stoichiometric requirements, such that it should not be a limiting factor. Continuous stirring and constant temperature of  $28 \pm 1^\circ\text{C}$  were maintained. Pure nitrogen was injected for about 5 minutes by a connection at the vessel bottom to strip dissolved oxygen and to replace the air from the space above the fluid. Gas exiting at the top was collected and directed by a hose to a water bath, sealing the space. Any additional nitrogen gas produced by denitrification followed the same path before entering the atmosphere. The operation of purging nitrogen was repeated after any sample collection (e.g., at the beginning, and then at 12-hour intervals over a period of 48 hours). TSS,

VSS, dissolved and total COD were measured according to Standard Methods (APHA et al., 1992) described previously, and  $\text{NO}_3^-$ -N was determined spectrophotometrically. Filters of 1.5  $\mu\text{m}$  pore diameter were used for collecting TSS, and for obtaining samples for dissolved COD determinations. The equations used to determine  $Y$  values (Grady et al., 1999) were:

$$Y_{VSS} = \frac{\Delta VSS}{\Delta \text{dissolved COD}} \left( \frac{\text{g}}{\text{g}} \right) \quad (3.17)$$

and:

$$Y_{\text{NO}_3^- - N} = \frac{\Delta VSS}{\Delta \text{NO}_3^- - N} \left( \frac{\text{g}}{\text{g}} \right) \quad (3.18)$$

$Y_{COD}$  was obtained by equation:

$$Y_{COD} = 1.42 \times Y_{VSS} \left( \frac{\text{g}}{\text{g}} \right) \quad (3.19)$$

Additionally,  $Y_{\text{NO}_3^- - N}$  was determined from data collected from DR, using Equation 3.18.

#### 3.5.4. Statistical analyses

To assess system performance for removing pollutants and improving water quality, key parameters were monitored and compared after each water treatment unit process, as well as after the entire water treatment train. The null hypothesis was that there was no difference in concentrations between different operating conditions (i.e., experimental stages). 95% confidence intervals were computed for the mean percent removal for different parameters after each of the five main units in the treatment train.

For each parameter of interest, data was gathered during three days at 2:00 p.m., 10:00 p.m., and 6:00 a.m. To get a better estimate of the daily percent removal of a certain parameter, a weighted average of the three measurements was used to obtain the daily mean. The weights were established based on the amount of water of different origin that entered the system; these weights were 18.4% for the 2:00 p.m. measurements, and 40.8% for each of the other two measurements.

ANOVA tests for differences of means compared parameter values among the four stages of operating conditions. When appropriate, values of parameters in the influent

stream were used as covariates in the ANOVA model. To observe the relationship between ozone dose and several water parameters, and between the reduction of  $\text{NO}_x^-$ -N concentration and the reaction rate coefficient  $k$ , linear regressions were performed.

Statistical analyses were conducted using SAS version 8.2 (SAS Institute, Cary, NC).

### **3.6. Results and discussion**

#### **3.6.1. BRA waste stream characterization**

The BRA waste stream was characterized over eight-hour sampling periods during 12 different days. The results indicated that solids, COD, and nitrate are the most significant waste components (by concentration and weight). All these forms of pollutants were targeted by unit processes in the treatment train. Depending which fish culture systems were being flushed or cleaned, waste stream characteristics varied significantly, made evident as differences between minimum and maximum values (Table 3.3). The table shows non-flow weighted average parameters for all samples, and the extreme concentrations that were recorded. Comparing the effluent characteristics with a criterion proposed by Metcalf and Eddy (1991) for the classification of untreated domestic wastewaters (Table 3.3), BRA effluent could be defined as having weak strength in the morning and medium strength in the afternoon and overnight. Stream source evaluations indicated that 12% of the wasted stream came from auxiliary systems such as fingerling and broodstock production units (e.g., morning waters), and the rest (88%) came from grow-out system water exchanges. Additionally, BRA effluent showed certain characteristics, such as a higher fixed solids fraction (60%), lower TAN (2.55 mg/L), and higher  $\text{NO}_3^-$ -N (43.0 mg/L) than is typical for a domestic wastewater. The high FSS was probably due to the accumulation of minerals in RAS waters, which enter the systems with feed (and then the water as metabolic byproducts), and from addition of various chemicals to the water in water quality management practices. The presence of low TAN and high  $\text{NO}_3^-$ -N concentrations was due to system nitrification. Tests conducted on settled samples also indicated a lower biodegradability index (e.g., a  $\text{cBOD}_5/\text{COD}$  ratio of 0.25 – 0.30). This could be explained by the presence in water of uneaten, fine-particle

feed components, and by poor digestibility of polysaccharides (about 50%) by fish that were subsequently released into systems with feces. Both of these forms are generally unsuitable for bacterial consumption.

Simple arithmetic showed that BRA released daily: 4150 kg of TS (1674 kg as volatile), 1278 kg TSS (1057 kg as volatile), 1312 kg COD, 5.84 kg TAN, 98.4 kg NO<sub>3</sub><sup>-</sup>-N, and 275 kg alkalinity. Below, I present and discuss in detail findings with regard to dynamics of key water quality parameters throughout the treatment train.

### 3.6.2. Solids removal

Results from settleable solids tests indicated that BRA effluent settles well, resulting in a separation of between 4 and 30 mL settled solids/L water. Not surprisingly, this index varied directly with the degree of solids loading. Despite this performance, a brownish color persisted after sedimentation, indicating a large number of colloidal particles and dissolved macromolecules suspended stably in the water column. This finding is in agreement with those of Wong and Piedrahita (2001), who reported only 72.4% solids removal in terms of mass concentration from aquaculture wastewater after 90 minutes of sedimentation.

In this study, raw influent non-flow weighted averages for TS, VTS and FTS were 1812, 731 and 1081 mg/L, respectively (Table 3.3). At these concentrations, the SB removed 21.7-27.7% of TS, 44.6-52.0% of VTS, and 8.7-10.8% of FTS. An explanation for higher percent VTS removal could be that a larger part of this fraction was trapped in solids that settled, while most of the fixed fraction was dissolved. The large fraction (60%) of FTS present in this water associated with the poor FTS settleability suggested that the stream was rich in dissolved minerals.

Along the treatment train, between 98.5 and 99.1% of the TSS was removed, with the highest performance corresponding to the largest ozone dose (Stage 3). This treatment yielded an average of less than 5 mg/L TSS in the final effluent. Figure 3.2 presents a treatment train profile of TSS and VSS dynamics in Stage 3, the values representing the means for tests conducted at 10:00 p.m. and at 6:00 a.m. Samples from 2:00 p.m. generally yielded much lower values for these parameters. Results for TSS removal in the main treatment units where TSS was reduced are shown in Table 3.4, each value

representing the non-flow weighted average for an entire experimental stage. In the raw influent, 81.7-83.7% of TSS was volatile, and the remainder represented the fixed fraction. These values are similar to those reported as typical for aquaculture sludge solids composition, e.g., 74.6-86.6% of TSS being volatile (Chen et al., 1996), and 82.8% VSS found by Summerfelt et al. (1999).

In the SB, TSS removal was between 70.9-72.9% and VSS between 68.8-72.0%. Under conditions of working flow reduction in Stages 3 and 4 (i.e., an increase in retention time from 55 to 75 minutes in SB), there was no statistically significant improvement in TSS removal. This unexpected finding could be due to the combined effects of discontinuous feeding of the SB, to solids floatation, and to a large mass percentage of fine, unsettleable solids in wastewater. There is an alternative hypothesis that most solids needed less than 55 minutes to settle. Persistent brownish color and high turbidity of water in the SB indicated that stable fine particles could notably impair the sedimentation performance of the SB. The problem of unsettleable colloids appears to be typical of aquaculture wastewaters. For example, Chen et al. (1994) found that of the particles in aquaculture wastewater, 40-70% are smaller than 20  $\mu\text{m}$  by weight, and 95% by number, while sedimentation becomes impractical for particles smaller than 50  $\mu\text{m}$ . Tetzlaff (2001) also reported difficulties in removing solids from recirculating aquaculture wastewaters, due to their small size and density near that of water. Additionally, pumping of the effluent twice before reaching the SB could have contributed to an increased number of fine, stable particles in this study.

In the MSF, percent removal of TSS ranged between 28.8 and 38.9% from the unit influent. An accumulation of solids was observed with time inside the microscreen basin, despite vacuuming of most solids from the rotating screen. The accumulation could be due to high density of some particles, especially those that should normally settle in the SB, but were driven into the water column by gas bubbles and passed onward to the MSF.

Further along the treatment train, there was a TSS increase with 18.2-29.4% in the DR (with largest addition occurring in the first two stages). The increase was probably due to the bacterial growth. In the OR, the values of TSS were reduced by 75.6-77.7% (Table 3.4). Quantitatively, 129-157 mg/L TSS in the OR influent were reduced to 31-35 mg/L

in the reactor effluent. There was a statistically significant difference only between Stages 1 and 3 with regard to TSS percent removal. Similar efficacy among stages for TSS percent removals during ozonation could be due to a rapid separation of solids at the top of the reactor, while the amount removed in the lower part of the OR (mineralization) represented a smaller portion. Hence, the residence times tested in this study exerted little influence on the amount of solids separated; neither did the ozone doses, which were probably sufficient to support the destructive solubility effect on suspended solids. The volumetric production of the condensed foam depended on the ozone dose and residence time, representing an equivalent of 3.6% and 6.9% of the working stream in Stages 1 and 3, respectively (Table 3.7). Balances between the mass removed and the mass recovered in foam showed that 58.3-66.3% of TS, 71.6-77.9% of VTS, 80.7-86.4% of TSS, and 81.2-86.6% of VSS were not recovered in foam. The significant reduction of the volatile solids could be due to partial mineralization of organic matter. The disappearance of the suspended fraction was probably due to the disintegration of cells and other solid structures. The destruction of bioflocs by ozonolysis also was reported by Weemaes et al. (2000) from a study of sewage sludge ozonation, and by Kamyia and Hirotsuji (1998) and Wang and Pai (2001) from studies of wastewater ozonation, who attributed an increase in wastewater DOC concentration following ozonation to solubilization of the sludge. Experimenting with aquaculture wastewaters, Rueter and Johnson (1995) and Krumins et al. (2001b) found that light use of ozone has a flocculent effect, increasing TSS concentration that can be separated afterward. The results collectively suggest that there is a critical ozone dose, determining how solids in wastewater are affected. Below this point, ozone acts as a flocculent, and above it the solids are solubilized. This interpretation was shown viable by Grasso and Weber (1988). The condensed foam observed in this study was, however, very turbid (Figure 3.4), due to presence of very fine light-scattering colloidal and dissolved compounds, which did not settle easily. Despite massive solids destruction, the foam still had a non-flow weighted average concentration of  $935 \pm 137$  mg/L VTS in all stages, which indicates solubilization, besides oxidation of the organic fraction of the solids.

Following ozonation, the TF added 7.7-12.5 % TSS to the stream due to cell growth. TSS values decreased with the organic loading of the stream (i.e., from Stages 1 and 2 to Stages 3 and 4).

Chemical flocculation treatment removed 78.9-86.0% of TSS (Table 3.4). A linear, positive correlation was observed between percent TSS removal in the CF and the ozone dose used (slope = 0.155;  $r^2 = 0.91$ ) (Figure 3.3), despite lack of correlation between these two parameters in the OR. Grasso and Webber (1988) suggested that improved TSS removal could occur because of the flocculent-enhancing effect of ozone upon colloidal and particulate matter remaining in wastewater, such that a larger number of destabilized particles and macromolecules resulting from higher doses of ozone become more prone to flocculation.

Results of this study indicated that due to inadequate settleability of BRA effluent, additional methods (e.g., air floatation) could be applied after sedimentation in order to improve the solids removal. This operation also could eliminate the need for microscreen filtration.

### 3.6.3. Turbidity

The raw effluent had many large, fast-settling particles, complicating an accurate determination of turbidity. However, repeated measurements resulted in approximate stage average values ranging between 131.9 and 139.5 NTU (Table 3.5). After sedimentation, the difference in turbidity between the two streams was slightly significant statistically ( $p = 0.046$ ), which indicates that longer sedimentation has a beneficial effect on turbidity, despite a smaller effect on TSS. Table 3.5 also shows the dynamics of turbidity among treatment units, and total percent removal over the treatment train. Total turbidity reduction was efficient, resulting in non-flow weighted averages between 5.7 NTU in Stage 1 and 4.2 NTU in Stage 3 after the CF.

An example of turbidity variation over the entire treatment train for Stage 3 is presented in Figure 3.4. In this figure, profiles were generated separately for data from greenhouses and from growout system effluents, because the two streams had significantly different pollutant loadings. For both water streams, sedimentation, ozonation and chemical flocculation appeared to have the greatest impact on turbidity

reduction. The initial turbidity of the growout facility stream was reduced sequentially in the SB, OR, and CF, by an average of 46.7, 73.0, and 75.0% respectively, relative to their influent values. The turbidity of greenhouse stream was reduced on average by 32.6, 65.7 and 81.8% in the same units. Qualitatively, the initial turbidity of the greenhouse effluent appeared to be less than one-third than of the growout facility, and the difference persisted (e.g., a two-fold difference) after chemical flocculation.

Figure 3.5 shows improvement of transparency of growout facility effluent after ozonation and chemical flocculation. The dark brownish color in the raw effluent was generated by presence of suspended solids, colloidal, and dissolved humic matter, but most of them disappeared after the combined physical and chemical treatment.

According to Grasso and Weber (1988), turbidity is the assay most commonly employed for determining the impact of ozone on particle stability. My results showed that ozone has the capacity to react with the complex matrix of the pollutants in BRA effluent, resulting in mineralization or destabilization of most organic matter, which then becomes prone to fractionation. An interesting observation to note in Figure 3.4 is that foam resulting from ozonation of the greenhouse stream had higher turbidity than foam resulting from ozonation of the growout systems effluent. The difference is probably due to higher concentration of solid and dissolved organics in the former, but also could imply a different degree of oxidation of these elements with influence on water's light-scattering capacity.

Efficient reduction of turbidity during chemical flocculation was likely a consequence of ozone interaction with pollutants in the previous step, as suggested by the correlation with TSS (Figure 3.3). The only mechanism that appears to be substantiated for improved flocculation after ozonation is polymerization of metastable organics and subsequent adsorption and inter-particle bridging or charge neutralization (Grasso and Weber, 1988). This results in stabilization, with the newly-formed polymers having the polyvalent ions from the flocculation agent at their core. With 95.7-97.0% average removal, turbidity was one of the parameters most dramatically improved in this study. The percent turbidity removed by ozonation in this study was equal or greater than those reported in studies of wastewaters of different nature (Millamena, 1992; Paraskeva et al.,

1998; Pena et al., 2003). This study confirmed the oxidizing power of ozone and the advantages of its applications for wastewater treatment.

#### 3.6.4. COD

COD in the raw influent was between 562.2 and 584.6 mg/L, decreasing to 44.4-68.2 mg/L after chemical flocculation. Table 3.6 shows COD concentration assessment for the SB, percent COD removal in different units, and total average COD percent removal for the entire train across the different experimental stages. The SB removed, on average, 59.2 and 62.7% for the two experimental flows, and the difference was statistically significant ( $p = 0.02$ ). These relatively low removal percentages indicate that a large portion of organics was dissolved or trapped in the unsettled colloids. However, subsequent treatment steps, such as ozonation, two forms of biological oxidation, and chemical flocculation removed most of the remaining COD. The highest average percent COD removal was 92.4%, achieved in Stage 3. A profile of COD dynamics through the treatment train in Stage 3 is presented in Figure 3.6, along with DOC values. The values represent averages only for samplings series of similar quality, i.e., those collected at 10:00 p.m. and 6:00 a.m.

Percent COD removal in the OR showed a positive, linear relationship with ozone dose applied (slope = 0.45;  $r^2 = 0.99$ ). A similar, but smaller correlation was observed between percent COD removal in the CF and ozone dose (slope = 0.12;  $r^2 = 0.74$ ) (Figure 3.7). Consequently, the degree of total COD removal,  $\eta(\text{CODt})$ , was more than double in Stage 3 (40.7%) than in Stage 1 (19.8%), under conditions of increasing ozone dose from 36.6 mg/L water in Stage 1 to 82.5mg/L in Stage 3 (Table 3.7). These differences proved statistically significant. The removal of approximately the same percentage of COD in Stages 2 and 4 (27.8 and 30.0%, respectively), indicates that a high ozonation rate could achieve the same degree of treatment in only a fraction of the time needed at low ozonation rates. These results agree with those of Paraskeva et al. (1998), who found that ozone dose was the main factor determining COD removal. However, foam removal was not addressed in these authors' study.

The TF also performed well in removing COD despite flow fluctuations to which it was exposed during operation. Average mass removals were 47.7 mg/L in Stage 1, 40.4

mg/L in Stage 2, 35.3 mg/L in Stage 3, and 41.3 mg/L in Stage 4. Comparing these mass-based COD removals to the percent COD removed in the TF, (Table 3.6), Stage 3 had both the lowest amount of COD removed, and the highest percent removal. A statistically significant difference was observed between Stages 1 and 3. This was presumably due to more efficient total COD removal during ozonation in Stage 3 (Table 3.7), which left less COD available to the TF.

Chemical flocculation removed a maximum of 50.1% of COD in average in Stage 3, while in the other three stages between 44.8 and 47.8% were removed (Table 3.6). There was a statistically significant difference between Stages 1 and 3, but not between Stages 2 and 4. Although the differences were not large, the results suggested that wastewaters treated with more ozone responded better to chemical flocculation. Grasso and Webber (1988) reported similar trends from a study on effects of ozone on flocculation. Another cause for better flocculation in Stage 3 could be that the lower COD content of the stream exiting the TF allowed more flocculating agent to be effectively available for reaction. This interpretation is supported by the larger percent TSS removal obtained by chemical flocculation for Stage 3 (Table 3.4).

#### 3.6.5. Reaction of ozone with COD

As previously discussed, ozone was suspected to be responsible for strong foam formation when interacting with BRA wastewater. This linkage is supported by the positive correlation (slope = 0.081;  $r^2 = 0.96$ ) between the ozone dose and the volume of foam removed (Figure 3.8), expressed as a percent of working stream flow in Table 3.7. Table 3.7 also shows other parameters characterizing ozonation and COD response during the four experimental stages with respect to system definition, experimental procedure, and assessment of results.

Despite the existence of a positive correlation between ozone dose and volume of foam produced, the COD concentration in foam did not appear to be dependent on the concentration of COD in the OR influent (Table 3.7). This was probably due to the different degree of dilution and COD mineralization,  $\eta(\text{COD})$ , % (equation 3.13), at various ozone doses.

Two ozone dose rates [ $F(\text{O}_3)$ , mg/L - s] (equation 3.6) were characteristic of this study at steady state (0.102 mg/L - s in Stages 1 and 4, and 0.153 mg/L - s in Stages 3 and 4), depending on the two initial ozone concentrations used in this study (i.e., 22 and 33 mg/L). The best ozone absorption [ $r_A(\text{O}_3)$ , mg/L - s] (equation 3.8) and consumption [ $r(\text{O}_3)$ , mg/L - s] (equation 3.7) rates were characteristic of Stage 1 [i.e.,  $r_A(\text{O}_3)=97.0\%$  and  $r(\text{O}_3)=96.0\%$  from  $F(\text{O}_3)$ ], under conditions of lowest ozone dose and longest hydraulic residence time ( $t_H$ ). At the other extreme set of conditions (i.e., Stage 3),  $r_A(\text{O}_3)$  was 86.9%, and  $r(\text{O}_3)$  was 83.0% of  $F(\text{O}_3)$ . Stages 2 and 4, in which the same dose of ozone was applied under different conditions of concentration and  $t_H$ , had close values of these two parameters, intermediate to the extreme. This indicates that  $t_H$  and ozone concentration could be equally important for the reaction under the conditions tested in this study. However, comparison of results from Stages 1 and 3 suggests that less ozone in gas and longer  $t_H$  could result in better ozone absorption and consumption. This interpretation is supported by the values of ozone transfer efficiency [ $\eta(\text{O}_3)$ , %] (equation 3.12), which represents a ratio between the specific ozone absorption ( $A^*$ , g  $\text{O}_3$ /g COD) and the specific ozone dose ( $I^*$ , g  $\text{O}_3$ /g COD).  $\eta(\text{O}_3)$  was determined to be 97.2% in Stage 1 and 87.2% in Stage 3, indicating closer values of  $A^*$  and  $I^*$  in the first case. These findings (e.g., better transfer at lower ozone concentration), are in agreement with results of the treatability study (Chapter 2). According to Gottschalk et al. (2000), a lower  $\eta(\text{O}_3)$  at high ozone concentration could be attributed to a faster depletion of COD from water, which reduces the chance of ozone reacting (i.e.,  $A^*$  becomes smaller relative to  $I^*$ ). The results from my study indicated that the near-zero dissolved ozone concentration in Stage 1 could be due to fast ozone reaction with pollutants, in which conditions the transfer rate limited the reaction rate. In contrast, in Stage 3 (and to a smaller extent, in Stages 2 and 4) residual dissolved ozone was observed, indicating that the reaction regime was kinetically controlled at this point. Besides faster COD reduction under conditions of intense ozonation at a certain  $t_H$ , Beltran et al. (2001b) suggested that the formation of compounds more refractory toward ozone also could contribute to  $\eta(\text{O}_3)$  decrease. However, despite a lower  $\eta(\text{O}_3)$  in Stage 3, in this stage the largest quantity of ozone was transferred. Such performance was attributed by Masschelein (1985) and by Mazzey et al. (1995) to Henry's law.

In this study, COD was used to assess reactions of organic pollutants with ozone. Parameters estimated were the rate of COD removal [ $r(\text{COD})$ , mg/L – s] (equation 3.9), the degree of COD removal  $\eta(\text{COD})$ , and the ozone yield coefficient [ $Y(\text{O}_3/\text{COD})$ ] (equation 3.14). Because of partial removal of COD in foam, these parameters were determined separately for total COD removed (COD<sub>t</sub>) and for COD oxidized (COD<sub>ox</sub>). This double determination allowed quantification of the advantages of foam removal for the ozonation process. Complete data for these parameters are presented in Table 3.7. For example, for the two extreme conditions tested in this study, total removal rates  $r(\text{COD}_t)$  of 0.125 and 0.169 mg/l – s in Stages 1 and 3 were determined, which corresponded to degrees of removal [ $\eta(\text{COD}_t)$ ] of 19.8 and 40.7%, respectively. In terms of COD oxidized,  $\eta(\text{COD}_{ox})$  was 7.7% in Stage 1 and 13.7% in Stage 3. If expressed as percentages of total COD removed, the amounts oxidized were 39.6% in Stage 1 and 33.5% in Stage 3, while the values for the other two stages fell between these values. The smaller percent of COD oxidized in Stage 3 could be explained by the fact that in this stage  $\eta(\text{O}_3)$  was lower by 10% than in Stage 1, and consequently, a smaller portion of the ozone dose reacted. Additionally,  $Y(\text{O}_3/\text{COD}_{ox})$  was larger in Stage 3 (2.23 g O<sub>3</sub>/g COD) than in the other stages (1.92-1.98 g O<sub>3</sub>/g COD), indicating decreased ozone efficiency with increased dose. An explanation for this phenomenon was provided by Beltran et al. (1999a), as referred above. When total COD removed was considered in determining  $Y$ , values between 0.70 and 0.77 g O<sub>3</sub>/g COD<sub>t</sub> were obtained. Values like those obtained for  $Y$  as a function of COD oxidized are typical of those from wastewater experiments. For example, in flow-through ozonation system with 7.5 minutes residence time, Beltran et al. (1999a) obtained an ozone yield between 0.87 and 1.36 g O<sub>3</sub>/g COD for domestic wastewater under steady state conditions, with higher performance at lower dosage. The results from my study suggest that by generating foam, the performance of ozone application could be enhanced up to three-fold with regard to COD removal. The maximum degree of COD removal (40.7%) under conditions of 87.2% ozone transfer efficiency, suggests that a larger dose of ozone (e.g., above that from Stage 3) can be used to reach the maximum limit of feasibility for ozone application to wastewater, suggested by Kirk et al. (1975) to be between 50 and 70% COD removal. However, establishing a “feasibility limit” must takes into account additional factors, and should be

determined in a case-by-case basis. Among these factors are the initial COD loading, the final effluent quality desired, pollutant composition and resistance to ozone attack, and the economic ability to afford a low ozone transfer efficiency.

#### 3.6.6. cBOD<sub>5</sub>

Starting from a concentration of 77.3-74.5 mg/L in the settled influent, cBOD<sub>5</sub> was reduced by a maximum of 88.1% in Stage 3, to 9 mg/L average concentration after chemical flocculation (Table 3.8). cBOD<sub>5</sub> increased because of methanol addition before denitrification, but was reduced to approximately its initial level in the DR effluent. The observation that cBOD<sub>5</sub> was not reduced to lower levels in that effluent indicates that methanol was added in sufficient amount (or occasionally in excess) to fully support denitrification, despite fluctuations of NO<sub>3</sub><sup>-</sup>-N concentration in the incoming influent. A treatment train profile of cBOD<sub>5</sub> dynamics in Stage 3 is presented in Figure 3.6. Results showed that the biodegradability index (i.e., the cBOD<sub>5</sub>:COD ratio) was between 0.24 and 0.29 in the SB effluent, which is much lower than that of a typical domestic wastewater of 0.5-0.6. Low biodegradability appears to be common in aquaculture effluents (Herbst, 1994; Easter et al., 1996), probably due to the presence of particulate and dissolved organic materials derived from uneaten feed, fish feces and mucus, mainly formed from molecules of proteins, lipids and polysaccharides. Those are substances with large molecular weight, which are not appropriate substrates for bacteria. To make them suitable for bacterial consumption, these substances should be hydrolyzed first, a common process being fermentation. However, in this study, the effluent from the fish systems had no time to ferment, because it entered the treatment train shortly after its release. Under these circumstances, the use of an external carbon source to support denitrifiers becomes not only legitimate, but absolutely necessary. In contrast to this situation, Grady et al. (1999) assessed that in sewage, most of the soluble organic matter is in the form of acetate and other short-chain fatty acids, because there is more time for fermentation as the wastewater flows to the treatment plant.

During ozonation, 19.0 – 27.6% of the cBOD<sub>5</sub> was removed (values from Stages 1 and 3, respectively), but the biodegradability index increased by 4.5 and 20.6% for the same stages (Table 3.8). The difference between Stages 1 and 2 was statistically significant.

The increase of biodegradability was technically due to a larger decrease of COD as compared to that of  $\text{cBOD}_5$  under certain conditions of reaction. For example, the difference can be observed in Figure 3.6, by comparing the values of these parameters at collection points ORi and ORe. Improvement of wastewater biodegradability by ozonation has been reported in other studies. Medley and Stover (1983) showed that upon absorption of  $50 \text{ mg O}_3/\text{L}$ ,  $\text{cBOD}_5/\text{COD}$  increased by 19% due to ozone creating biodegradable products and destroying compounds' inhibiting proprieties. More recently, Rivas et al. (2000), Beltran et al. (2001a), and Tosik and Wiktorowski (2001) attributed this phenomenon to the formation of smaller, oxygenated species under ozone attack, which were more suitable for microbial consumption in biological filters. However, Rosenthal and Kruner (1985) observed that at high ozonation levels,  $\text{cBOD}_5$  removal from aquaculture wastewater ceases. In a similar situation, Beltran et al. (1999b) suggested that the biodegradability increase becomes limited at higher (excessive) ozone doses, because chemical oxidation results in highly oxidized products with little metabolic value for microorganisms. Therefore, large amounts of ozone could be wasted upon easily-biodegradable reactions intermediates, and hence, system efficiency is decreased. However, in this study, the increase of ozone dose and its effect upon biodegradability improvement proved beneficial for removing  $\text{cBOD}_5$  in the TF, and even in chemical flocculation. Stage 3, in which the highest ozone dose was applied, showed the best results in this respect, i.e., 48.6%  $\text{cBOD}_5$  removed in TF, and 58.1%  $\text{cBOD}_5$  removed by CF. This suggests that none of the ozone doses tested was above the critical level at which effluent biodegradability could be impaired, despite the observation of Beltran et al. (1999b) of an optimum ozone dose at approximately  $40 \text{ mg/L}$  for domestic wastewater with similar COD concentrations.

An interesting phenomenon was observed in foam, where the biodegradability index had values between 0.46 and 0.58 (Figure 3.9). This higher degradability of organics was probably due to a more efficient contact and reaction of ozone with organics in the thin walls of the bubbles of the foam, even after they rise above the water surface in the reactor. Intense reaction in foam before condensing could also help explain the destruction of TSS and COD, which were missing in significant amounts from the condensed foam. Regardless of the reason, the foam may prove useful as a substrate for

controlled fermentation, generating volatile fatty acids that could replace methanol in the DR. This may require additional installations (e.g., fermentation reactors), but certainly would reduce the costs of operation by considerable savings on methanol and on condensed foam hauling.

### 3.6.7. DOC

Tests for DOC showed no significant difference between influent and effluent concentrations (e.g., all values were between 20.8 and 24.5 mg/L). This appeared to be an exceptional parameter, because water quality was improved dramatically with regard to most pollutants during treatment. In SB effluent, DOC represented 11.3% of COD concentration in Stage 1 and 10.7% in Stage 3, but after CF the ratios increased in the same stages to 32.9% and 49.6%, respectively, as shown by the example in Figure 3.9. The change of ratio obviously was due to a significant decrease of COD, with little overall variation of DOC. This also suggests that an increase in the oxidation state of organics occurred as the stream passed through the treatment units, notably during ozonation, as shown by Yu and Yu (2000). Further, large fluctuations of DOC were observed through the treatment train. Table 3.9 shows DOC concentrations dynamics throughout different units of the treatment train in each experimental stage. Average DOC concentrations in the MB are also presented, in order to assess the consumption of added methanol in DR. Methanol addition to the MB increased the organic substrate accounted for as DOC by an average of 198.7 and 159.6% for each of the two working flows. The addition of methanol at a constant flow rate was probably in excess when the  $\text{NO}_3^-$ -N was at a low concentration in the influent stream. Nevertheless, there were cases (i.e., at high  $\text{NO}_3^-$ -N concentration in the influent) in which the denitrification process used some of the effluent DOC in addition to the added methanol, resulting in lower DOC in the DR effluent. These findings contradict those of Balderston and Siueburth (1976) and Arbiv and van Rijn (1995), who suggested that organics from aquaculture wastewaters include mostly long-chain carbon molecules, proving unsuitable to sustain denitrification. Overall, on a non-flow weighted average basis, between 59.2 and 54.4% of the DR influent DOC (natural plus methanol) was removed by cell growth during denitrification from each working stream. There was no statistically significant difference

between the two concentrations or percent removals, which suggests that the stoichiometric ratio of 3.6 mg CH<sub>3</sub>OH/mg NO<sub>3</sub><sup>-</sup>-N was sufficient. Also, in situations when the stream NO<sub>3</sub><sup>-</sup>-N was around 50 mg/L, the concentrations of COD before entering MB and after DR were approximately the same. The ratio used in this study was in agreement with those of Jeris and Owens (1975), Jeris et al. (1977) and Semon et al. (1997), ranging between 2.9 and 4.2 mg CH<sub>3</sub>OH/mg NO<sub>3</sub><sup>-</sup>-N. Authors that recommended a ratio of 3:1 conducted their studies on wastewaters with much higher DOC, but they did not specify the role of this form of DOC in denitrification. Therefore, it can be assumed that at least in part, the natural DOC was suitable for bacterial growth, which could explain the near complete denitrification achieved in this study when higher NO<sub>3</sub><sup>-</sup>-N concentrations (e.g., above 50 mg/L) were present in the influent. Alternatively, some of the NO<sub>3</sub><sup>-</sup>-N could have been converted to ammonia through assimilative reduction for use in cell synthesis, thereby increasing the ratio by making less NO<sub>3</sub><sup>-</sup>-N available for denitrification.

Following treatment with ozone, stream DOC increased by 14.4-27.7% (i.e., from OR average influent of 27.2-28.5 mg/L, to 33.3-37.6 mg/L in the effluent). DOC appears to be a byproduct of the ozonation process, its generation also being reported by Summerfelt et al. (1997b) and Kamiya and Hirotsuji (1998). Wang and Pai (2001) attributed an increase of DOC by up to 120% following wastewater ozonation to decomposition of microorganisms and other unsettled solids. Further, by filtering the same wastewater before ozonation, they obtained an 80% reduction of DOC after two hours of ozonation. Because in this study, the results were obtained from a similar wastewater (i.e., rich in microorganisms and solid biofloc), it can be assumed that the effluent DOC increased through a similar mechanism.

The degree of DOC generation in OR was not linear with increase in ozone dose. The largest average percent increases were found in Stages 2 and 4, the lowest increase in Stage 1, and an intermediate increase in Stage 4 (Table 3.9). Percent increases in Stages 1 and 3 were statistically different, but those in Stages 2, 3 and 4 were not. Figure 3.10 shows these values plotted against the ozone dose applied in the OR. The shape of the regression line suggests that there was more DOC generation than destruction at lower ozone doses. As ozone dose increased, DOC reached a maximum as the two processes

rates equalized, at approximately the ozone dose used in Stages 2 and 4 (i.e., 55 mg O<sub>3</sub>/L water). Beyond this point, DOC destruction became predominant, resulting in a decline of DOC accumulation. Wang and Pai (2001) reported similar results, suggesting that the peak of DOC generation during ozonation coincided with the moment when all microorganisms were decomposed. Following that moment, the organic compounds accounted for as DOC become removed more rapidly by mineralization.

DOC removal in the TF ranged between 20.6 and 23.1%, and did not appear to depend on the ozone dose used in the previous step. This finding indicated that at this point, a major part of DOC was not amenable to bacterial consumption. Studying post-ozonation DOC characteristics, Carlson and Amy (1997) showed that only a part of DOC is readily biodegradable (called DOC<sub>rapid</sub>), while the remainder biodegrades more slowly (called DOC<sub>slow</sub>). Further, they reported that the formation of DOC<sub>slow</sub> was not sensitive to ozone dose, which is consistent with the results from this study.

DOC was reduced by 6.9 – 7.9% during chemical flocculation, but the final effluent had an overall DOC approximately equal to that of the stream that entered the treatment train. However, there was probably a compositional difference, in that the final DOC was less biodegradable. Establishing the impact of this final DOC on fish if the stream is reused, remains a subject for further investigation.

#### 3.6.8. Alkalinity

Water quality management practices at BRA include alkalinity control, which is accomplished by addition of industrial grade sodium bicarbonate (NaHCO<sub>3</sub>). The addition of alkalinity buffers the pH, and replaces alkalinity lost by nitrification and water exchange. The operation of the pilot station showed that alkalinity lost by water exchange can be recovered, and further that the treated effluent would be enriched by up to one-third of the initial amount of alkalinity. Table 3.10 shows the averages of alkalinity variation through the treatment units.

In the DR, alkalinity was generated, increasing by 39.7-40.4% in all stages. Despite a significant increase in percentage terms, the net production represented only about 2 mg/mg NO<sub>3</sub><sup>-</sup>-N reduced. Because the stoichiometry of denitrification reactions indicates a ratio of about 3.6, it can be inferred that some of the NO<sub>3</sub><sup>-</sup>-N was transformed into

ammonia by assimilative reduction and used in cell synthesis when ammonia was lacking, as suggested by Grady et al. (1999). The inference is supported by the observation that the effluent subjected to treatment was poor in TAN, having a ratio of about 1:20 with  $\text{NO}_3^-$ -N.

During ozonation, between 6.4-11.9% of the alkalinity was lost. The loss of alkalinity could be due to the scavenging effect of its ions on ozone, considering that the stream entering the OR was at an alkalinity level of 175.5-187.5 mg/L. Wang and Pai (2001) indicated that  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  ions compete in wastewater with organic matter for reaction with  $\text{OH}^\circ$  radical, and that a high alkalinity could impair the reaction of ozone with targeted organics. They also suggested that alkalinity depletion increases with ozone dose increase, due to an increased probability of  $\text{OH}^\circ$  radical formation as a consequence of faster organics removal. However, the relatively low percent removal of alkalinity in this study was probably due to the pH, which at a value slightly over 8.0 was not high enough for carbonate ion formation, while ozone is less reactive with bicarbonate ion. For example, Buxton et al. (1988) found the reaction rate constants for the reaction of hydroxyl with inorganic carbon to be  $0.85 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$  for  $\text{HCO}_3^-$ , and  $39 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$  for  $\text{CO}_3^{2-}$  ions.

In the TF, some more alkalinity was removed, which probably was due to nitrification. This was not surprising, considering that stoichiometrically 1g of TAN can destroy 8.62 g  $\text{HCO}_3^-$  during oxidation to  $\text{NO}_3^-$ -N (Grady et al., 1999). However, because TAN concentration was relatively low and because the nitrification was not complete (i.e.,  $\text{NO}_2^-$ -N was produced in the biofilter), the final effluent still had 26.0 – 32.6% more alkalinity than the stream entering the treatment train. This suggests that the reuse of this effluent could result in significant savings on supplemental alkalinity addition to the RAS.

### 3.6.9. pH

The pH of the discharged effluent was neutral or slightly basic, close to that from the fish tanks. According to Millamena (1992), a neutral pH in wastewater could be due to the presence of inorganic salts, while Medley and Stover (1983) attributed the maintenance of a neutral pH to the heterogeneous composition of the organic matter.

BRA effluent proved to have both of these characteristics. After entering the treatment train, pH increased slightly in the storage tanks and SB, and then more significantly during denitrification, reaching values between 8.22 and 8.26 in the DR effluent (Table 3.11). The pH increase was probably due to intense biological activity in these units, especially in DR where the increase was also promoted by alkalinity generation. During ozonation the pH decreased, probably because some of the alkalinity was also lost. Nevertheless, the loss of alkalinity caused a reduction in water buffering capacity. Observation of pH shift during ozonation is in agreement with Kirk et al. (1975), who found that whether the ozonation feed water is acidic or basic, the product water always shifts toward neutrality, and the pH change is greater for higher COD feeds. Further, Wang and Pai (2001) suggested that the best results for organics removal by ozonation are obtained at low pH, which could indicate that the high pH-waters in this study could be a disadvantage. A lower pH would likely affect the composition of the  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  in water by reducing their concentrations and the composition of the functional groups on the organic compounds, and hence affecting the oxidation process.

The pH increased again in the TF, which was somehow surprising, considering that some nitrification occurred in this filter and alkalinity was partially lost. It is possible that the large amounts of organics in the feed influent and their overwhelming compositional diversity had a dominant influence over pH change, overshadowing the effects of nitrification of relatively small amounts of ammonia. Additionally,  $\text{CO}_2$  stripping could contribute to the pH increase. Further decline of pH was found to occur during the chemical flocculation, reaching final values of about 7.60, which is considered safe for fish.

#### 3.6.10. Hardness

Hardness comes into the BRA systems with the replacement spring water and feed, and supports biomass development in both fish and microorganisms in biofilters. Average hardness concentrations between 148.4 and 151.5 mg/L as  $\text{CaCO}_3$  place BRA effluent into the category of medium-hard wastewaters. Table 3.12 presents the fate of hardness (concentration and percent) as the effluent passed through different units of the treatment station. The results showed that small amounts of hardness were lost between the SB and

DR effluents, which suggests that it was probably removed by the MSF vacuum system after bonding with dissolved and colloidal organics, or was assimilated during bacterial growth in the DR. The hardness decreased further during ozonation, the amount removed being proportional to the amount of ozone used in the process, (e.g., 4.1% removal in Stage 1 and 7.8% removal in Stage 3, respectively). There was a statistically significant difference between hardness removals in these two stages. The mechanism for ozone-induced hardness removal was probably the one described by Grasso and Weber (1988), who stated that increase in water carboxyl acid content due to ozonation may lead to greater magnesium and calcium association, resulting in precipitation of metal-humate complexes. The mechanism also helps explain the different hardness removals at different ozone doses in this study, suggesting that higher ozone doses would produce more destabilized carboxyl acids, which bond with a larger amount of hardness ion species. Additionally, noting that Rueter and Johnson (1995) reported that increasing hardness up to 150 mg/L CaCO<sub>3</sub> in ozonated water continuously improves the removal of TSS, it can be concluded that BRA effluent had ideal conditions from this point of view. After being formed in the aqueous medium, the complexes likely were caught on bubble surfaces and buoyed to the top as foam. This mechanism also could explain the great removal efficiency of TSS during ozonation. However, once in the foam, the associations of hardness with TSS appeared to have been severely attacked by ozone from the trapped gas, which this time broke and dissolved these particles, as discussed in Section 3.6.2.

Smaller amounts of hardness then were lost in the TF unit, probably due to bacterial assimilation and bonding into the solids.

#### 3.6.11. TKN

In the raw influent, TKN ranged between 41.8-42.3 mg/L, most of it being organic nitrogen. Following chemical flocculation, average TKN was 2.0 mg/L or less in effluents from all stages. This indicates that TKN removal was more efficient than that of COD overall. Table 3.13 shows TKN removal in different treatment units, and through the entire treatment train. TKN was removed in approximately the same proportion with COD in SB, which indicates that nitrogen-containing organics were distributed similarly between the solid and dissolved forms. A slight decrease in TKN was observed in the

DR, which could be attributed to ammonia consumption in this reactor. The ozonation process removed up to 54.3% of the influent TKN (under the highest ozone dose), which is more than the corresponding percent COD removal under the same conditions. There was a statistically significant difference in TKN removal between Stages 1 and 3, which could indicate that TKN removal rate depended on the ozone dose applied. According to Razumovskii and Zaikov (1984), nitrogen-containing compounds are more prone to destabilization when interacting with ozone than many other organics, which facilitates bonding with opposite electrical charges. In this case, they probably bonded directly to charges at the surface of gas bubbles (fractionation effect) or with polyvalent ions, and subsequently were removed with foam. Some of these molecules also were mineralized, which is reflected in the ammonia increase during ozonation. In turn, a part of the ammonia was further oxidized to  $\text{NO}_3^-$ -N, due to favorable conditions provided by an alkaline environment and pH higher than 8, which is reflected by a slight  $\text{NO}_3^-$ -N increase during ozonation. This interpretation would be in agreement with Lin and Wu (1996), who suggested that it is possible for TKN to be reduced during ozonation in alkaline conditions, mainly due to ammonia oxidation. These results show in general a better removal of TKN than was reported in other studies. For example, Beltran et al. (2001a) found a 26% TKN removal at ozone doses between 40-60 mg/L from domestic wastewater preliminarily treated biologically. The better performance in this study could be attributed to higher alkalinity in BRA wastewater, and to different composition of organics in the two wastewaters. In the TF, TKN was reduced by 15.-30.8%. The percent removal does not appear to depend on the ozone dose used in the previous treatment step. Although according to Beltran et al. (2001a) an increase in ozone dose should promote better TKN removal, in this study the situation was different probably due to the presence of a great part of TKN in ammonia form after ozonation. Under these circumstances, TKN removal efficiency was rather dependent on the nitrification performance of the TF.

TKN removal by the CF was higher than that from SB, in terms of percent removal. However, comparing the averages of 1.7-2.0 mg/L TKN after the CF to those of TAN from the same effluent (1.5-1.7 mg/L), it is clear that the organic part of TKN was almost entirely removed by the end of the treatment.

### 3.6.12. TAN

The average influent TAN concentration ranged between 2.53 and 2.58 mg/L in all experimental stages. These concentrations increased slightly during sedimentation presumably due to bacterial activity, but were significantly reduced during denitrification. According to Grady et al. (1999), ammonia is utilized preferentially as a nitrogen source by the heterotrophic bacteria during this process, which could explain the reduction of TAN by 48.4-50.2% when the stream passed through the DR. Table 3.14 shows details about TAN dynamics through different treatment units and over the entire treatment train. However, during ozonation, TAN concentration rose to levels higher than those in the initial (SB) influent by a stage average of 29.1-39.9%. At the same time, these TAN concentrations were at least twice those in the DR effluent, as was observed in Stages 2 and 3. The increase of TAN concentration during ozonation appeared to be in a positive, linear relationship with ozone dose (slope = 0.012;  $r^2 = 0.93$ ) (Figure 3.11). The increase of TAN during ozonation was probably due to amino acid and protein oxidation by ozone. Ammonia is a byproduct of these reactions, especially when they are complete (e.g., mineralization). The basic pH of the OR influent appeared to promote partial oxidation of ammonia to nitrate, because  $\text{NO}_3^-$ -N increased by more than should be produced from influent  $\text{NO}_2^-$ -N oxidation. However, the reaction was insignificant, and ammonia accumulation predominated. Rosenthal and Otte (1979) and Wang and Pai (2001) reported similar findings: partial oxidation of ammonia to  $\text{NO}_3^-$ -N during ozonation in alkaline conditions, but at the same time, TAN accumulated due to oxidation of organic nitrogen.

In the TF, partial nitrification (53.6 to 63.9% TAN removal) occurred, along with organic removal. The organic loading of the TF was estimated at 0.43 kg cBOD<sub>5</sub>/m<sup>3</sup>-d during Stages 1 and 2, and 0.65 kg cBOD<sub>5</sub>/m<sup>3</sup>-day during Stages 3 and 4. At these loadings, conditions were not permissive for nitrifiers to grow and compete with heterotrophs, which made the nitrification performance of the TF surprisingly good. By comparison, Metcalf and Eddy (1979, cited in Karnchanawong and Polprasert, 1990) obtained 75 – 85% TAN removal in a TF at volumetric loading of between 0.1 to 0.16 kg BOD<sub>5</sub>/m<sup>3</sup>-day. Additionally, Parker and Richards (1986) found a relationship between nitrification and the soluble BOD<sub>5</sub> in a TF, suggesting a maximum threshold of 27 mg/L

BOD<sub>5</sub> in order for any nitrification to occur. The results from this study showed the same relationship, TAN removal occurring more efficiently when the stream had less organics, as in Stage 3. However, in this study I measured cBOD<sub>5</sub> instead of sBOD<sub>5</sub>.

The final effluent had TAN stage averages between 1.52 and 1.69 mg/L, which is generally undesirable in water used for exchange in RAS. However, it was determined that were this treated water used for exchange at BRA, only 0.84% of the daily TAN production would be reintroduced to the systems. The RBCs should be able to remove these amounts, as is demonstrated in the next chapter.

### 3.6.13. NO<sub>2</sub><sup>-</sup>-N

Nitrite is a byproduct resulting from incomplete nitrification in the systems' RBCs. The average concentration was between 0.92 and 0.96 mg/L NO<sub>2</sub><sup>-</sup>-N in BRA effluent. Along the treatment train, NO<sub>2</sub><sup>-</sup>-N concentration fluctuated, as presented in Table 3.15. First, in the DR, between 71.7 and 76.0% of the influent NO<sub>2</sub><sup>-</sup>-N was reduced. According to van Rijn and Rivera (1990), this could indicate that the external carbon source was supplied in sufficient amount to support the completion of denitrification; therefore, influent nitrite also was reduced in this process. Another reason for nitrite reduction could be its utilization as a source of nitrogen by heterotrophic organisms (e.g., in the upper part of the biofilter), considering the relatively low concentration of TAN in the stream. It would be impossible to conclude which of these factors determined nitrite reduction, but this was certainly a positive outcome, since accumulation of NO<sub>2</sub><sup>-</sup>-N in denitrification filters often is reported. At the end, NO<sub>2</sub><sup>-</sup>-N reduction in the DR was a positive outcome from methanol use in excess during operation. Even if NO<sub>2</sub><sup>-</sup>-N resulted as a byproduct of denitrification, it would be oxidized to NO<sub>3</sub><sup>-</sup>-N during ozonation, but this would be at the expense of a higher NO<sub>3</sub><sup>-</sup>-N in the final effluent. Although the excess methanol posed a higher load of dissolved organics on the OR, this may be preferred to poorer removal of nitrogenous compounds.

Next, the remaining NO<sub>2</sub><sup>-</sup>-N then was oxidized totally to NO<sub>3</sub><sup>-</sup>-N in the OR, regardless of the ozone dose used in this study. These results are in agreement with Rosenthal and Otte (1979), who found that even with light ozonation, NO<sub>2</sub><sup>-</sup>-N from aquaculture wastewaters could be oxidized efficiently to NO<sub>3</sub><sup>-</sup>-N.

Although the stream entering the TF had zero or only trace  $\text{NO}_2^-$ -N, the effluent had an average of 0.45-0.61 mg/L  $\text{NO}_2^-$ -N, varying among experimental stages. This concentration represented between 52.9 and 63.8% of the treatment train influent concentration. The generation of  $\text{NO}_2^-$ -N in the TF was probably due to incomplete nitrification of ammonia. One of the causes could be the lack of  $\text{NO}_2^-$ -N itself as substrate in the influent, which did not support the growth of bacteria converting  $\text{NO}_2^-$ -N to  $\text{NO}_3^-$ -N (i.e., *Nitrobacter sp.*). Summerfelt (2003) suggested that lack of these species in nitrification biofilters can be a drawback of integrating an ozonation step in an RAS treatment loop, although the decrease of nitrite levels in a RAS is a substantial benefit. According to Parker and Richards (1986), another cause of nitrite generation could be that growth of *Nitrobacter sp.* is suppressed by faster-growing heterotrophs under conditions of abundant organic material. Grady et al. (1999) confirmed that *Nitrobacter sp.* are the slowest-growing nitrifiers and are, indeed, the first to be eliminated by heterotrophs in a biofilter, if competing for space. Considering the TF influent nitrite and organic concentration, both scenarios appear plausible causes for nitrite accumulation.

As in the case for ammonia, presence of nitrite is undesirable in waters used for RAS exchange because of its toxicity to fish, though concentrations like those in the final effluent do not represent a threat to fish. Further, it is expected that the RBCs would be able to remove the amounts of  $\text{NO}_2^-$ -N returned with exchange water.

#### 3.6.14. $\text{NO}_3^-$ -N

Nitrate was the most abundant form of nitrogenous pollutant present in BRA effluent, resulting from nitrification and accumulation in the system. Stage averages were between 42.8 and 43.2 mg/L  $\text{NO}_3^-$ -N in the influent (Table 3.16), but large diurnal variations were observed due to different origins of the wastewater from greenhouses and from grow-out systems. Figure 3.12 shows the nitrate concentrations across the DR, as well as the entire system experiencing diurnal variation. The DR was able to remove on average 95.8-97.0%  $\text{NO}_3^-$ -N in different experimental stages, suggesting that the biofilter was able to adapt rapidly to the nitrate fluctuations. This is in agreement with Jeris and Owens (1975), who suggested that under conditions of nitrate variation, it is sufficient to supply

the right amount of carbon source at any time in order to obtain satisfactory denitrification. Nitrate removal performance also appeared to be independent of the recycled stream fraction used in different stages.

Finally, the efficiency of the overall treatment system was slightly lower than that of the DR unit, because  $\text{NO}_3^-$ -N was produced during ozonation and nitrification in the TF. However, the final concentration was in general less than 3 mg /L. This concentration poses no issue for reuse of the recovered wastewater for fish production.

### 3.6.15. Kinetics of denitrification

A half-order kinetic model was used to determine the rate constant ( $k$ ) (equation 3.16) and the removal rate of  $\text{NO}_x^-$ -N (equation 3.15). The assumption was based on Stephenson and Murphy's (1980) findings. They determined that for a FBBR with sand media, the half - order model coupled with an Arrhenius temperature dependency relationship had the highest probability of being correct (i.e., about 88%). In this study,  $k$  was between 0.82 and 1.69 for the first working flow (Stages 1 and 2), and between 0.57 and 1.26 for the second (Stages 3 and 4) (Table 3.17). It appeared that the values of  $k$  were dependent on the amount of  $\text{NO}_x^-$ -N fed, which suggested that the reaction is kinetically controlled, with  $\text{NO}_x^-$ -N being a limiting factor. This is supported by the observation that the reduction of  $\text{NO}_x^-$ -N had a very close, positive linear relationship with  $k$  for both flow rates (slope = 0.039 for the first working flow and slope = 0.044 for the second;  $r^2 > 0.99$  for both cases) (Figure 3.13). This finding was in agreement with Coelho et al. (1992), who demonstrated that a sand FBBR works on the kinetically-controlled regime at concentrations comparable to those in this study, despite small  $\text{NO}_x^-$ -N residuals in the effluent. The results showed that  $k$  values found in this study were specific to this type of reactor at an operating temperature of around 28°C. For example, Jeris et al. (1977), and Stephenson and Murphy (1980) found  $k$  to be between 1.4 and 1.8 at 23 - 30°C and about 20 mg/L  $\text{NO}_x^-$ -N influent. The latter authors also demonstrated that  $k$  does not depend greatly on hydraulic flux through the reactor, but rather on temperature and on influent  $\text{NO}_x^-$ -N. In addition, they suggested that a FBBR can provide denitrification rates more than ten-fold larger than suspended growth and RBC reactors, which could be due to a higher biomass concentration and to a longer residence time of

solids. In practical terms, the reaction rate values found in this study (Table 3.17) may suggest that shallower beds could be used for this application, or that the FBBR can operate efficiently without any recycling (effectively feeding it with higher  $\text{NO}_x^-$ -N concentrations).

From tests on the batch reactor, cell yield ( $Y_{\text{NO}_3^- - \text{N}}$ ) was found to be 0.69 g VSS cells produced/g  $\text{NO}_3^-$ -N consumed. This  $Y$  value is in agreement with values reported in literature from tests with similar  $\text{NO}_3^-$ -N concentrations (Table 3.18). However, Moore and Schroeder (1970) showed that under steady state flow through conditions and  $\text{NO}_3^-$ -N feed variation,  $Y_{\text{NO}_3^- - \text{N}}$  decreases linearly with increased  $\text{NO}_3^-$ -N concentration to about 35 mg/L  $\text{NO}_3^-$ -N, and remains constant thereafter. They attributed this relationship to a saturation effect, based on the fact that some species of bacteria synthesize polysaccharide storage materials under nitrogen-limited conditions. Consequently, the process slows as more  $\text{NO}_3^-$ -N is utilized, resulting in  $Y_{\text{NO}_3^- - \text{N}}$  dropping until  $\text{NO}_3^-$ -N reaches the saturation level. Above 35 mg/L  $\text{NO}_3^-$ -N,  $Y_{\text{NO}_3^- - \text{N}}$  was found to be around 0.60 g VSS cells produced/g  $\text{NO}_3^-$ -N consumed (Moore and Schroeder, 1970), which is consistent with the value found in this study. Therefore, the inference would be that the tests in this study were conducted under saturation conditions. Indeed, the batch tests started from a  $\text{NO}_3^-$ -N concentration of 207 mg/L, and were interrupted close to the alleged saturation limit. In addition,  $Y_{\text{VSS}}$  and  $Y_{\text{COD}}$  also were determined, having values of 0.29 and 0.41 g/g, respectively. These values agree only with those reported by Grady et al. (1999), and are larger than the values reported by other authors (Table 3.18). For example, Jeris and Owens (1975) suggested that between 15 and 20% of the methanol consumed is expected to be converted into cell mass, while Karnchanawong and Polprasert (1990) found this conversion to be between 20 and 28%. The difference could be explained by noting that Grady et al.'s (1999) values were obtained under similar conditions to those in this study, such as excess  $\text{NO}_3^-$ -N, and batch continuously-stirred tank reactors (CSTR). It was therefore exploiting the maximum potential of ATP formation under anoxic conditions, which resulted in a higher yield for anoxic growth. In contrast, other authors (i.e., those cited in Table 3.18) derived their results from steady state operating conditions in FBBRs, which have much larger denitrification rates than continuously stirred tank reactors (CSTRs). Lower denitrification rates coincide with

high solids production rates (Stephenson and Murphy, 1980), which could help explain the larger  $Y_{VSS}$  and  $Y_{COD}$  observed in CSTR tests. Indeed, in my batch study, denitrification rate constants were no larger than  $0.015 \text{ mg}^{1/2} \text{ L}^{-1/2} \text{ min}^{-1}$ .

$Y_{NO_3^- - N}$  also was determined in this study from the DR data, in order to confirm the above results and interpretations, along with the behavior of DR under conditions of diurnal  $NO_3^- - N$  variations. The findings (Table 3.19) confirmed that this reactor worked at an  $NO_3^- - N$  dose smaller than saturation, had large reaction rates, and resulted in larger  $Y_{NO_3^- - N}$ . Additionally, the largest yields were obtained for the afternoon measurements (e.g., the lowest  $NO_3^- - N$  influent), regardless of experimental stage or working stream flow.

The weighted average biomass production of DR (as VSS) was estimated at  $20.203 \text{ kg VSS m}^3 \text{ day}^{-1}$  for the first working flow rate, and  $15.8 \text{ kg VSS m}^3 \text{ day}^{-1}$  for the following one. The difference was probably due to the different percent of recirculation, which resulted in different working streams. As was suggested by the biofilter  $Y_{NO_3^- - N}$  (i.e., approximately one), nitrogen removal had values close to those of VSS removal. The nitrogen removal was between  $23.4 \text{ kg NO}_3^- - N \text{ m}^3 \text{ day}^{-1}$  for the first working flow, and  $16.2 \text{ kg NO}_3^- - N \text{ m}^3 \text{ day}^{-1}$  for the second. In this study, maximum nitrogen removal performance of the DR was better than what is generally expected from this technology for domestic wastewater treatment. For example, Coelho et al. (1992) obtained nitrogen removal of  $5.4$  to  $10.4 \text{ kg NO}_3^- - N \text{ m}^3 \text{ day}^{-1}$ . Semon et al. (1997) suggested a maximum design loading of  $6.4 \text{ kg NO}_3^- - N \text{ m}^3 \text{ day}^{-1}$ . Jeris and Owens (1975) reported nitrogen removal of  $20.7 \text{ kg NO}_3^- - N \text{ m}^3 \text{ day}^{-1}$  in sand FBBR. The removal was better in this study probably because of the higher operating temperature, which determined higher reaction rates. The reaction rate values suggested that even higher nitrogen removal could be obtained in a DR operated under these conditions if recycling were not employed in order to assure bed fluidization. Additionally, the reactor was able to maintain a high VSS biomass concentration, which was found to be around  $38,000 \text{ mg/L}$ . Under these conditions, the sand from the settled media represented on average only 17% of the settled bed volume in the reactor.

#### 3.6.16. Statistical results

The relationships between ozone dose and parameters TSS, COD, foam flow rate, and TAN were modeled using linear regression. The slopes and the  $r^2$  values quantifying direction and strength of any relationships are shown in Figures 3.3., 3.7., 3.8, and 3.11, respectively. The linear regression between the reduction of  $\text{NO}_x^-$ -N concentration and the reaction rate coefficient,  $k$ , is given in Figure 3.13.

95% simultaneous confidence intervals were determined to compare means of water quality parameters after passage through each of the five main units of the train. The model used was one-way ANOVA with the four stages as the factor in the model; the values of the parameters in the influent water added as covariates for ANOVAs performed for the SB and the DR. For the units following the DR, covariates were not necessary because they did not improve the model. Comparisons between the four stages are given in individual tables for each parameter.

### **3.7. Estimation of cost for consumable materials for wastewater treatment at BRA**

For operating a scaled-up wastewater treatment station based on the pilot-scale design evaluated in this study, treatment of the entire BRA effluent of 2260 m<sup>3</sup>/day would need the following supply of consumable materials:

#### **3.7.1. Electricity for ozone production**

The dose of ozone assumed is 0.1 g/L wastewater, which is about 15% larger than the dose used in the best experimental stage during the pilot station experiments (i.e., Stage 3). Results of the pilot-scale study showed that such an increase should be economically feasible. This dose represents a total of 226 kg O<sub>3</sub>/day. At an average of 12.14 kWh consumed for 1 kg of ozone produced, the required daily amount of energy will be 2743.6 kWh. At a market price of \$0.04/kWh, the costs of producing ozone will be \$110/day. Assuming that the amount of energy required to operate the station (e.g., pumps) is 10% of the energy required to produce ozone, the total costs for electricity would be \$120.75/day.

#### **3.7.2. Oxygen for ozone production and delivery**

With current technology, which is capable of transforming 12% of oxygen into ozone, 1652.87 m<sup>3</sup> of oxygen will be necessary to produce the daily amount of ozone required (i.e., 1.17 m<sup>3</sup> O<sub>2</sub>/min). This volume is equivalent to 2257.6 kg O<sub>2</sub> (at 1.34 g O<sub>2</sub>/m<sup>3</sup>). Assuming oxygen recirculation and a supplemental loss of oxygen due to dissolution in water, the oxygen consumption should be approximately 10% of the amount used. Hence, the cost of 225.8 kg of oxygen consumed daily will be \$15.50/day, at a bulk price of \$0.092/m<sup>3</sup>.

### 3.7.3. Methanol for denitrification

BRA releases 97 kg NO<sub>3</sub><sup>-</sup>-N with the effluent, at an average concentration of 43 mg/L. At an assumed methanol:nitrate-nitrogen ratio of 3.2:1 required by denitrification, 310.4 kg methanol per day will be necessary. At a methanol density of about 0.8 kg/L, 388L methanol will be necessary. The industrial grade methanol has 98% concentration, which results in a total of 396L. At a price of \$0.50/L, the cost of methanol will be \$198/day.

### 3.7.4. Ferric chloride for chemical flocculation

Jar test showed that treatment with an average of 50 mg FeCl<sub>3</sub>/L wastewater, followed by sand filtration, could reduce the TSS in the treated effluent well below 10 mg/L. At this dose, 113 kg FeCl<sub>3</sub>/day would be necessary. At an average market price of \$300/metric ton FeCl<sub>3</sub>, the daily cost should be \$34.

### 3.7.5. Labor

The operation of a full-scale wastewater treatment station was estimated to require labor equivalent to a full-time position, including weekends. However, on a daily basis, the tasks of monitoring and maintaining the installations and operations probably could be handled among employees that work in shifts in adjacent sections of the facility. This will add up to 56 hrs of labor per week, or 240 hrs per mo. At an estimated wage of \$10/hr, the labor costs will add \$2,400/mo to total costs.

### 3.7.6. Savings from not heating exchange water

Operation of the pilot station indicated that water temperature did not decrease in effluent by more than 5% during the 3-4 hrs of treatment. However, the pilot station was operated during the summer, and high environmental temperature could have affected the rate of temperature loss. Considering that the treatment station also will function during the winter and the water will spend time in a storage tank after treatment before being reutilized, an estimated temperature decrease by an average of 25% might be assumed. Because BRA spends between \$10,000 and \$28,000/month (depending on the season) on combustible material (oil) to heat the water, 75% heat recovery will represent \$14,250 per month in savings, from the current average of \$19,000. That is, only \$4,750/mo (\$158.33/day) will be spent to bring replacement water to culture temperature and \$14,250/mo will represent savings.

The summation of all the above costs results in total estimated expenses of \$606.60/day (\$18,198/month) for consumable materials. It may be concluded that considering the maximum of \$14,250 that could be saved on water heating combustible (i.e., if the entire effluent is reused), the wastewater treatment at BRA would cost only \$3,948/mo.

### **3.8. Summary, conclusions and recommendations**

The work with the pilot station showed that the treatment strategy employed could support effective recovery and recycling of BRA effluent. Most of the water quality parameters were dramatically improved, although to reach satisfactory levels for parameters such as dissolved organics and ammonia, refinement of methods will be needed. These aspects can be addressed if a full-scale treatment station based on this design is placed into operation.

Up to 99% of TSS was removed by the treatment process. However, the experience with the pilot station showed that it is important to provide effective solids removal by sedimentation, because it would be harder and more expensive to remove TSS from the subsequent units. Further, unremoved solids would interfere with the operation of these units, negatively affecting their performance. For example, high solids concentration

would divert the ozonation process from its main purpose of removing dissolved and colloidal organics, and also would affect the normal operation of the DR. Under these circumstances, it is important to employ a sedimentation basin that not only will settle solids, but also remove them fast enough to prevent floatation. TSS removal of 85% should be a minimum target for sedimentation alone. Such performance could eliminate the need for microscreen filtration.

Another important aspect that should be considered in sedimentation basin design is the intermittent nature of wastewater release from the facility. Large amounts of wastewater are discharged for several-hour periods, alternating with similar periods of sporadic or no effluent release. Consequently, the need for sedimentation could be overshadowed by the need for storing excess effluent. Because a sufficient and continuous source of effluent is paramount for the operation of the treatment station, it is likely that the need for storage will determine the size of the sedimentation basin. Alternatively, a better distribution of water release over time could reduce the need for a large storage space.

COD was removed throughout the treatment system, by sedimentation, ozonation, biological oxidation, and chemical flocculation. Performance of the ozonation unit was significantly different between the treatment stages, as a function of ozone doses applied (e.g., 19.8 and 40.7% COD removal at doses of 36.6 and 82.5 mg O<sub>3</sub>/L water). The tests also showed that BRA effluent is rich in organics that react easily with ozone, and which form abundant foam. Between 60.4-66.5% of COD was removed as foam, and between 35.5-39.6% was completely oxidized by ozone. Additionally, a larger dose of ozone showed beneficial effects on the performance of TF and CF processes. Ozone utilization decreased with ozone dose, but better overall COD removal was achieved at higher doses due to stronger foam formation. At 82.5 mg O<sub>3</sub>/L water, the ozone reactivity was 83.7%. The ozone yield coefficient  $Y(O_3/COD_{ox})$  for COD oxidized was between 1.92 and 2.23 g O<sub>3</sub> g<sup>-1</sup> COD, and  $Y(O_3/COD_t)$  for total COD removed was between 0.70 and 0.78. The difference indicates that due to foam elimination, the efficiency of the ozonation process could be enhanced by up to three-fold. Ozone utilization probably could be enhanced if diffusers producing smaller bubbles are used. Improved removal of COD as foam also could be expected with this modification. The results of the tests suggested that high

ozone doses are necessary in order to achieve a lower final COD concentration and better organic biodegradability. A minimum of 0.5 mg O<sub>3</sub>/mg COD should be provided to achieve complete disinfection and to remove more than 1g CODt/1g O<sub>3</sub>.

The biodegradability of BRA effluent, in terms of COD/cBOD<sub>5</sub> ratio, is less than one half that of domestic wastewater. A maximum of 88.1% cBOD<sub>5</sub> removal was obtained during this study, corresponding to a concentration of 9 mg BOD<sub>5</sub>/L. Increasing ozone dose could reduce this cBOD<sub>5</sub> concentration further. A greater ozone dose also could reduce the loading of TF and favoring nitrification, thereby having beneficial effects on the final effluent.

Tests for DOC showed no significant difference between the influent and the effluent concentration (20-24 mg/L). Instead, larger fluctuations were observed among the different treatment units. DOC was increased by methanol addition intended to sustain denitrification, and also during the ozonation process where DOC was a byproduct. In both biological filters, DOC was reduced significantly. DOC management probably could be enhanced with automated synchronization between nitrate concentration and the dosage of methanol provided to the system (i.e., 3.6 mg CH<sub>3</sub>OH:1 mg NO<sub>3</sub><sup>-</sup>-N).

The operation of the pilot station showed a 26.0-32.4% increase of alkalinity in the final effluent. Alkalinity was increased by up to 40% during the denitrification process, but some of it was lost during ozonation and during nitrification in the TF. Reusing this effluent would not eliminate entirely the need for alkalinity adjustment in RASs, but a significant reduction in the amount of bicarbonate addition should be expected.

The pH increased to more than 8.2 during denitrification, but decreased to an average of 7.6 in the final effluent due to ozonation and to nitrification. This value fits within the normal range of tolerance for most aquaculture species, including tilapia.

Hardness decreased by 8.9-12.7% in the treatment train, mainly by bacterial assimilation in the DR and by involvement of divalent ions in the flocculation process during ozonation. Although hardness was not lost in a major proportion during treatment, it could be depleted below the critical limits after repeated treatments, and may require periodic adjustment.

TKN was removed in proportion of 95.2-96.0% throughout the treatment train. It was removed with the settled solids in SB, vacuumed with solids from MSF, removed with

foam or destroyed in ammonia form in OR, and removed with settled solids after chemical flocculation. The proportion of TKN removed was higher than that of COD, suggesting that the system performed well in removing organic nitrogen.

The system removed 34.5-39.9% of the incoming TAN. After a significant reduction of TAN in the DR, more TAN was generated in the OR, probably due to oxidation of nitrogen-containing organics. Further, TAN was partially removed in the TF by nitrification, reaching average final concentrations of 1.52-1.69 mg/L. These concentrations are generally undesirable in water used for RAS exchange, but it was determined that were this treated water used for 100% exchange at BRA, only 0.84% of daily TAN production would be reintroduced to the systems. The RBCs should be able to remove these amounts, as is demonstrated in the next chapter.

The pilot station removed between 36.2 and 47.1% of the influent  $\text{NO}_2^-$ -N, which is an equivalent of between 0.45 and 0.61 mg/L in the final effluent. The DR and OR removed all influent  $\text{NO}_2^-$ -N, but some  $\text{NO}_2^-$ -N was generated in the TF. Like ammonia, this amount should be removed by the RBCs if the effluent is recycled.

94.0-95.4% of  $\text{NO}_3^-$ -N was removed, i.e., from a stage average of 42.8-43.2 mg/L in the influent, to 2.0-2.7 mg/L  $\text{NO}_3^-$ -N in the final effluent). These final concentrations are well below the level of toxicity, and should not be a concern if the effluent is reused for exchange. The DR produced 20.2 kg VSS  $\text{m}^3 \text{ day}^{-1}$  at 6 Lpm working stream flow rate (Stages 1 and 2), and 15.8 kg VSS  $\text{m}^3 \text{ day}^{-1}$  at 4 Lpm working stream flow rate (Stages 3 and 4). In the same reactor was found a biological yield ( $Y$ ) of 0.69 g biomass VSS/ g  $\text{NO}_3^-$ -N, and the maximum nitrogen removal was 23.4 kg  $\text{NO}_3^-$ -N  $\text{m}^3 \text{ day}^{-1}$ , above that expected from this technology in domestic wastewater treatment. The biofilm developed represented an average of 83% of the settled sand bed volume.

### **3.9. Potential problems**

The pilot-station study addressed only the parameters presented and discussed above. The approach employed to remove the main pollutants from the waste stream proved feasible, establishing it as a potential solution. However, the pilot-scale study indicated that some of the units and management practices would work successfully on a scaled-up

version of the treatment station after only refinement of management procedures. A potential problem not addressed in this study was accumulation of dissolved minerals and salts. Once a system becomes operational, a rapid increase in concentrations of some of these compounds should be expected. It would not be the case for phosphorus, considering literature sources claiming that phosphorous is 97% removed from wastewater by chemical flocculation alone. Rather, accumulation of different salts probably cannot be avoided. Salts come into the system with feed and also by particular management practices. For example, sulfate, manganese, iron, and eventually heavy metals, halogens, and other possible toxins could come into the systems in small amounts with feed. Additionally, sodium bicarbonate ( $\text{HCO}_3^-$ ) is added daily to maintain alkalinity and sodium chloride ( $\text{NaCl}$ ) is added to promote slime production by fish prior to handling. Organics refractory to ozone treatment could be generated and accumulate inside the system. The accumulation of these pollutants or a combination of them could become toxic to the fish. There is not much information suggesting maximum allowable concentrations for fish, less for combinations of stressors, and even less specific information for tilapia. Considering this situation, the suggestion would be to control the levels of these constituents by periodically exchanging water to dilute the concentrations experienced by the fish. The volumes required and the frequency of exchanges will be determined by adaptive management once the system becomes operational.

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Table 3.1. Controlled parameter conditions applied to the pilot station for the four experimental stages.

| Stage | Water flow<br>Lpm | Gas flow <sup>1</sup><br>Lpm | O <sub>3</sub> conc.<br>mg O <sub>3</sub> /L gas | O <sub>3</sub> dose<br>mg O <sub>3</sub> /L water | Oz. time<br>min | Recircul. DR <sup>2</sup><br>Lpm (%) | TF used <sup>3</sup><br>% |
|-------|-------------------|------------------------------|--|---|-----------------|--------------------------------------|---------------------------|
| 1     | 6                 | 10                           | 22   | 36.6  | 6               | 4 (40%)                              | 100                       |
| 2     |                   |                              | 33   | 55.0  |                 |                                      |                           |
| 3     | 4                 |                              | 33   | 82.5  | 9               | 6 (60%)                              | 50                        |
| 4     |                   |                              | 22   | 55.0  |                 |                                      |                           |

<sup>1</sup> Flow of the O<sub>2</sub>/O<sub>3</sub> mixture.

<sup>2</sup> Recirculation rate in the fluidized bed biological reactor.

<sup>3</sup> Proportion of cross section of trickling filter used.

Table 3.2. Sampling points and water quality analyses performed on samples collected throughout the study. BOD<sub>5</sub> analyses were performed twice per stage.

| Sample Location | TS | VS | TSS | VSS | COD | DOC | BOD <sub>5</sub> | TAN | NO <sub>2</sub> | NO <sub>3</sub> | pH | T°C | Alk. | Hard. | FTU | DO | DO <sub>3</sub> | TKN | Volume |
|-----------------|----|----|-----|-----|-----|-----|------------------|-----|-----------------|-----------------|----|-----|------|-------|-----|----|-----------------|-----|--------|
| SBi             | x  | x  | x   | x   | x   |     |                  |     |                 |                 | x  | x   |      |       | x   | x  |                 | x   |        |
| SBe             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    | x     | x   | x  |                 | x   |        |
| MSe             | x  | x  | x   | x   | x   |     | x                |     |                 |                 | x  | x   |      |       | x   | x  |                 |     |        |
| DRi             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    | x     | x   | x  |                 | x   |        |
| ORi             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    | x     | x   | x  |                 | x   |        |
| ORe             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    | x     | x   |    | x               | x   |        |
| TFi             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    |       | x   | x  |                 | x   |        |
| TFe             | x  | x  | x   | x   | x   | x   | x                | x   | x               | x               | x  | x   | x    | x     | x   | x  |                 | x   |        |
| FE              | x  | x  | x   | x   | x   | x   | x                |     |                 |                 |    |     |      |       | x   |    |                 | x   |        |
| SSB             | x  | x  | x   | x   | x   |     |                  |     |                 |                 |    |     |      |       |     |    |                 |     | x      |
| SMF             | x  | x  | x   | x   | x   |     |                  |     |                 |                 |    |     |      |       |     |    |                 |     | x      |
| F               | x  | x  | x   | x   | x   | x   | x                |     |                 |                 |    |     |      |       | x   |    |                 | x   | x      |

SBi = sedimentation basin influent (raw influent),  
 SBe = sedimentation basin effluent,  
 MSe = microscreen filter effluent,  
 DRi = denitrification reactor influent (mixing basin),  
 ORi = ozonation reactor influent (denitrification reactor effluent),  
 ORe = ozonation reactor effluent (stripping chamber influent),  
 TFi = trickling filter influent (stripping chamber effluent),  
 TFe = trickling filter effluent,  
 FE = final effluent, after chemical flocculation,  
 SSB = sludge from sedimentation basin,  
 SMF = sludge from microscreen filter, and  
 F = foam.

Table 3.3. Waste stream characteristics for BRA effluent, based on samples from 12 different days, collected at 6:00 a.m., 2:00 p.m., and 10:00 p.m. each day, and composition of typical untreated domestic wastewater of weak and medium strength (Metcalf and Eddy, 1991). Average values represent non-flow weighted averages (12% for samples from 2:00 p.m., and 44% each for the other two). Minimum and maximum represent the observations for the extreme events.

| Parameter                              | Average | Minimum | Maximum | Domestic (medium) | Domestic (weak) |
|--|---------|---------|---------|-------------------|-----------------|
| COD (mg/L)                             | 573     | 88      | 1102    | 500               | 250             |
| TS (mg/L)                              | 1812    | 108     | 2426    | 720               | 350             |
| VTS (mg/L)                             | 731     | 118     | 1363    | 200               | 105             |
| FTS (mg/L)                             | 1081    | 844     | 1299    | 300               | 145             |
| TSS (mg/L)                             | 558     | 53      | 993     | 220               | 100             |
| VSS (mg/L)                             | 461     | 44      | 824     | 165               | 80              |
| FSS (mg/L)                             | 97      | 8       | 169     | 55                | 20              |
| TAN (mg/L)                             | 2.55    | 1.72    | 4.11    | 25                | 12              |
| NO <sub>3</sub> <sup>-</sup> -N (mg/L) | 42.98   | 6.8     | 68.8    | 0                 | 0               |
| NO <sub>2</sub> <sup>-</sup> -N (mg/L) | 0.91    | 0.45    | 1.53    | 0                 | 0               |
| TKN (mg/L)                             | 31.5    | 5.11    | 47.36   | 15                | 8               |
| pH                                     | 7.21    | 6.95    | 7.62    | -                 | -               |
| T (°C)                                 | 28.6    | 27.8    | 30.5    | -                 | -               |
| DO (mg/L)                              | 0.45    | 0.06    | 3.2     | -                 | -               |
| Alk. (mg/L)                            | 118     | 76      | 141     | 100               | 50              |
| Hard. (mg/L)                           | 150     | 139     | 170     | -                 | -               |
| S.S. (mL/L)                            | 24.0    | 4.0     | 30.0    | 10                | 5               |

Table 3.4. TSS concentrations after unit process designed to remove solids and percent removal over the entire treatment train. The values represent non-flow weighted averages for an entire experimental stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | TSS (mg/L)      |     | TSS removal per treatment unit (%) |                    |                    | Total removal (%)  |
|-------|-----------------|-----|------------------------------------|--------------------|--------------------|--------------------|
|       | RI <sup>1</sup> | TE  | SB                                 | OR                 | CF                 |                    |
| 1     | 552.8           | 8.3 | 70.9 <sup>a</sup>                  | 75.6 <sup>a</sup>  | 78.9 <sup>a</sup>  | 98.5 <sup>a</sup>  |
| 2     |                 | 7.3 |                                    | 76.6 <sup>ab</sup> | 80.7 <sup>ab</sup> | 98.7 <sup>ab</sup> |
| 3     | 563.3           | 4.7 | 72.9 <sup>a</sup>                  | 77.7 <sup>b</sup>  | 86.0 <sup>b</sup>  | 99.1 <sup>b</sup>  |
| 4     |                 | 6.0 |                                    | 76.9 <sup>ab</sup> | 82.9 <sup>ab</sup> | 98.9 <sup>ab</sup> |

<sup>1</sup>RI = raw influent, TE = treated effluent, SB = sedimentation basin, OR = ozonation reactor, and CF = chemical flocculation.

Table 3.5. Turbidity variation among different treatment units and percent removal over the entire treatment train. The values represent non-flow weighted averages for an entire experimental stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | Turbidity (NTU) |                   |                   |                    |                   | Total removal (%)  |
|-------|-----------------|-------------------|-------------------|--------------------|-------------------|--------------------|
|       | RI <sup>1</sup> | SB                | DR                | OR                 | CF                |                    |
| 1     | 131.9           | 69.8 <sup>a</sup> | 59.1 <sup>a</sup> | 23.9 <sup>b</sup>  | 5.7 <sup>a</sup>  | 95.7 <sup>a</sup>  |
| 2     |                 |                   |                   | 21.0 <sup>ab</sup> | 4.5 <sup>ab</sup> | 96.6 <sup>ab</sup> |
| 3     | 135.5           | 67.7 <sup>a</sup> | 52.1 <sup>a</sup> | 18.3 <sup>a</sup>  | 4.2 <sup>b</sup>  | 97.0 <sup>b</sup>  |
| 4     |                 |                   |                   | 20.8 <sup>a</sup>  | 4.3 <sup>ab</sup> | 96.8 <sup>ab</sup> |

<sup>1</sup>RI = raw influent, SB = sedimentation basin, DR = denitrification reactor, OR = ozonation reactor, and CF = chemical flocculation.

Table 3.6. Raw influent, sedimentation basin, and chemical flocculation COD mass non-flow weighted average concentration, percent COD removal in different units, and total percent COD removal across the treatment train for each stage of the experiment. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | COD (mg/L)      |       |      | %COD removal / unit |                    |                   |      | Total removal (%) |
|-------|-----------------|-------|------|---------------------|--------------------|-------------------|------|-------------------|
|       | RI <sup>1</sup> | SB    | CF   | SB                  | OR                 | TF                | CF   |                   |
| 1     | 562.2           | 229.1 | 68.2 | 59.2                | 19.8 <sup>b</sup>  | 28.9              | 44.9 | 87.9              |
| 2     |                 |       | 54.6 |                     | 27.8 <sup>ab</sup> |                   |      |                   |
| 3     | 584.6           | 218.0 | 44.4 | 62.7                | 40.7 <sup>a</sup>  | 34.4 <sup>b</sup> | 50.1 | 92.4              |
| 4     |                 |       | 54.9 |                     | 30.0 <sup>a</sup>  |                   |      |                   |

<sup>1</sup>RI = raw influent, SB = sedimentation basin, CF = chemical flocculation, OR = ozonation reactor, and TF = trickling filter.

Table 3.7. Parameters characterizing ozonation tests and COD variation during the four stages with respect to system definition, experimental procedure, and results assessment.

| Parameter                    | Units                                 | Stage #     |             |             |             |
|------------------------------|---------------------------------------|-------------|-------------|-------------|-------------|
|                              |                                       | 1           | 2           | 3           | 4           |
| $t_H$                        | min                                   | 6           | 6           | 9           | 9           |
| $V_L$                        | L                                     | 36          | 36          | 36          | 36          |
| $Q_L$                        | L min <sup>-1</sup>                   | 6           | 6           | 4           | 4           |
| Q foam                       | % of $Q_w$                            | 3.6         | 4.0         | 6.9         | 5.4         |
| COD in                       | mg L <sup>-1</sup>                    | 230         | 221         | 224         | 221         |
| COD out                      | mg L <sup>-1</sup>                    | 185         | 160         | 133         | 155         |
| COD <sub>t</sub> rmvd.       | mg L <sup>-1</sup>                    | 45          | 61          | 91          | 66          |
| Foam COD                     | mg L <sup>-1</sup>                    | 756         | 928         | 899         | 792         |
| COD rmvd. as foam            | mg L <sup>-1</sup> (%)                | 27.2 (60.4) | 37.0 (60.6) | 60.5 (66.5) | 42.5 (64.4) |
| COD ox.                      | mg L <sup>-1</sup> (%)                | 17.8 (39.6) | 24.5 (39.4) | 30.7 (33.5) | 23.8 (35.6) |
| O <sub>3</sub> dose in water | mg O <sub>3</sub> L <sup>-1</sup>     | 36.6        | 55.0        | 82.5        | 55.0        |
| CG <sub>0</sub>              | mg L <sup>-1</sup>                    | 22          | 33          | 33          | 22          |
| CG <sub>e</sub>              | mg L <sup>-1</sup> (%)                | 0.62 (2.8)  | 3.73 (11.3) | 4.22 (12.8) | 2.32 (10.5) |
| CL <sub>e</sub>              | mg L <sup>-1</sup> (%)                | 0.07 (0.32) | 0.55 (1.66) | 1.17 (3.54) | 0.64 (2.91) |
| tO <sub>3</sub> residual     | %                                     | 3.14        | 12.96       | 16.32       | 13.41       |
| O <sub>3</sub> reacted       | %                                     | 96.86       | 87.04       | 83.68       | 86.59       |
| $F(O_3)$                     | mg L <sup>-1</sup> s <sup>-1</sup>    | 0.102       | 0.153       | 0.153       | 0.102       |
| $r(O_3)$                     | mg L <sup>-1</sup> s <sup>-1</sup>    | 0.098       | 0.131       | 0.127       | 0.086       |
| $r_A(O_3)$                   | mg L <sup>-1</sup> s <sup>-1</sup>    | 0.099       | 0.136       | 0.133       | 0.091       |
| $r(COD_t)$                   | mg L <sup>-1</sup> s <sup>-1</sup>    | 0.125       | 0.169       | 0.169       | 0.122       |
| $r(COD_{ox})$                | mg L <sup>-1</sup> s <sup>-1</sup>    | 0.049       | 0.068       | 0.057       | 0.044       |
| $I^*$                        | g O <sub>3</sub> /g <sup>-1</sup> COD | 0.159       | 0.249       | 0.368       | 0.249       |
| $A^*$                        | g O <sub>3</sub> /g <sup>-1</sup> COD | 0.155       | 0.221       | 0.321       | 0.223       |
| $\eta(O_3)$                  | %                                     | 97.18       | 88.70       | 87.21       | 89.45       |
| $\eta(COD_t)$                | %                                     | 19.8        | 27.8        | 40.7        | 30.0        |
| $\eta(COD_{ox})$             | %                                     | 7.74        | 11.09       | 13.71       | 10.77       |
| $Y(O_3/COD_t)$               | g O <sub>3</sub> /g <sup>-1</sup> COD | 0.78        | 0.77        | 0.75        | 0.70        |
| $Y(O_3/COD_{ox})$            | g O <sub>3</sub> /g <sup>-1</sup> COD | 1.98        | 1.92        | 2.23        | 1.94        |

$t_H$  = hydraulic residence time,  $V_L$  = volume of reactor occupied by liquid,  $Q_L$  = wastewater flow rate,  $Q_{foam}$  = foam stream flow rate expressed as percent from  $Q_L$ , COD in = COD concentration in reactor influent, COD out = COD concentration in reactor effluent, COD<sub>t</sub> rmvd. = total reduction of COD concentration, Foam COD = COD concentration in foam, COD rmvd. as foam = amount of COD (percent of influent) removed as foam from 1L of wastewater, COD ox. = the amount of COD mineralized (percent of influent) by ozone from 1L of wastewater, O<sub>3</sub> = the amount of ozone applied per liter of wastewater; CG<sub>0</sub> = ozone concentration in the influent gas, CG<sub>e</sub> = ozone concentration (mass percent of influent) in the effluent gas, CL<sub>e</sub> = ozone concentration (mass percent of influent) in the effluent wastewater, tO<sub>3</sub> residual = total percent of ozone dose measured as residual (water and gas), O<sub>3</sub> reacted = percent of ozone dose that reacted,  $F(O_3)$  = ozone dose of feed rate,  $r(O_3)$  = ozone consumption rate,  $r_A(O_3)$  = ozone absorption rate,  $r(COD_t)$  = total COD removal rate,  $r(COD_{ox})$  = rate of COD removed by oxidation,  $I^*$  = specific ozone dose or input,  $A^*$  = specific ozone absorption,  $\eta(O_3)$  = ozone transfer efficiency,  $\eta(COD_t)$  = degree of total COD removal,  $\eta(COD_{ox})$  = degree of COD removed by oxidation,  $Y(O_3/COD_t)$  = ozone yield coefficient as a function of total COD removal, and  $Y(O_3/COD_{ox})$  = ozone yield coefficient as a function of COD oxidized.

Table 3.8. cBOD<sub>5</sub> dynamics through the pilot plant: initial and final cBOD<sub>5</sub> mass non-flow weighted average concentrations, percent cBOD<sub>5</sub> removal in different treatment units, total removal for the treatment train, and values and biodegradability assessment for the OR. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | cBOD <sub>5</sub> (mg/L) |      | % cBOD <sub>5</sub> removal/unit |      |      |       | cBOD <sub>5</sub> /COD |                 |                    |
|-------|--------------------------|------|----------------------------------|------|------|-------|------------------------|-----------------|--------------------|
|       | SB <sup>1</sup>          | CF   | OR                               | TF   | CF   | Total | OR <sub>i</sub>        | OR <sub>e</sub> | % increase         |
| 1     |                          | 15.0 | 19.0                             | 45.7 | 42.6 | 79.9  | 0.32                   | 0.33            | 4.5 <sup>b</sup>   |
| 2     | 77.3                     | 15.5 | 24.8                             | 41.1 | 41.4 | 80.6  | 0.27                   | 0.29            | 5.2 <sup>ab</sup>  |
| 3     |                          | 9.0  | 27.6                             | 48.6 | 58.1 | 88.1  | 0.25                   | 0.32            | 20.6 <sup>a</sup>  |
| 4     | 74.5                     | 12.5 | 20.4                             | 38.0 | 47.2 | 82.9  | 0.25                   | 0.29            | 12.2 <sup>ab</sup> |

<sup>1</sup>SB = sedimentation basin, CF = chemical flocculation, OR = ozonation reactor, TF = trickling filter, OR<sub>i</sub> = ozone reactor influent, and OR<sub>e</sub> = ozone reactor effluent.

Table 3.9. Dissolved organic carbon dynamics through the pilot plant: initial DOC mass non-flow weighted average concentrations, average DOC generation (+) or loss (-) and percent DOC variation in different treatment units. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB <sup>1</sup> | MB   |          | DR   |                      | OR   |                       | TF   |                      | CF   |                     |
|-------|-----------------|------|----------|------|----------------------|------|-----------------------|------|----------------------|------|---------------------|
|       | (mg/L)          | mg/L | (%)      | mg/L | (%)                  | mg/L | (%)                   | mg/L | (%)                  | mg/L | (%)                 |
| 1     | 23.4            | 69.9 | (+198.7) | 28.5 | (-59.2) <sup>a</sup> | 33.3 | (+14.4) <sup>a</sup>  | 23.3 | (-26.8) <sup>a</sup> | 21.7 | (-6.9) <sup>a</sup> |
| 2     |                 |      |          |      |                      | 38.4 | (+25.8) <sup>b</sup>  | 24.8 | (-29.1) <sup>a</sup> | 23.1 | (-7.3) <sup>a</sup> |
| 3     | 23.0            | 59.7 | (+159.6) | 27.2 | (-54.4) <sup>a</sup> | 35.6 | (+23.6) <sup>ab</sup> | 23.1 | (-30.6) <sup>a</sup> | 21.3 | (-7.9) <sup>a</sup> |
| 4     |                 |      |          |      |                      | 37.6 | (+27.7) <sup>b</sup>  | 22.1 | (-33.9) <sup>a</sup> | 20.6 | (-7.2) <sup>a</sup> |

<sup>1</sup>SB = sedimentation basin, CF = chemical flocculation, MB = mixing basin, DR = denitrification reactor, OR = ozonation reactor, and TF = trickling filter.

Table 3.10. Alkalinity dynamics through the pilot plant: alkalinity initial mass non-flow weighted average concentrations, average alkalinity generation (+) or loss (-) and percent alkalinity removal in different treatment units, and total percent alkalinity increase for the treatment train in each stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB <sup>1</sup> | DR                         | OR                         | TF                        | Total increase <sup>2</sup> |
|-------|-----------------|----------------------------|----------------------------|---------------------------|-----------------------------|
|       | mg/L            | mg/L (%)                   | mg/L (%)                   | mg/L (%)                  |                             |
| 1     | 114.5           | 192.1 (+40.4) <sup>a</sup> | 175.5 (-6.6) <sup>a</sup>  | 163.6 (-6.8) <sup>a</sup> | 30.0 <sup>a</sup>           |
| 2     |                 |                            | 179.9 (-6.4) <sup>a</sup>  | 169.3 (-5.9) <sup>a</sup> | 32.4 <sup>a</sup>           |
| 3     | 121.7           | 201.8 (+39.7) <sup>a</sup> | 187.5 (-7.1) <sup>a</sup>  | 179.5 (-4.2) <sup>a</sup> | 32.2 <sup>a</sup>           |
| 4     |                 |                            | 177.8 (-11.9) <sup>a</sup> | 164.3 (-7.5) <sup>a</sup> | 26.0 <sup>a</sup>           |

<sup>1</sup>SB = sedimentation basin, DR = denitrification reactor, OR = ozonation reactor, and TF = trickling filter.

<sup>2</sup>Indicates ratio between SB (initial) and TF (final) concentrations.

Table 3.11. Mean pH values in different treatment units of the treatment train for each experimental stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | RI <sup>1</sup> | SB                | DR                | OR                | TF                | CF                |
|-------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 1     | 7.22            | 7.30 <sup>a</sup> | 8.26 <sup>a</sup> | 8.09 <sup>a</sup> | 8.32 <sup>a</sup> | 7.61 <sup>a</sup> |
| 2     |                 |                   |                   | 7.89 <sup>a</sup> | 8.24 <sup>a</sup> | 7.63 <sup>a</sup> |
| 3     | 7.20            | 7.37 <sup>a</sup> | 8.22 <sup>a</sup> | 7.93 <sup>a</sup> | 8.32 <sup>a</sup> | 7.58 <sup>a</sup> |
| 4     |                 |                   |                   | 7.89 <sup>a</sup> | 8.24 <sup>a</sup> | 7.58 <sup>a</sup> |

<sup>1</sup>RI = raw influent, SB = sedimentation basin, DR = denitrification reactor, OR = ozonation reactor, TF = trickling filter, and CF = chemical flocculation.

Table 3.12. Hardness dynamics through the pilot plant: hardness initial mass non-flow weighted average concentrations, average hardness concentrations and percent hardness removal after different treatment units, and total percent hardness decrease for the treatment train in each stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB <sup>1</sup>    | DR                       | OR                        | TF                       | Total decrease <sup>2</sup> |
|-------|--------------------|--------------------------|---------------------------|--------------------------|-----------------------------|
|       | mg/L               | mg/L (%)                 | mg/L (%)                  | mg/L (%)                 | (%)                         |
| 1     | 151.5 <sup>a</sup> | 145.7 (3.8) <sup>a</sup> | 139.7 (4.1) <sup>a</sup>  | 138.0 (1.2) <sup>a</sup> | 8.9 <sup>a</sup>            |
| 2     |                    |                          | 137.4 (5.7) <sup>b</sup>  | 135.9 (1.1) <sup>a</sup> | 10.3 <sup>a</sup>           |
| 3     | 148.4 <sup>a</sup> | 141.7 (4.5) <sup>a</sup> | 130.6 (7.8) <sup>ab</sup> | 129.5 (0.8) <sup>a</sup> | 12.7 <sup>a</sup>           |
| 4     |                    |                          | 134.6 (5.0) <sup>ab</sup> | 132.6 (1.5) <sup>a</sup> | 10.6 <sup>a</sup>           |

<sup>1</sup>SB = sedimentation basin, DR = denitrification reactor, OR = ozonation reactor, and TF = trickling filter.

<sup>2</sup>Indicates ratio between SB (initial) and TF (final) concentration values.

Table 3.13. Total Kjeldahl nitrogen (TKN) dynamics through the pilot plant: TKN initial mass non-flow weighted average concentrations, average TKN concentrations after different treatment units and percent TKN removal, and total TKN percent removal over the treatment train in each stage. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | RI <sup>1</sup> | SB   |        | OR   |                      | CF   |                     | Total removal <sup>2</sup><br>(%) |
|-------|-----------------|------|--------|------|----------------------|------|---------------------|-----------------------------------|
|       | mg/L            | mg/L | (%)    | mg/L | (%)                  | mg/L | (%)                 |                                   |
| 1     | 41.8            | 16.9 | (59.6) | 9.2  | (45.6) <sup>a</sup>  | 2.0  | (78.7) <sup>a</sup> | 95.2 <sup>a</sup>                 |
| 2     |                 |      |        | 8.2  | (51.5) <sup>ab</sup> | 1.9  | (76.8) <sup>a</sup> | 95.5 <sup>a</sup>                 |
| 3     | 42.3            | 16.2 | (61.7) | 7.4  | (54.3) <sup>b</sup>  | 1.7  | (77.0) <sup>a</sup> | 96.0 <sup>a</sup>                 |
| 4     |                 |      |        | 8.2  | (49.4) <sup>ab</sup> | 1.9  | (76.8) <sup>a</sup> | 95.5 <sup>a</sup>                 |

<sup>1</sup>SB = sedimentation basin, OR = ozonation reactor, and CF = chemical flocculation.

<sup>2</sup>Indicates ratio between RI (initial) and TF (final) concentrations.

Table 3.14. Total ammonia nitrogen (TAN) dynamics through the pilot plant: initial TAN mass average concentrations, TAN mass average concentration after different treatment units and percent TAN removal (-) or generation (+) in each unit, percent TAN increase after ozonation, and percent TAN removal over the treatment train in all stages. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB <sup>1</sup> | DR                        | OR                         | OR %<br>increase <sup>2</sup> | TF                        | Total <sup>3</sup><br>(%) |
|-------|-----------------|---------------------------|----------------------------|-------------------------------|---------------------------|---------------------------|
|       | mg/L            | mg/L (%)                  | mg/L (%)                   |                               | mg/L (%)                  |                           |
| 1     | 2.58            | 1.33 (-48.4) <sup>a</sup> | 3.64 (+173.6) <sup>a</sup> | 29.1                          | 1.69 (-53.6) <sup>a</sup> | 34.5 <sup>a</sup>         |
| 2     |                 |                           | 3.92 (+194.7) <sup>a</sup> | 34.2                          | 1.59 (-59.4) <sup>a</sup> | 38.4 <sup>a</sup>         |
| 3     | 2.53            | 1.26 (-50.2) <sup>a</sup> | 4.21 (+234.1) <sup>a</sup> | 39.9                          | 1.52 (-63.9) <sup>a</sup> | 39.9 <sup>a</sup>         |
| 4     |                 |                           | 4.00 (+217.5) <sup>a</sup> | 36.8                          | 1.63 (-59.3) <sup>a</sup> | 35.6 <sup>a</sup>         |

<sup>1</sup>SB = sedimentation basin, TF = trickling filter, OR = ozonation reactor, and DR = denitrification reactor.

<sup>2</sup>Indicates percent increase of TAN concentration after ozonation over the initial TAN concentration in sedimentation basin.

<sup>3</sup>Indicates total percent decrease of TAN concentration after ozonation over the initial TAN concentration in sedimentation basin.

Table 3.15. Nitrite-nitrogen ( $\text{NO}_2^-$ -N) dynamics through the pilot plant: initial and final  $\text{NO}_2^-$ -N mass average concentration,  $\text{NO}_2^-$ -N mass average concentration after different treatment units and percent  $\text{NO}_2^-$ -N removal (-) or generation (+) in each unit, and percent total  $\text{NO}_2^-$ -N removed over the treatment train in all stages. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB <sup>1</sup> | DR   |        | OR   |        | TF   |                      | Total removal (%) |
|-------|-----------------|------|--------|------|--------|------|----------------------|-------------------|
|       | mg/L            | mg/L | (%)    | mg/L | (%)    | mg/L | (%) <sup>2</sup>     |                   |
| 1     | 0.96            | 0.23 | (76.0) | 0.00 | (24.0) | 0.45 | (+55.6) <sup>a</sup> | 44.4 <sup>a</sup> |
| 2     |                 |      |        |      |        | 0.54 | (+52.9) <sup>a</sup> | 47.1 <sup>a</sup> |
| 3     | 0.92            | 0.26 | (71.7) | 0.00 | (28.3) | 0.61 | (+58.7) <sup>a</sup> | 41.3 <sup>a</sup> |
| 4     |                 |      |        |      |        | 0.51 | (+63.8) <sup>a</sup> | 36.2 <sup>a</sup> |

<sup>1</sup>SB = sedimentation basin, TF = trickling filter, DR = denitrification reactor, and OR = ozonation reactor.

<sup>2</sup>The percentages for the TF express the mass generated as a fraction of the treatment train influent concentrations.

Table 3.16. Nitrate-nitrogen ( $\text{NO}_3^-$ -N) dynamics through the pilot plant: initial and final  $\text{NO}_3^-$ -N mass average concentration, percent  $\text{NO}_3^-$ -N removal in DR, and overall percent  $\text{NO}_3^-$ -N removal through the treatment train. Means with the same superscript are not significantly different ( $p>0.05$ ).

| Stage | SB   | DR                      | TF               | Total removal (%) |
|-------|------|-------------------------|------------------|-------------------|
|       | mg/L | mg/L (%)                | mg/L             |                   |
| 1     | 43.2 | 1.8 (95.8) <sup>a</sup> | 2.6 <sup>a</sup> | 94.0              |
| 2     |      |                         | 2.7 <sup>a</sup> |                   |
| 3     | 42.8 | 1.3 (97.0) <sup>a</sup> | 2.0 <sup>a</sup> | 95.4              |
| 4     |      |                         | 2.4 <sup>a</sup> |                   |

<sup>1</sup>SB = sedimentation basin, TF = trickling filter, and DR = denitrification reactor.

Table 3.17. Oxidized nitrogen species reaction kinetics through the denitrification reactor: recycling flow rates (QL), influent  $C_0(\text{NO}_x^- \text{-N})$ , effluent  $C(\text{NO}_x^- \text{-N})$ , and removal  $\Delta(\text{NO}_x^- \text{-N})$  (weighted average concentrations) for each experimental flow, rate constant ( $k$ ), and reduction rates ( $r_{\text{NO}_x^- \text{-N}}$ ) determined in steady state conditions for each test time during experimental flows. Hydraulic residence time was 5.5 minutes.

| Stage<br>(Testing time) | QL <sup>1</sup> recycle<br>(Lpm) | $C_0(\text{NO}_x^- \text{-N})$<br>(mg/L) | $C(\text{NO}_x^- \text{-N})$<br>(mg/L) | $k$<br>( $\text{mg}^{1/2} \text{L}^{-1/2} \text{min}^{-1}$ ) | $r_{\text{NO}_x \text{N}}$<br>( $\text{mg L}^{-1} \text{min}^{-1}$ ) |
|-------------------------|----------------------------------|--|--|--|--|
| 1 & 2 (2:00 p.m.)       | 4                                | 7.80                                     | 0.31                                   | 0.82   | -2.27  |
| 1 & 2 (10:00 p.m.)      | 4                                | 28.04                                    | 1.05                                   | 1.56   | -8.24  |
| 1 & 2 (6:00 a.m.)       | 4                                | 30.11                                    | 0.71                                   | 1.69   | -9.27  |
| 3 & 4 (2:00 p.m.)       | 6                                | 4.48                                     | 0.30                                   | 0.57   | -1.21  |
| 3 & 4 (10:00 p.m.)      | 6                                | 20.82                                    | 1.25                                   | 1.25   | -5.72  |
| 3 & 4 (6:00 a.m.)       | 6                                | 21.19                                    | 1.28                                   | 1.26   | -5.81  |

<sup>1</sup>Recycling flow rate in denitrification reactor.

Table 3.18. Comparison of biological yield ( $Y$ ) estimates from the literature and from this experiment from batch reactor tests under  $\text{NO}_3^-$ -N saturation conditions.

| Source                      | $Y\left(\frac{\text{Biomass VSS}}{\text{NO}_3^- - \text{N}}\right)$ | $Y\left(\frac{\text{Biomass VSS}}{\text{Dissolved COD}}\right)$ | $Y\left(\frac{\text{Biomass COD}}{\text{Dissolved COD}}\right)$ |
|-----------------------------|---|---|---|
| This experiment             | 0.69  | 0.29  | 0.41  |
| Semon et al., 1997          | 0.62  | 0.17 - 0.18   | 0.24 - 0.26   |
| Jeris et al., 1977          | 0.57  | 0.17  | 0.24  |
| Moore and Schroeder, 1971   | 0.53 - 1.4  | 0.14 - 0.29   | 0.17 - 0.35   |
| Coelhoso et al., 1992       | 0.5 - 1.3   | -   | -   |
| Stephenson and Murphy, 1980 | 1.0   | -   | -   |
| Grady et al., 1999          | -   | 0.27  | 0.39  |

Table 3.19. Biological yields ( $Y_{NO_3^- - N}$ ) data from denitrification reactor (steady state conditions) for each experimental working flow, VSS produced, and  $NO_3^-$ -N removal.

| Stage #<br>(Testing time) | $\Delta VSS$<br>(mg/L) | $\Delta NO_x-N$<br>(mg/L) | $Y(\Delta VSS/$<br>$\Delta NO_3^- - N)$<br>(g/g) | Parameter weighted average per stage |                      |                     |                      |
|---------------------------|------------------------|---------------------------|--|--------------------------------------|----------------------|---------------------|----------------------|
|                           |                        |                           |  | VSS produced                         |                      | $NO_3^-$ -N removal |                      |
|                           |                        |                           |  | $Kg\ d^{-1}$                         | $Kg\ m^{-3}\ d^{-1}$ | $Kg\ d^{-1}$        | $Kg\ m^{-3}\ d^{-1}$ |
| 1 & 2 (2:00 p.m.)         | 9.17                   | 7.49                      | 1.24   |                                      |                      |                     |                      |
| 1 & 2 (10:00 p.m.)        | 23.65                  | 26.99                     | 0.88   | 0.303                                | 20.203               | 0.351               | 23.407               |
| 1 & 2 (6:00 a.m.)         | 27.50                  | 29.40                     | 0.94   |                                      |                      |                     |                      |
| 3 & 4(2:00 p.m.)          | 6.50                   | 4.18                      | 1.58   |                                      |                      |                     |                      |
| 3 & 4 (10:00 p.m.)        | 18.84                  | 19.57                     | 0.96   | 0.237                                | 15.797               | 0.243               | 16.198               |
| 3 & 4 (6:00 a.m.)         | 18.67                  | 19.91                     | 0.95   |                                      |                      |                     |                      |

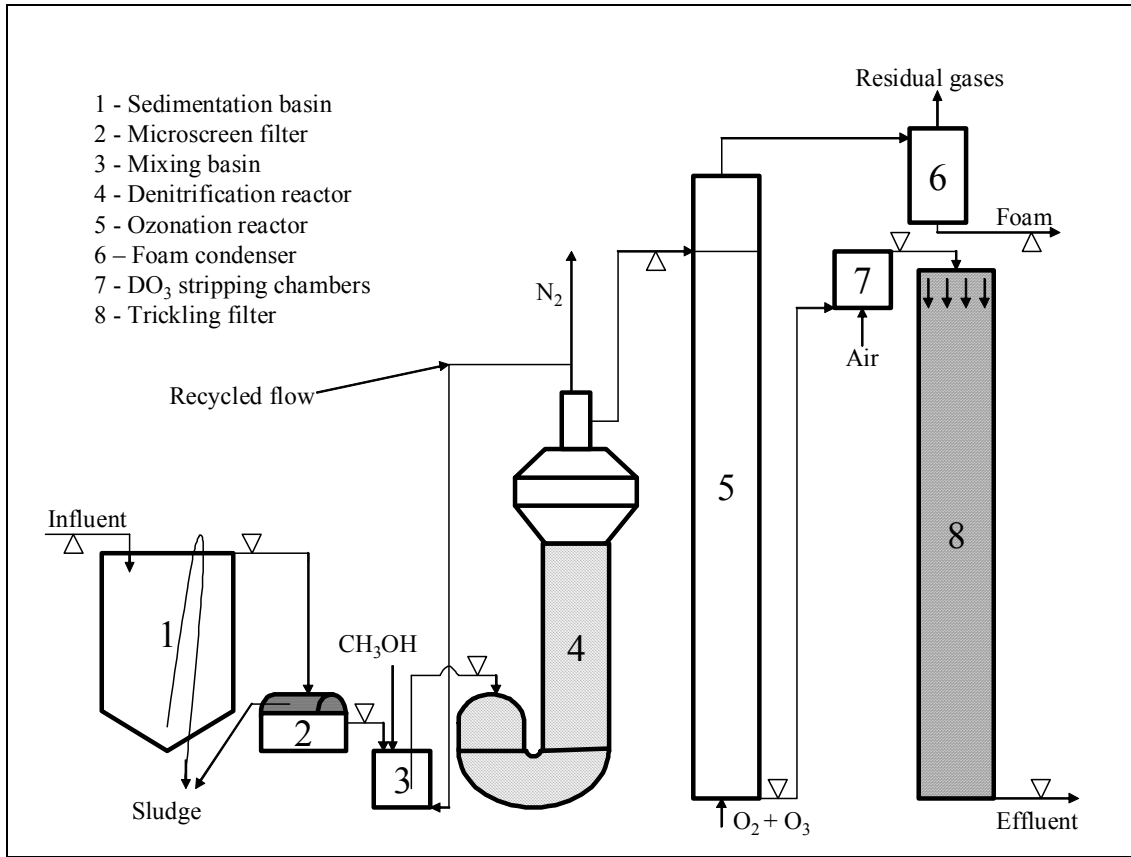


Figure 3.1. Schematic diagram of pilot-scale wastewater treatment train at Blue Ridge Aquaculture. The details are not drawn to scale. Δ represents sample collection points.

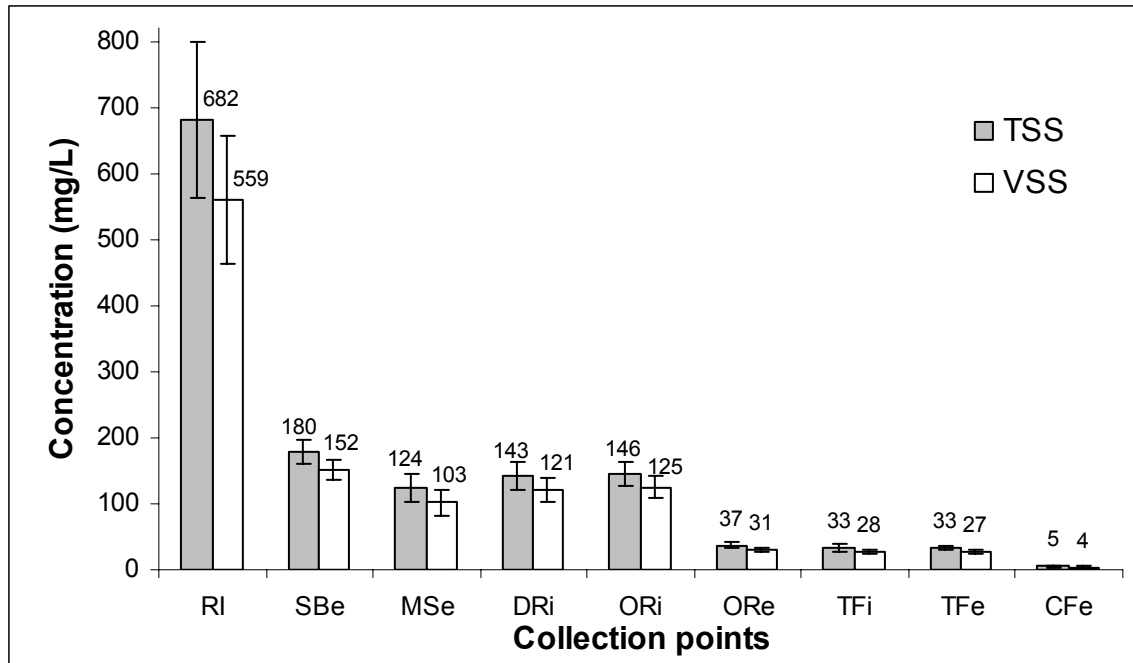


Figure 3.2. Treatment train profile for total suspended solids (TSS) and volatile suspended solids (VSS) variation in Stage 3 (10:00 p.m. and 6:00 a.m. samplings). Treatment conditions: working stream flow rate = 4 Lpm, ozone reactor residence time = 9 min, ozone dose = 82.5 mg O<sub>3</sub>/L water. Mean values ± standard deviations shown (*n* = 6).

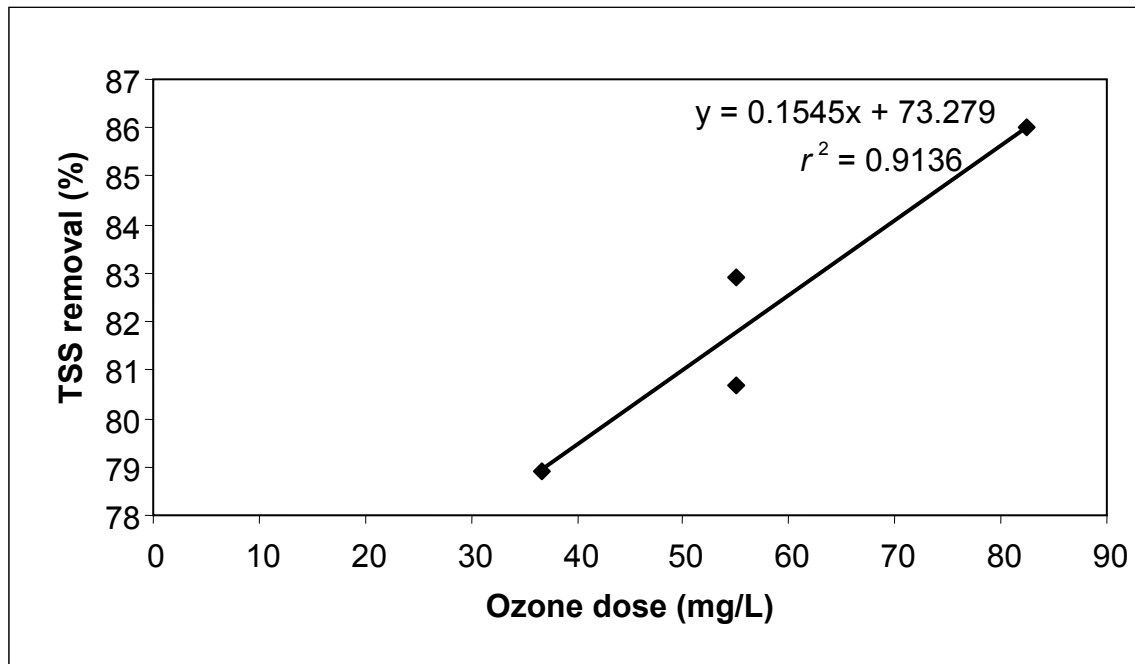


Figure 3.3. Relationship between TSS percent removal during chemical flocculation and ozone dose.

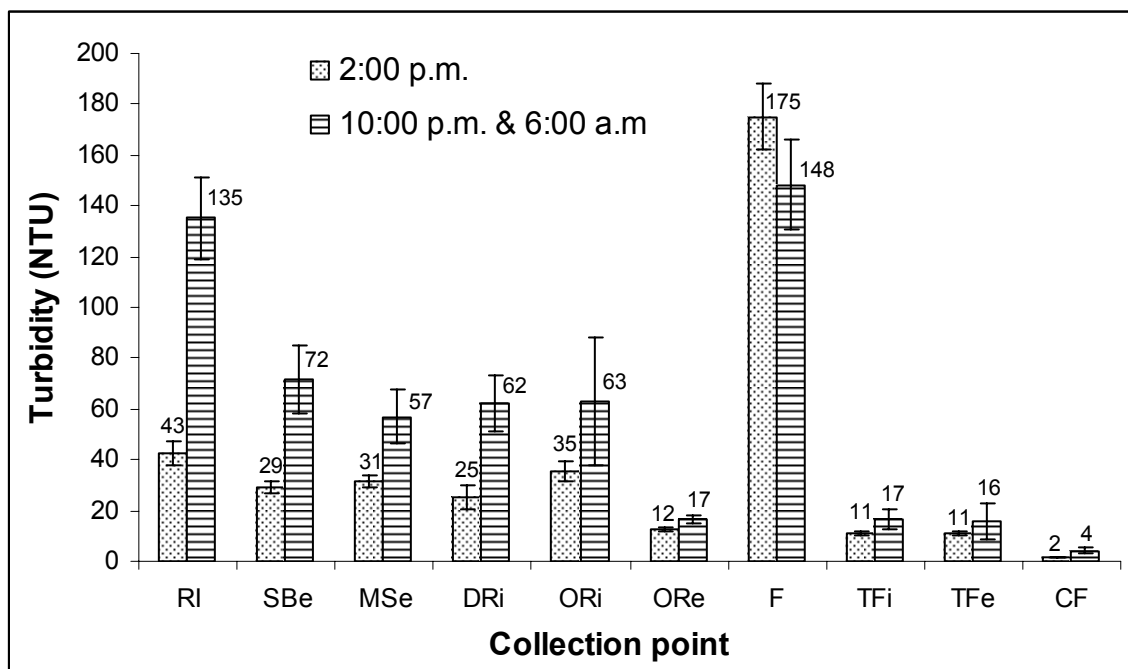


Figure 3.4. Treatment train profile for turbidity variation in Stage 3 (2:00 p.m. and 10:00 p.m. and 6:00 a.m. samplings). Treatment conditions: working stream flow rate = 4 Lpm, ozone reactor residence time = 9 min, ozone dose = 82.5 mg O<sub>3</sub>/L water. Mean values ± standard deviations shown ( $n = 3$  for 2:00 p.m., and  $n = 6$  for 10:00 p.m. and 6:00 a.m.).

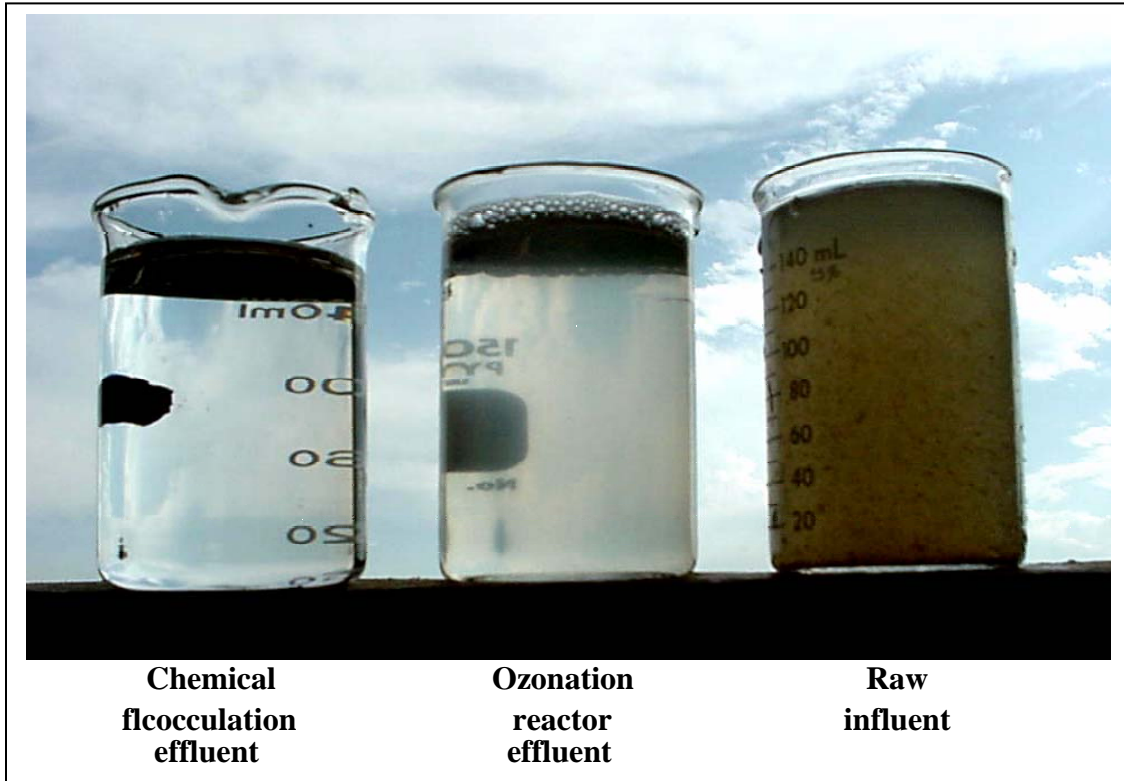


Figure 3.5. Improvement of wastewater turbidity and color by ozonation and chemical flocculation. Samples are from Stage 3.

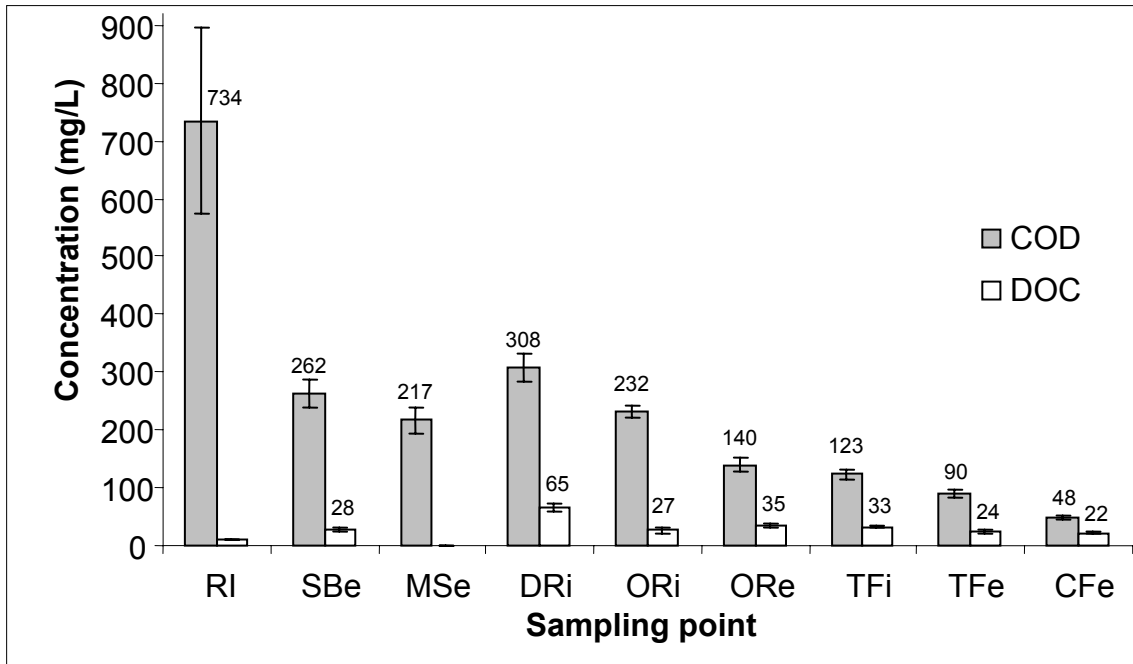


Figure 3.6. Treatment train profile for COD and DOC variation in Stage 3 (10:00 p.m. and 6:00 a.m. samplings). Mean values  $\pm$  standard deviations shown ( $n = 6$ ).

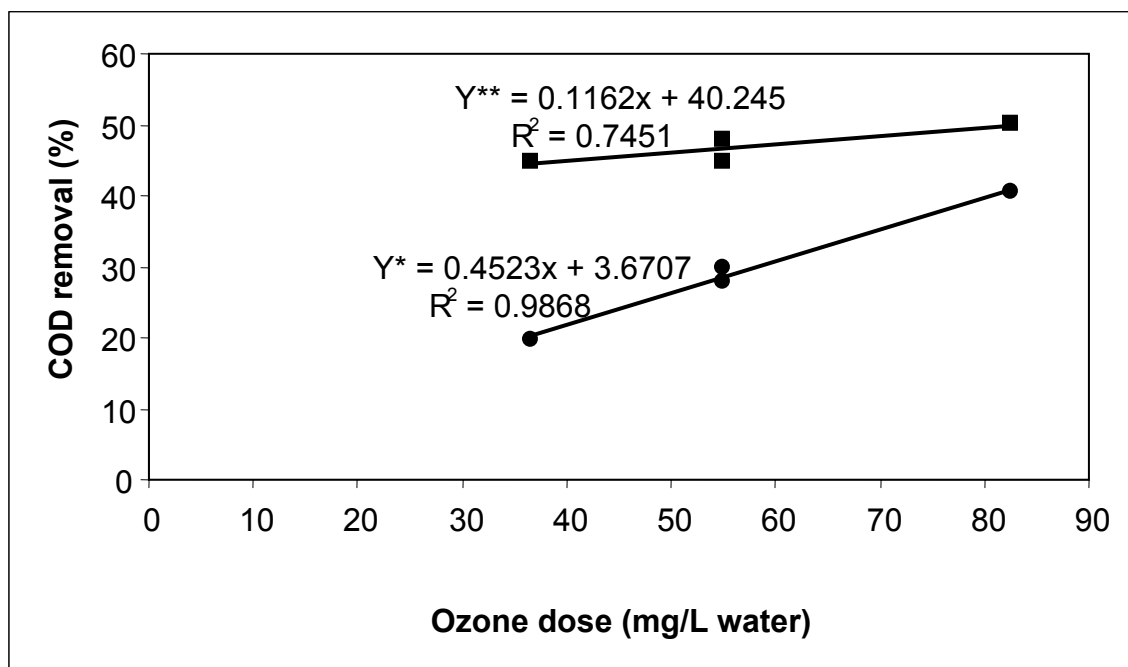


Figure 3.7. Relationship between COD percent removal in OR and CF and ozone dose.

\*Indicates values for % COD removed by OR.

\*\*Indicates values for % COD removed by CF.

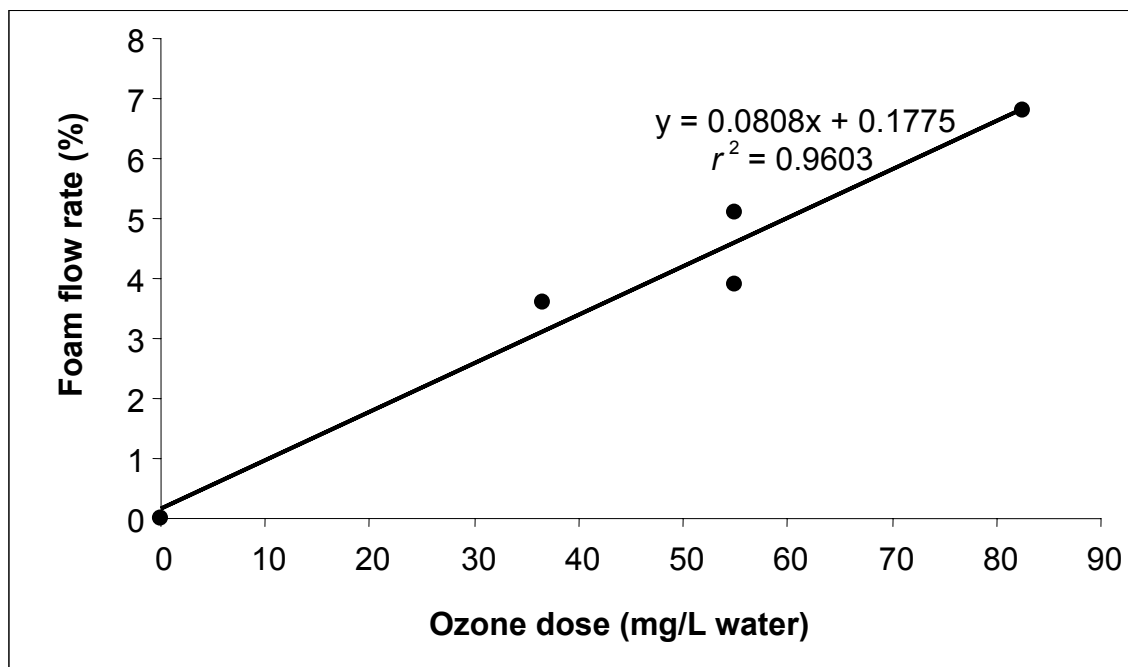


Figure 3.8. Relationship between foam flow rates (expressed as percentage of water flow through the system) and ozone dose.

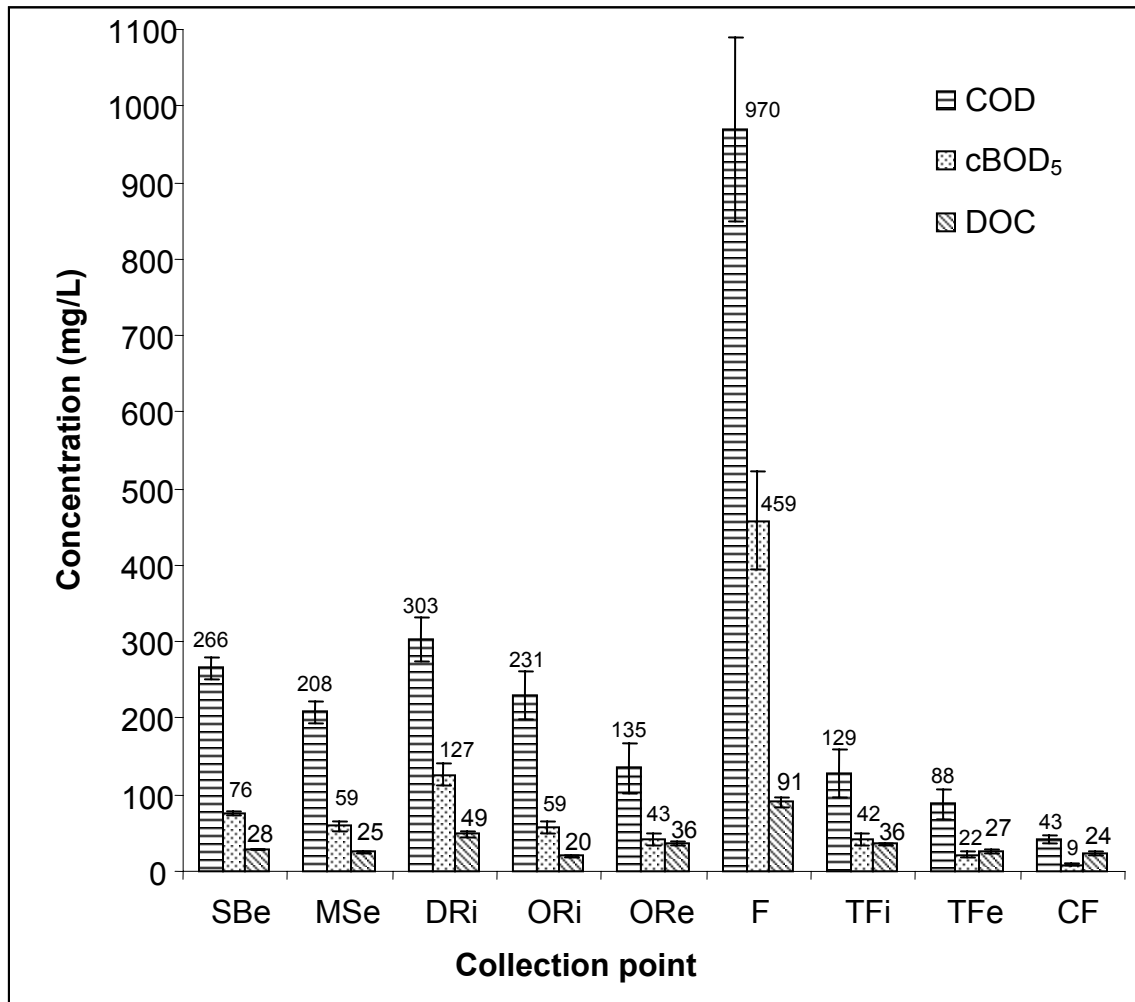


Figure 3.9. Treatment train profile for COD, cBOD<sub>5</sub> and DOC variation in Stage 3. Mean values ± standard deviations shown ( $n = 2$ ).

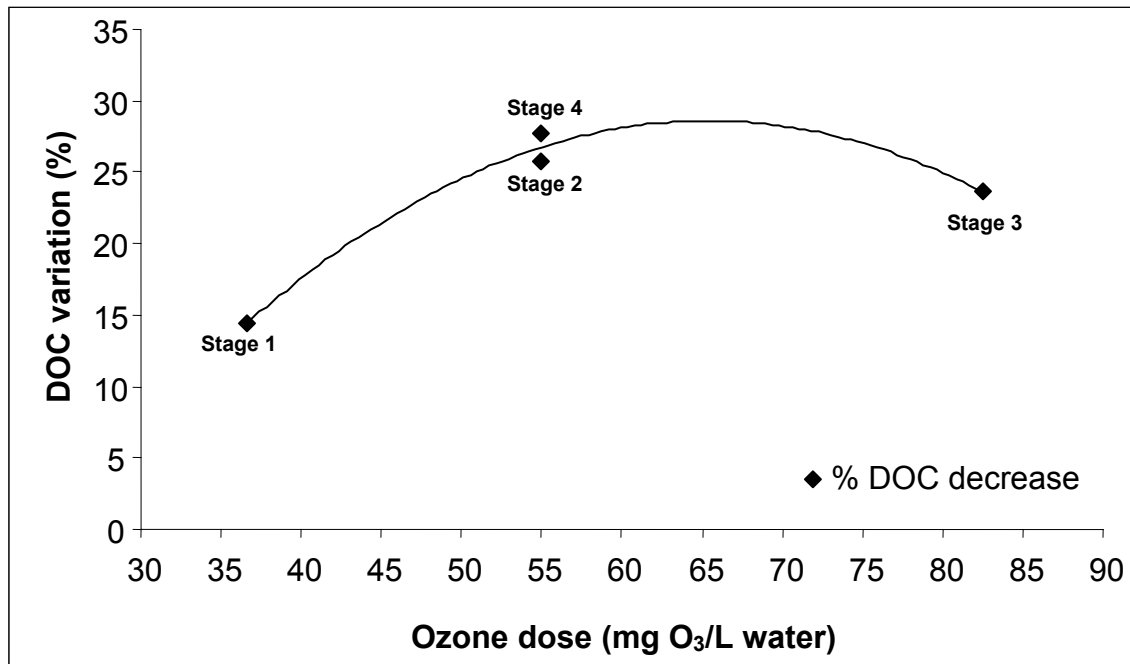


Figure 3.10. DOC generation as a function of ozone dose applied. The curve represents the quadratic regression line between the four stages' non-flow weighted averages.

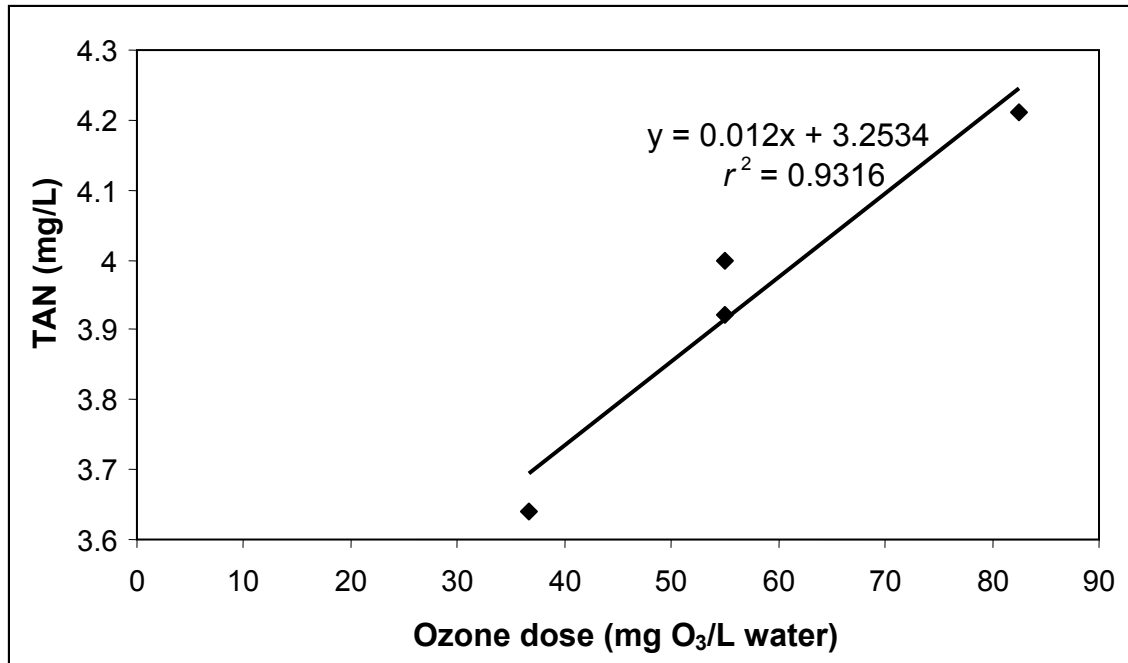


Figure 3.11. Relationship between ozone reactor effluent TAN concentration and ozone dose.

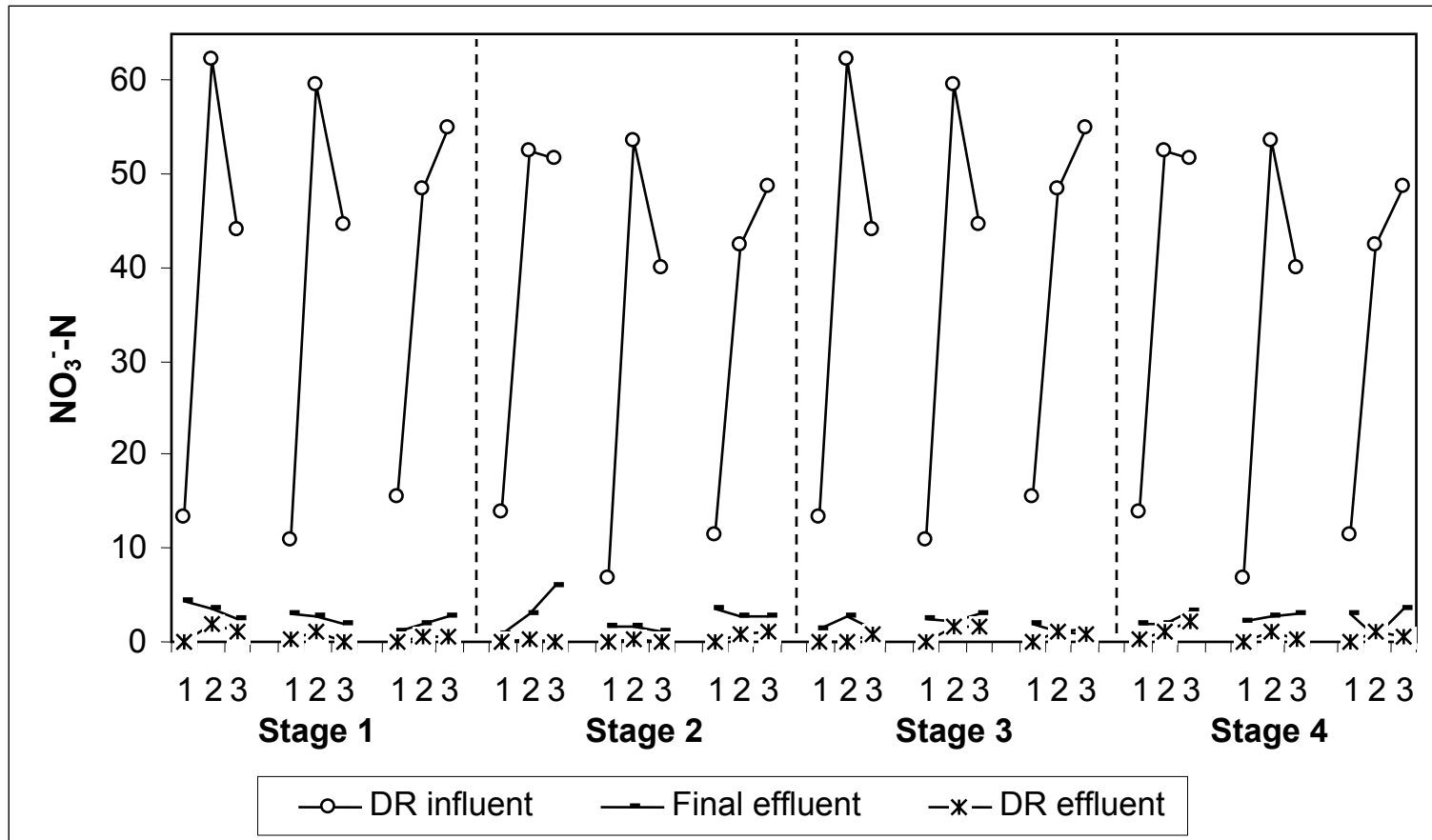


Figure 3.12. Effect of diurnal flow variation on nitrate concentration. DR influent, DR effluent and final effluent NO<sub>3</sub><sup>-</sup>-N concentrations were measured under steady-state conditions. Values represent measurements from 2:00 p.m. (1), 10:00 p.m. (2), and 6:00 a.m. (3) for three different days of each stage.

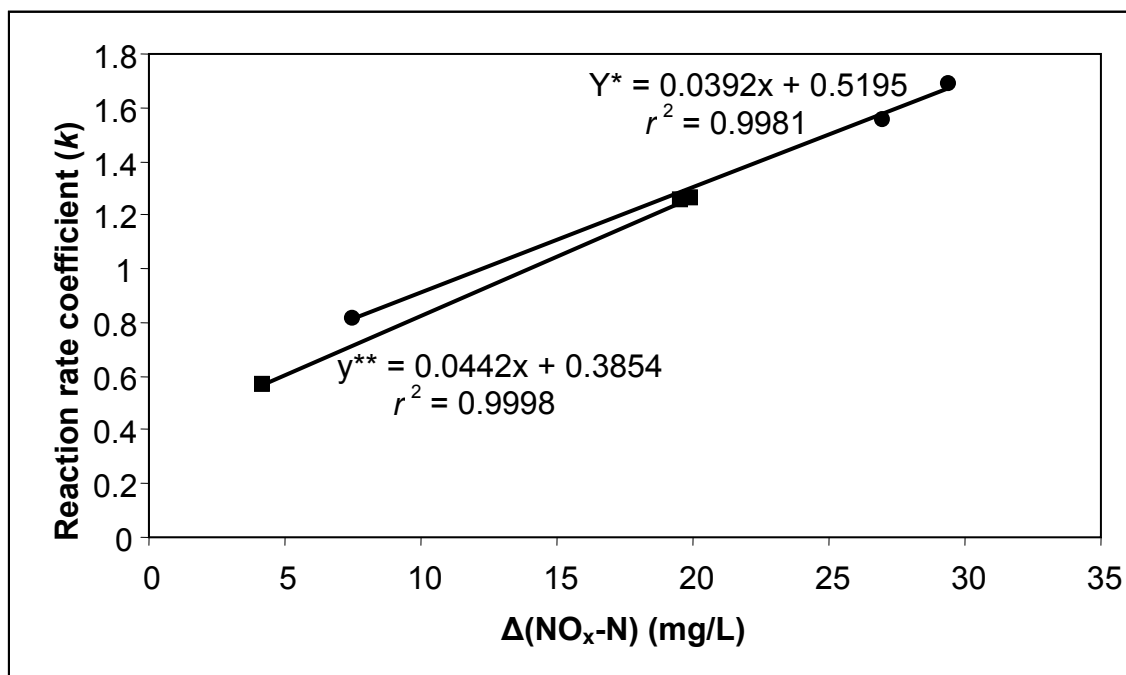


Figure 3.13. Relationship between reduction of NO<sub>x</sub>-N concentration and reaction rate coefficient, *k*.

\* Indicates values for the 6 Lpm working flow.

\*\* Indicates values for the 4 Lpm working flow.

## Chapter 4. NITROGEN BUDGET FOR BLUE RIDGE AQUACULTURE

### ABSTRACT

In this study, the dynamics of nitrogen through the Blue Ridge Aquaculture (BRA) facility was examined by developing a nitrogen budget. The model evaluates total ammonia nitrogen (TAN) production and removal in biofilters, identifying and quantifying the fate of nitrate-nitrogen ( $\text{NO}_3^-$ -N) and determining the systems' maximum carrying capacity. The facility was divided in five subsystems, and a nitrogen budget was derived for each. The subsystems were the growout facility (PS), broodstock subsystem (BS), hatchery subsystem (HS), and two fingerling subsystems ( $\text{FS}_1$  and  $\text{FS}_2$ ). For the PS and BS subsystems, steady state conditions were assumed. Due to cyclical fish production, for the HS,  $\text{FS}_1$ , and  $\text{FS}_2$ , a batch model was adopted for deriving the nitrogen budget. Of the nitrogen input into PS via feed, 85.7% was recovered from different pools: 34.4% as fish flesh, 0.9% as mortalities, 31.5% as dissolved inorganic forms of nitrogen, and 18.9% as total (solid and dissolved) organic nitrogen. The remaining 14.3% of nitrogen unaccounted for likely was lost as nitrogen gas due to passive denitrification and as volatilization of ammonia. In the BS, fish apparently assimilated 79.5% of the nitrogen input with artificial feeds, although some proportion of this high assimilation was mediated by consumption of algae that had taken up dissolved nitrogen. In the HS, 88.6% of nitrogen was accounted for in the nutrient budget, but only 7.9% of the nitrogen was assimilated by fish; a larger proportion (32.5%) was accounted for as organic nitrogen from water, because of the poor feed consumption by larvae. From the  $\text{FS}_1$  and  $\text{FS}_2$ , only 73.5 and 54.8% of the input nitrogen were recovered, respectively. These relatively low percentages were due to recirculation through sand filters in  $\text{FS}_1$ , and to accumulation of uneaten feed on the bottom of the basins, which could not be quantified accurately. Finally, an overall nitrogen budget for BRA was quantified, in which values from the PS accounted for 96.6%. Most of the recovered nitrogen was in fish (34.5%), followed by nitrate-nitrogen (28.2%) and total organic nitrogen (20.6%). The total nitrogen recovered or accounted for totaled 86.3%. In terms of mass, 308.3 kg nitrogen was accounted for of a daily input of 352.2 kg.

Average TAN (over four selected systems) in the PS fish tanks was 2.06 mg/L. The model predicted that the systems could operate safely at up to 3.15 mg/L. Under current conditions, system loading with fish biomass was 56.7-91.5% of the maximum predicted. The production of TAN ( $P_{TAN}$ ) was 36.7 and 40.6 g kg feed<sup>-1</sup> for feeds with 36% and 40% crude protein content, respectively. The biofilters' removal efficiency averaged 54.4% TAN per pass. The biofilters' areal conversion rate (ACR) averaged 442.5 mg m<sup>-2</sup> day<sup>-1</sup>, and could be 634 mg m<sup>-2</sup> day<sup>-1</sup> under conditions of maximum biomass loading; i.e., the biofilters should be able to remove the excess TAN. From  $P_{TAN}$ , 84% was removed in biofilters, 14.9% by passive nitrification and 1.1% by water exchange. Under conditions of reusing treated effluent with residual TAN, the biofilters should work normally, while TAN in the systems should not increase noticeably. NO<sub>3</sub><sup>-</sup>-N accumulated slowly in the systems (9.1-17.2 mg/L in 24 hours), which represented in average 44% of the total nitrate production. The balance (55%) was removed by passive denitrification. NO<sub>2</sub><sup>-</sup>-N was not a parameter of concern, always remaining below 0.3 mg L<sup>-1</sup>.

The high nitrogen utilization by fish indicated that using high-energy feed for tilapia is worthwhile. With better management, fish production could be increased in the existing infrastructure. In situ denitrification could reduce by more than 50% the investments necessary for effluent nitrogen removal.

#### **4.1. Introduction**

Effluents from aquaculture operations pose environmental and operational cost concerns. Aquaculture effluents may contribute to eutrophication of aquatic environments through direct addition of reactive organic matter and by stimulation of organic matter production through addition of nutrients (Cho et al., 1991; Phillips et al., 1993). Effluents originating from RAS can raise production costs if they are discharged to municipal sewer systems for treatment. In many cases, recirculation or effluent treatment technologies are required to achieve desired discharge reduction onsite (Eikebrokk et al., 1995). One way to reduce treatment costs is through the reduction of nutrients in the aquaculture effluent. Substantial reduction in the environmental impacts and costs of fish farm effluents can be obtained by improvements in feed composition and feeding

regimes. Nitrogen is associated with protein, the most expensive component of feeds, and feeds constitute over half of the variable costs of production. Hence, the performance and efficiency of an aquaculture system can be evaluated through analysis of the conversion of nitrogen to fish biomass (Thoman et al., 2001). In turn, it is important to understand the nitrogen budget in aquaculture systems in order to minimize potential environmental or production costs and to maximize possible benefits.

The nutrient discharge from a fish farm (nutrient load) can be described by a mass balance equation, in its most simple form as the difference between feed supply and fish utilization (Eikebrokk et al., 1995). Nitrogen is found dissolved in fish culture tanks as ammonia, nitrite, and nitrate, and as organic molecules such as amino acids and proteins. In addition, it is contained in suspended organic particles. During biological degradation, the organic nitrogen is transformed to ammonia. In operating a recirculating fish culture system, care must be taken to prevent ammonia concentration from reaching toxic levels within the culture tanks. Therefore, it is important to estimate the total ammonia loading on the water treatment system to ensure that ammonia removal units and water exchange rates will remove ammonia at a rate such that concentration design goals are met (Thomas and Piedrahita, 1998). By estimating total nitrogen budgets for the particular species cultured and culture conditions, it should be possible to determine the amount and nature of the dissolved nutrient load both within the aquaculture facility and downstream, to institute appropriate treatment action, and to prevent or at least mitigate the effects of pollutants downstream (Porter et al., 1987). A nitrogen budget is also necessary to determine how fish at various stocking densities utilize nitrogen (Suresh and Kwei Lin, 1992; Siddiqui and Al-Harbi, 1999), to identify and to quantify the major processes affecting water quality, and to understand the role of each nitrogenous compound, such as the amount and nature of the nutrient release into the water column by the dissolved and particulate excretion of pellet-fed fish (Krom and Neori, 1989).

Therefore, a total budget for nitrogen on aquaculture systems and the rate of fish waste production (loading size) over time could provide crucial information for the design and the optimization of recirculation, feeding strategies, and water and effluent treatment technologies. It also helps to identify and quantify the role of each unit process in the

production system, to suggest changes in operating practices, and to predict the impact of these changes on system water quality.

#### 4.2. Literature review

Recent studies documenting nitrogen dynamics in aquaculture (Porter et al., 1987; Schroeder, 1987; Krom and Neori, 1989; Acosta-Nassar et al., 1994; Boyd, 1997) primarily targeted pond systems and involved a variety of species. Nitrogen budgets of ponds systems are difficult to evaluate because these systems are open to exchange through rainfall, infiltration or exfiltration, overflow, ammonia volatilization, and effects from exchange with pond sediments (Thoman et al., 2001). Several other nitrogen budget studies focused on arctic char *Salvelinus alpinus* and Atlantic salmon *Salmo salar* reared in raceways (Fivelstad et al., 1990), and in recirculating fish culture systems for African catfish *Clarias gariepinus* (Bovendeur et al., 1987), brown trout *Salmo trutta* (Eikebrokk et al., 1995), and red drum *Sciaenops ocellatus* (Thoman et al., 2001). Studies also have estimated nitrogen balances for aquarium mesocosm (Parent and Morin, 2000) and for wastewaters generated in large ecosystems (Lauver and Baker; 2000). Although tilapia culture is becoming popular for intensive aquaculture, few studies of nutrient budgets in tanks stocked with tilapia have been made (Suresh and Kwei Lin, 1992; Siddiqui and Al-Harbi, 1999). Because the particular response of a species with regard to feed (protein requirement and assimilation), environmental requirements (e.g., stocking density, temperature, pH, dissolved oxygen, salinity), and the nature and the amount of nitrogenous compound production, nitrogen dynamics must be estimated for each particular case (Eikebrokk et al., 1995).

Data from different studies on different species showed a linear relationship between protein fed and ammonia production of fish (Thomas and Piedrahita, 1998; Paulson, 1980; Gershanovich and Pototskij, 1992). Thomas and Piedrahita (1998) strengthened this correlation in a recent study involving data from several California white sturgeon *Acipenser transmontanus* farms, aimed at determining daily ammonia production rates as a function of the tank operating parameters and examining the diel variations in ammonia rates in response to feeding. They found that 87% of the variation in ammonia values

could be attributed to variations in protein ration, and the protein ration proved to be the only variable which was significant in predicting the mean daily ammonia production rates. The daily mean  $\text{NH}_3\text{-N}$  production rate was found between 1.5 and 27.6  $\text{mg/kg fish}^{-1} \text{ h}^{-1}$ , corresponding to protein rations of 0.08 and 1.14  $\text{g/g fish}^{-1} \text{ day}^{-1}$ , (%), respectively.

Eikebrokk et al. (1995) calculated the TAN production rates from  $\text{NO}_x$  mass balance, the total effluent discharge and the discharge distribution during normal tank operation with tank flushing from brown trout and Atlantic salmon production tanks, and showed a decrease in TAN production rate with increasing fish size. However, they found no indication of effects on growth rates from differences in stocking densities, specific water use, or feeding regimes in two brown trout tests. Fivelstad et al. (1990) examined the metabolite production rate in single pass, land-based rearing units for arctic char and Atlantic salmon. Balancing the nitrogen inputs with the partitioned nitrogen outputs, they found for both species that the average production rates ( $\text{mg nitrogen/kg fish}$ ) of total nitrogen, total ammonia, and urea increased linearly with specific growth rate. The production rates of total ammonia, when expressed as  $\text{g/kg food}$ , decreased with increasing conversion factor. Total nitrogen excreted as total ammonia was in the range 61 – 67%, while 7 – 10% excreted as urea. Krom and Neori (1989) derived a total nitrogen budget in an experimental intensive fishpond with circularly moving seawater, water retention time of two days, and gilthead seabream *Spratus aurata* as the fish produced. They estimated that fish food accounted for more than 95% of the nitrogen input. Fish assimilated 26% of the nitrogen inputs, 10% was found in the drained settled detritus, 46% in the particulate phase of the water column, 13% in the outflow stream as dissolved organic matter, and the difference of 5% was attributed to the measurement error or to denitrification.

Suresh and Kwei Lin (1992) reared red tilapia *Oreochromis niloticus* x *O. aureus* in RAS, studying the effect of stocking density on water quality. The tanks were stocked with 50 or 200  $\text{fish/m}^3$  at an average size of 75 g. They showed that although individual fish growth rate and feed utilization efficiency were inversely related to stocking density, there were no significant differences between these parameters at the medium and high densities. The nitrogen budget constructed for the experimental periods suggested that less than 20% of the nitrogen consumed was converted into useful body nitrogen and the

rest was converted to solid or soluble waste. Siddiqui and Al-Harbi (1999) estimated nitrogen and phosphorus budgets in an experiment with hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) reared in indoor fiberglass tanks. They found that feed consumption rate significantly decreased with increasing density. Of the feed input, 21.4% of the nitrogen and 18.8% of the phosphorus were incorporated into the fish harvested.

Thoman et al. (2001) studied the efficiency of nitrogen conversion from food to fish biomass by constructing mass and isotopic balances of all major nitrogen pools for closed, independent recirculating systems producing red drum. They demonstrated that wet weight gain per fish and percent dry nitrogen assimilated by the fish were not significantly different. The nitrogen mass balance and isotopic data demonstrated occurrence of denitrification in recirculating mariculture systems, resulting in a nitrogen loss of 9 – 21% of the total nitrogen input. Bovendeur et al. (1987) determined the loading rates of the separate water treatment units (as part of high-density RAS producing African catfish) from the distribution of organic matter and nitrogen compounds between the dissolved and solid phases. Starting from the assumption that all wastes produced originated from the feed ration, they demonstrated that the quality and the quantity of the waste depended on feeding level, feed composition, feed digestibility, and utilization of digested feed. From the mass balances concerning nitrogen compounds, they determined that 1 kg feed resulted in a loading of the biological reactor with approximately 37 g N at a feeding level of  $8.4 \text{ g d}^{-1} \text{ kg}^{-0.8}$ . A feeding level of  $16.8 \text{ d}^{-1} \text{ kg}^{-0.8}$  resulted in a lower loading of 32 g N per kg feed, due to the smaller proportion of dissolved fecal matter in combination with the application of a primary clarifier. They also found that the pilot reactor removed 87% of the daily ammonia load by nitrification, and that denitrification in the lamellar separator removed 40 – 80% of the nitrate produced by nitrification. The remaining amounts of both ammonium and nitrate were removed by water exchange.

Parent and Morin (1999) computed an annual nitrogen budget to examine how the increase of nitrate could be attenuated or stopped in the St. Lawrence Mesocosm (SLM) in Montreal, Canada. They found that nitrogen accumulated in the mesocosm mainly as nitrate (92%), but also as fish and invertebrate biomass (8%). The nitrogen budget was effective in identifying the role of each component in the system, suggesting changes in

the operating practices, and predicting the impact of these changes. The conclusion was that without integrated dissimilation or assimilation process, marine aquaria similar to the SLM must be opened after 5 – 7 years of operation unless N inputs can be reduced or N outputs increased.

In summary, studies reported to date demonstrated that nitrogen budgets can be derived for a variety of aquaculture or aquatic systems, and the accuracy of these determinations increases with the degree of the control over these systems. Therefore, RAS, regarded as well-controlled systems, are at the top of such a classification. For these systems, a nitrogen budget offers useful information with regard to nitrogen assimilation by fish, to metabolic production rates as a function of nitrogen content in the feed, and to water quality throughout the system, allowing better design and management of treatment units or prediction of the environmental impact of effluents. However, little effort has addressed the nitrogen budget for tilapia production, and no attempts have been made for estimating the nitrogen budget for a commercial-scale RAS facility.

### **4.3. Mass balance approach and problem statement**

From both engineering and economic perspectives, goals of an aquaculture system are the efficient conversion of nitrogen in feed into healthy, marketable biomass (Thoman et al., 2001), maintaining high biological carrying capacity in relatively little space (Quillere et al., 1993; Twarowska et al., 1997), and keeping a healthy environment for fish, by eliminating toxic metabolites and growth-inhibiting substances (Otte and Rosenthal, 1979). To reach these goals, developments of mass balance analysis are appropriate for designing aquaculture tank production systems, estimating carrying capacities, optimizing operations, or designing effluent treatment units. This is particularly important, since all too often, aquaculture tank production systems based on water reuse or recycle are designed by the trial and error approach (Losordo and Westers, 1994).

The method of mass balance analysis is based on the physical Law of Conservation of Mass that states that mass cannot be created or destroyed, but only transformed. In this approach to problem solving, the following steps are suggested (Tchobanglous and Schoeder, 1985):

- 1) Define system boundaries,
- 2) Isolate and identify flow streams crossing the boundaries,
- 3) Isolate the material to be balanced, and
- 4) Identify transformation processes taking place inside the system boundaries that affect the mass balance.

After these steps have been taken, a mass balance equation can be written, whether for existing steady state conditions (i.e., a system with water exchange or flow-through) or for non-steady state conditions (i.e., batch type of production mode). RAS have daily water exchange and slight variation of biomass with time. These characteristics make RAS fit well into the steady state conditions mode (Losordo and Westers, 1994). The equations that apply to materials to be balanced follow the model:

$$\text{Inputs} + \text{Generation} = \text{Output} + \text{Consumption} \quad (4.1)$$

Blue Ridge Aquaculture (BRA) is a facility that operates continuously, producing 1360 metric tons per year of tilapia *Oreochromis niloticus*. BRA employs typical RAS, with fish tanks, multi-tube clarifier basins and RBCs for nitrification, and work with daily water exchange for controlling solids, dissolved organics and nutrient accumulation. An exception involves hatchery installations and greenhouses for fingerlings that are operated in cycles of production (i.e., in batch-type mode). A part of the fingerling systems has solids separation units (sand filters), but along with the spawning broodstock basins, have no biofiltration installations. Additionally, these systems are subject to periodical exchange of water. Together, these auxiliary systems represent only a small part of the facility volume and exchange flow.

No information exists about the partitioning of nutrients once entering the systems via feed at BRA, except for very general estimations on feed conversion rates. By deriving a nitrogen budget, the form and proportion of nitrogen ingested as food that is bound as flesh protein by tilapia, excreted as metabolites and released in the effluent, or utilized further by microorganisms inside the system and lost as gas (e.g., by denitrification) could be characterized and determined. Knowledge about nitrogen partitioning would help with optimization of operations, thereby improving facility efficiency and

maximizing production. The same purposes would be also served by predicting the maximum system carrying capacity with respect to different critical factors, such as the total ammonia nitrogen. This goal could be achieved by using a model that combined acknowledged and experimentally derived data. Then, the predictions could be compared to the existing conditions, in order to evaluate the state of the art and to maximize the utilization of space. At the same time, the removal efficiency and areal conversion rate of biofilters could be determined, determining their maximum potential. Additionally, the fate of ammonia and nitrate nitrogen throughout the RAS could be determined. These data would constitute the prerequisites for the design of effluent treatment capacity with regard to these pollutants.

#### **4.4. Objectives**

Against this background, the objectives of this study were to:

- 1) Examine nitrogen dynamics for the BRA facility, estimating nitrogen flows and nitrogen budgets for five major subsystems by using mass balance equations,
- 2) Determine how the total nitrogen budgets relate to water quality observations and predictions at BRA,
- 3) Evaluate ammonia production, loading, and removal efficiency of RBCs at BRA, and
- 4) Evaluate and predict, by using a model, the RAS maximum carrying capacity and the impact of returning remnant nitrogen compounds with treated effluent on the systems' nitrogen dynamics.

#### **4.5. Methods**

The objectives above were approached using the culture systems, sampling procedures, analytical techniques, procedures, and statistics described below.

##### 4.5.1. Culture conditions

BRA is a large commercial-scale facility producing 1,360 metric tons of tilapia per year. The facility includes systems for food-fish production in RAS, fish breeding, egg incubation/hatching, fingerling rearing, and broodstock holding. The main building houses 42 RASs for growout to market size and the hatchery. Each RAS is rectangular in shape, built from concrete, and holds 215 m<sup>3</sup> of water consisting of a fish rearing tank (119 m<sup>3</sup>), a multi-tube clarifier sedimentation basin (37 m<sup>3</sup>), an air-driven RBC biofilter (59 m<sup>3</sup> basin volume, and 13,366 m<sup>2</sup> surface area per shaft), and a U-tube oxygenation system. The total volume of the growout unit is 9030 m<sup>3</sup>. The water surface is at the same level in fish tank, clarifier and RBC compartments, and water passes freely from one section into another through large pipes or apertures. A pump receives water from the RBC compartment and pushes it through U-tubes and then to the fish tank, at the far end, driving the recirculation. The filtration rate is 3.8 m<sup>3</sup>/min, and the turnover time is about once per hour. The hatchery is in a side room of the main building, housing one battery of six incubators, and 13 small basins of different sizes (43 m<sup>3</sup> total) for post-hatch development of the larvae. The first greenhouse holds 42 small above-ground basins (21 concrete and 21 fiberglass, with a total volume of 113 m<sup>3</sup>), where fingerlings are reared through early stages. The second greenhouse holds four lined in-ground basins of the same size, two for rearing fingerlings in the late stages, and two for broodstock maintenance. Each basin has a volume of 65 m<sup>3</sup>. The third greenhouse holds only broodstock in two in-ground basins, similar in size to those in the second greenhouse.

Water is exchanged daily from the growout unit in the interval between 2:00 p.m. and 8:00 a.m. Management practice is to completely flush the sedimentation basin after each instance that 227 kilograms of feed has been administered. During other hours of the day, all the water is replaced in the hatchery and fingerling tanks, and around 90% from the two fingerling basins in the second greenhouse. The four tanks with broodstock are flushed weekly (about 50% of the total volume), in the meantime allowing algal development. All three greenhouses have oxygen diffusion systems. The first greenhouse also has a recirculation system through sand filters that retain solids. The exchange rate is on average 22.3% per day over the BRA facility, but the percentage varies from system to system, as a function of the life stage of fish, water quality requirements, and the amount of feed delivered to the system. The total daily volume of wastewater generated

is approximately 2,290 m<sup>3</sup> per day, which is discharged to the City of Martinsville sewage system.

The exchange water originates from the wells, and is supplemented with municipal tap water when necessary (i.e., during drought periods). Exchange water is used to remove settled particulate material, and to dilute dissolved organic materials, dissolved nutrients and salts in the various systems. Before utilization, the exchange water is heated to 30°C. This temperature is maintained well throughout the systems, reaching 26 to 29°C in the effluent.

Fish are fed commercially prepared diets containing minimum crude protein and fat levels ranging between 36 to 50% and 8 to 16%, respectively, varying with the age category of fish at issue. In the nursery, feed is distributed every 20 minutes over a 24-hour period. Fingerlings receive feed every hour between 8:00 a.m. and 5 p.m. every day. In the production tanks, the feed is distributed hourly over the 24-hour period. As part of the management strategy, fish production is organized so that approximately the same amount of fish reaches marketable size each week for harvest and shipment to the live market (i.e., 21 – 27 metric tons, at an average of 600 grams/fish).

#### 4.5.2. Systems boundary delimitation

For the purpose of this study, the systems' boundaries were delimited as a function of the management strategy and the possibility of influent-effluent sampling and measurement, allowing the development of mass balance equations for the nitrogen budget. Based on this strategy, the 42 RASs for growout were delimited within the same boundaries as “production subsystem” (PS), as shown in Figure 4.1.

The tanks from the hatchery constituted the “hatchery subsystem” (HS). For this subsystem and for those that follow, it was assumed that the tanks are completely mixed, their water therefore having the water quality characteristics as the effluent at any time during water exchange.

The greenhouse complex was studied as three other subsystems. “Fingerling subsystem 1” included all tanks from greenhouse 1 (FS<sub>1</sub>), “fingerling subsystem 2” included the two fingerling basins from greenhouse 2 (FS<sub>2</sub>), and the “broodstock subsystem” included two broodstock basins from greenhouse 2 and two from greenhouse

3 (BS). The different management strategy for the different age groups allowed quantification of nitrogen and water flux for each subsystem. In practice, waters from subsystems FS<sub>1</sub> and FS<sub>2</sub> were exchanged daily and the broodstock basins were flushed on Tuesday, each at a different time.

#### 4.5.3. Inputs, outputs and nitrogen pools

BRA has a single, measurable flow stream that provides the water input for all subsystems. It originates from local phreatic sources (wells), and is backed up by an emergency tapwater line. No measurable amounts of dissolved inorganic nitrogen [i.e., total ammonia-nitrogen ( $N_{TAN}$ ), nitrite-nitrogen ( $N_{NO2}$ ), and nitrate-nitrogen ( $N_{NO3}$ ) (g L<sup>-1</sup>)] were identified in the replacement water. Hence, the feed provided to the fish was the sole nitrogen source for each subsystem in the form of organic nitrogen [ $N_{feed}$ , (g kg<sup>-1</sup> of feed)]. The multiplication of  $N_{feed}$  by the total amount of feed provided the mass of total nitrogen input ( $TNI$ ). The removal of nitrogen was accounted for in a variety of known pools, as follow:

- 1) Nitrogen fixed in fish biomass as organic nitrogen [ $N_{fish}$ , (g kg<sup>-1</sup> fish produced)];
- 2) Nitrogen fixed in dead fish biomass as organic nitrogen [ $N_{mort}$ , (g kg<sup>-1</sup> fish removed)];
- 3) Dissolved inorganic nitrogen [ $N_{DIN}$ , (g L<sup>-1</sup>)], including  $N_{TAN}$ ,  $N_{NO2}$ , and  $N_{NO3}$ ;
- 4) Total organic nitrogen in the effluent [ $N_{TON}$ , (g L<sup>-1</sup>)];
- 5) Nitrogen gas removed from the system by passive denitrification [ $N_{denit}$ , (g L<sup>-1</sup>)], and by ammonia volatilization [ $N_{NH3\ vol}$ , (g L<sup>-1</sup>)].

Some physical, biological and biochemical processes caused movement of nitrogen among pools inside of each subsystem. The initial forms of output nitrogen undergo partial physical, chemical, and biochemical transformation through the nitrogen cycle, moving within and among the pools. Processes affecting  $N_{TON}$  pool included solubilization of organic fecal components in water, assimilation of ammonia into bacterial cells as  $N_{TON}$ , ammonia release following the bacterial lyses and decay, and uptake of nitrogenous species by phytoplankton. Transformation affecting  $N_{DIN}$  included nitrification of ammonia in biofilters, and loss of nitrogen due to dissimilatory nitrate reduction, passive denitrification, and volatilization of ammonia. Nitrogen uptake by

phytoplankton also affects  $N_{DIN}$ . Dynamics of these processes depend on numerous factors, such as system design, mode of operation, management strategy, size and biomass of fish, type and ration of feed, luminosity, and exchange rate of water. The large number of variables makes it impossible to identify the magnitude of an individual transformation throughout a subsystem. For this reason, all transformations were assumed to be in a dynamic equilibrium over a definite period of time, which allowed determination of the forms of nitrogen for each pool. Consequently, the mass balances presented the status quo of each pool under steady state conditions for the case of PS, or as a subsystem's cycle average for the other subsystems.

The mass fractions of nitrogen from the Pools 1 to 4 (i.e., measurable pools) were accounted for as the total nitrogen recovered ( $TNR$ ), while the difference between  $TNI$  and  $TNR$  constituted the mass fraction of total nitrogen unaccounted for ( $TNUA$ , Pool 5). Hence, analysis of nitrogen content of each composite pool provided insights into partition and flows of nitrogen throughout subsystems. That is, the nitrogen budget is expressed as a mass balance equation of all nitrogen forms (inputs and outputs through the pools). From a nitrogen budget point of view, the total inputs plus generation is equal to the total outputs plus consumption.

#### **4.6. Analytical techniques**

Samples were collected as necessary for the assay at issue or preserved for later analyses, as follows. Analyses of fish and of feed for protein content were carried out according to Thiex et al. (2002), who indicated that by dry weight, 16% of protein is nitrogen. Samples were processed at the Forage Testing Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Analyses for inorganic dissolved nitrogen forms (e.g., TAN,  $\text{NO}_2^-$ -N, and  $\text{NO}_3^-$ -N) were conducted on site using a Hach DR 2400 spectrophotometer (Hach Company, Loveland, CO). TKN was determined using macro-Kjeldahl (Standard Method 4500 –  $\text{N}_{\text{org}}$  B, APHA et al., 1998). Samples were acidified below pH 2 using  $\text{H}_2\text{SO}_4$ , refrigerated with ice, and transported to the Department of Environmental Engineering at Virginia Polytechnic Institute and State University, Blacksburg, Virginia, for analysis. Temperature and pH were measured

directly on site using a pH/mV/°C meter (Acorn Meter Kit Model pH 6, Oakton, Vernon Hills, IL). Alkalinity was determined on-site using the Hach Permachem<sup>®</sup> Method. Dissolved oxygen (DO) was measured using a YSI (Model 550, Yellow Springs, Ohio) instrument.  $N_{TON}$  was determined as the difference between TKN and TAN.

## 4.7. Procedures

### 4.7.1. Procedures for nitrogen budget determination

Steady state conditions of nitrogen flow were assumed in order to determine the nitrogen budget for the PS. Under such conditions, a subsystem's fish biomass does not fluctuate significantly over time (i.e., harvest equals growth), and the daily rations of feed of the various types are constant. Based on these assumptions, the nitrogen budget was derived by determining the nitrogen input introduced with feeds and the output of nitrogenous compounds from the known pools. Daily  $N_{feed}$ ,  $N_{fish}$ , and  $N_{mort}$  were derived from yearly numbers for feed consumption, fish production and mortalities that were provided by BRA management, divided by 365. The components of  $N_{DIN}$  and  $N_{TON}$  pools were determined experimentally from the effluent, and the average findings were extrapolated (by multiplication) to the entire exchange volume from a day, in order to determine the mass of nitrogen recovered in these forms. The amount of nitrogen missing from the balance was assumed to be lost by passive denitrification (as  $N_{denit}$ ) and by ammonia volatilization ( $N_{NH3\ vol}$ ).

Two different types of feed used in the PS (with 36% and 40% standard protein content) were considered for determination of  $N_{feed}$ . To identify the true  $N_{feed}$ , three samples were collected from different points of silos storing the two types of feed, and processed for nitrogen content determination. Subsequently,  $N_{feed}$  was calculated as a composite, using the equation:

$$N_{feed} = \Sigma(FA \times PC \times 0.16) \quad (4.2)$$

where:

$FA$  = amount of feed (kg);

$PC$  = protein content of the feed (decimal fraction);

$0.16$  = percent of nitrogen in protein (decimal fraction).

$PC$  in equation 4.2 was determined by laboratory analyses, based on the conjecture that they could differ from the standard percentage claimed by the feed producer. The concentration of nitrogen in protein (16%) was assumed to agree with levels suggested by Thiex et al. (2002). When  $FA$  was considered the daily ration of feed, the total mass of nitrogen originating from the feed input ( $TNI$ ) was obtained by multiplying  $N_{feed}$  by that amount.

To determine fish utilization of nitrogen ( $N_{fish}$ ), samples of muscle tissue from fish from three size classes were analyzed for protein content (in triplicate). The proportions of fish in each size class were estimated as 5% juveniles (i.e., newly introduced to the PS), 60% intermediate, and 35% marketable size. Data on protein content of each fish size class allowed determination of  $N_{fish}$  as a composite, using the equation:

$$N_{fish} = \Sigma(FB \times FP \times 0.16) \quad (4.3)$$

where:

$FB$  = biomass of fish (kg);

$FP$  = protein content of the fish (decimal fraction).

About 3.5% of the fish production (by number) was lost as mortalities.  $N_{mort}$  was assumed to have the same nitrogen content as  $N_{fish}$ . However, in order to determine the weight biomass of  $N_{mort}$ , mortalities were collected daily from the PS for a two-week period, selected by size and weighted. These data were used to determine  $N_{mort}$  using equation 4.3.

All nitrogen from the  $N_{feed}$  which was not accounted for as  $N_{fish}$  or  $N_{mort}$  was quantified as nitrogen load to the water ( $L_N$ , g N kg<sup>-1</sup> fish produced), and was determined using equation:

$$L_N = [N_{feed} - (N_{fish} + N_{mort})] / FB \quad (4.4)$$

The use of the total values determined for the terms in the right hand side of equation 4.4 (i.e., not divided by  $FB$ ) provided the daily  $L_N$  of the PS.  $L_N$  also represented the amount of nitrogen that sustained the nitrogen cycle throughout the systems, supplying all other non-fish effluent nitrogen pools.  $L_N$  entered the water column in dissolved form ( $N_{DIN}$  pool) as ammonia, and as organic nitrogen bound in feces ( $N_{TON}$  pool).

$N_{TON}$  was determined as the difference between TKN and TAN from the effluent. Values for these parameters (as well as for  $NO_2^-$ -N and  $NO_3^-$ -N) were obtained from analyzing samples collected between 2:00 p.m. and 8:00 a.m. at three-hour intervals from the effluent discharge pipe. The effluent originated from the PS only during that period of the day. The tests were repeated twice (on different days), and results were averaged. The daily production of these nitrogen forms was determined by multiplying the average concentration ( $mg\ L^{-1}$ ) by the volume of wastewater released from PS during a one-day period.

All  $N_{feed}$  that was not recovered as  $N_{fish}$ ,  $N_{mort}$  or  $N_{TON}$  represented the dissolved inorganic fraction that entered the water as TAN. Hence, it was possible to determine ammonia production ( $P_{TAN}$ ,  $g\ N\ kg^{-1}\ feed$ ) using the equation:

$$P_{TAN} = N_{feed} - (N_{fish} + N_{mort} + N_{TON}) / FA \quad (4.5)$$

The sum of TAN,  $NO_2^-$ -N and  $NO_3^-$ -N found in the effluent represented the recovered fraction of  $N_{DIN}$ . The summation of this fraction,  $N_{fish}$ ,  $N_{mort}$  and  $N_{TON}$  provided the value for  $TNR$ . Expressed as a percentage of  $TNI$  (considered 100%),  $\%TNR$  was determined by using the equation:

$$\%TNR = \%N_{fish} + \%N_{mort} + \%N_{NH3} + \%N_{NO2} + \%N_{NO3} + \%N_{TON} \quad (4.6)$$

The nitrogen deficit ( $TNUA$ ) then was determined using the equation:

$$\%TNUA = 100 - \%TNR \quad (4.7)$$

The subsequent nitrogen mass balance was:

$$TNI = TNR + TNUA \quad (4.8)$$

Alternatively, the mass balance was determined based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system:

$$N_{feed} = \%N_{fish} + \%N_{mort} + \%N_{HN3} + \%N_{NO2} + \%N_{NO3} + \%N_{TON} + \%N_{denit} + \%N_{NH3\ vol} \quad (4.9)$$

Steady state conditions also were assumed for nitrogen budget determination in the BS with regard to the feeding rate and total biomass of broodstock fish. This assumption represented a simplification for modeling purposes, because some management practices interfered with the nitrogen flow equilibrium through the BS. Among the complicating factors were the long periods of time between the removal/replacement of individual broodstock, long periods of time between water exchanges, and routinely replacement of water lost by evaporation with nutrient-rich effluent water from fingerling subsystems. These actions led to fish biomass accumulation, and sustained algal development, but also diminished the chances for deriving an accurate nitrogen budget. The strategy adopted was to select a two-week period as the testing time, during which it was known that fish biomass in the subsystem was comparable to the annual average. The broodstock received feed with 36% standard protein, the same type used in the PS. Their size also was approximately the same with that of the largest class of fish from the PS. Hence,  $N_{feed}$  and  $N_{fish}$  were assumed to be known. Daily feed ration and feed conversion factor also were known (on the basis of data from BRA management), and fish growth was determined from them, using the equation:

$$FB = FA \times FC \quad (4.10)$$

where:

$FC$  = feed conversion factor (kg feed  $\text{kg}^{-1}$  fish biomass produced).

$N_{mort}$  was considered to be zero, because mortalities in BS were negligible, and if they occurred the dead individuals were replaced with new specimens. Next, analyses of TAN,  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N and TKN were conducted on three sets of samples collected before and after weekly exchange of water from each basin for.  $L_N$ ,  $P_{TAN}$ , %TNR, %TNUA and the nitrogen budget were determined equations corresponding to those used for the PS, with the difference that  $N_{mort}$  was eliminated from equations 4.6 and 4.9.

In HS, FS<sub>1</sub>, and FS<sub>2</sub>, a steady state mode of production could not be assumed due to the cycling nature of fish growth in these subsystems. Consequently, fish biomass was accumulating concurrent with continuous increase of feed rations. However, daily average  $N_{feed}$  and  $N_{fish}$  were determined for each subsystem from the total feed and cumulative fish growth (data from BRA management), by dividing these values by the number of days in each cycle. In terms of numbers,  $N_{mort}$  was estimated at 5, 3, and 1% in HS, FS<sub>1</sub>, and FS<sub>2</sub>, respectively. The mortalities were transformed into mass units, and considered in the nitrogen balance equations.

To evaluate nitrogen partitioning in the water pools, analyses were conducted during an entire growth cycle in each of these subsystems. The cycles lasted for 16 days in HS, 42 days in FS<sub>1</sub>, and 28 days in FS<sub>2</sub>. During each cycle five tests were conducted at equal intervals of time, and samples collected before and after the daily exchange were analyzed for TAN,  $\text{NO}_2^-$ -N,  $\text{NO}_3^-$ -N and TKN. The subtraction final from initial values provided the average concentration of the volume displaced. The production of each nitrogen form was determined by multiplying the difference of concentration by the daily volume of wastewater replaced in the subsystem. Because the production of nitrogen compounds was not a linear function over time, a simple average of the concentrations determined experimentally could not provide the average concentration over the entire cycle. Consequently, I used a numerical integration technique to determine mean production of nitrogen species. The integration technique was Simpson's Parabolic Rule, which states:

$$\int_a^b f(x)dx \approx \frac{\Delta x}{3} (y_0 + 4y_1 + 2y_2 + 4y_3 + \dots + 2y_{n-2} + 4y_{n-1} + y_n) \quad (4.11)$$

where:

$a$  = the initial day of the cycle;

$b$  = the last day of the cycle;

$\Delta x$  = time between the measurements (days);

$y$  = parameter value at time  $t$  ( $\text{mg L}^{-1}$ );

$n+1$  = number of measurements.

The average concentration over the entire cycle resulted from dividing the value provided by the function by the number of days in the cycle. The daily mass average of each parameter then was obtained by multiplying the average concentration by the volume of water exchanged daily. Afterward,  $L_N$ ,  $P_{TAN}$ , %TNR, %TNUA, and the nitrogen budget were determined by the corresponding equations used for the previous subsystems.

Finally, the nitrogen budget equations of all subsystems were used to create a composite nitrogen budget over the entire facility, based on weighted averages of flows through individual components.

#### 4.7.2. Procedure for estimating RAS carrying capacity, RBC design, TAN and $\text{NO}_3^-$ -N removal evaluation

A simplified version of a model proposed by Losordo and Timmons (1994) was used to determine the maximum system carrying capacity of RAS from the production subsystem. I considered only the parts of the model that pertain to determination of maximum system carrying capacity with respect to TAN. Modeling of the flow rate through biofilters was unnecessary because the flow rate was fixed among all RASs at  $3.78 \text{ m}^3 \text{ min}^{-1}$ . Consequently, flow rate was included in the set of known parameters.

Four RAS – holding different age groups of fish from juvenile to marketable size – were chosen for tests, and considered representative for the overall population in the facility. Total fish biomass, fish size, feeding rate, type of feed (crude protein content), daily percent body weight fed, flow rate through the system, and daily rate of exchange were known for each selected system (data from BRA management, Table 4.2). Other parameters, such as TAN,  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, pH, temperature, dissolved oxygen, and alkalinity were determined experimentally, according to standard methods as described in

Section 4.6 (APHA et al., 1998). Analyses for determination of these parameters were performed in samples collected as composites from the fish rearing tanks, and from the RBCs influent and effluent at four-hour intervals. These locations allowed determining the effects of fish tanks, biofilters or sedimentation basins on each parameter. Specifically, I determined parameter concentrations in fish tanks (proven homogenous by preliminary tests), which also were the concentrations of influents into the sedimentation basins. In turn, the sedimentation basin effluents were determined at RBCs influent sampling points. Finally, concentrations of the RBCs effluents were the same as those of fish tank influents.

The experiments extended over the interval of time between two consecutive water exchanges. However, the data were processed and presented for 24-hour intervals, which provided daily averages or variation for each parameter. As necessary, hourly values were obtained by division by 24 (e.g., flow rate through the system, L hr<sup>-1</sup>).

Determination of the maximum system carrying capacity with respect to TAN was determined as follows:

Calculation of the maximum allowable TAN concentration ( $A_{TAN}$ , mass volume<sup>-1</sup>):

$$A_{TAN} = A_{NH3-N} / a \quad (4.12)$$

where:

$A_{NH3-N}$  = concentration of unionized ammonia nitrogen (mass N volume<sup>-1</sup>)

$a$  = mole fraction of unionized ammonia nitrogen (decimal fraction)

The value of  $a$  was selected from the mole fraction of unionized ammonia nitrogen (Huguenin and Colt, 1989) based on the pH and temperature found during the tests.

Maximum feed rate ( $FR_{mTAN}$ , mass feed time<sup>-1</sup>) was calculated based on the assumption that the TAN concentration of a fish tank equals  $A_{TAN}$ , using the equation:

$$FR_{mTAN} = [A_{TAN} \times Q_f \times E_a + Q(C_{TAN} - C_{TANi})] / (0.092 \times PC) \quad (4.13)$$

where:

$Q_f$  = recirculating flow rate, or flow rate to the RBC (volume time<sup>-1</sup>) (known to be 227100 L/hr)

$Q$  = flow rate through system (volume time<sup>-1</sup>)

$E_a$  = RBC removal efficiency (%)

$C_{TAN}$  = TAN concentration of fish tank (mass N volume<sup>-1</sup>)

$C_{TANi}$  = TAN concentration of new water (mass N volume<sup>-1</sup>)

0.092 = model constant coefficient

$PC$  = protein content of feed (decimal fraction)

$E_a$  was determined using the equation:

$$E_a = [(C_{TAN} - C_{TANe})/C_{TAN}] \times 100 \quad (4.14)$$

where:

$C_{TANe}$  = TAN concentration in the effluent from the filter (mass N volume<sup>-1</sup>)

The maximum biomass that could be sustained within the system ( $SBM_{mTAN}$ , kg fish) was determined using the equation:

$$SBM_{mTAN} = FR_{mTAN} / \%BW \quad (4.15)$$

where:

$\%BW$  = time unit rate of fish feeding, expressed as a percent of body weight

The production rate of TAN ( $P_{TAN}$ , mass N time<sup>-1</sup>) refers to the rate of production of TAN in the system as a result of the metabolism of the fish and the microbial degradation of uneaten feed.  $P_{TAN}$  was estimated as a function of the feed rate and the percentage of protein in feed such that:

$$P_{TAN} = (FA * PC * 0.102) / t \quad (4.16)$$

where:

$FA$  = amount fed (mass)

$PC$  = protein content of the feed (decimal fraction)

$t$  = period of time from the onset of feeding to the next feeding (time)

The equation is based on the following assumptions:

- a) 16% of feed protein is nitrogen,
- b) 80% of the nitrogen is assimilated,
- c) unassimilated nitrogen in fecal matter is removed rapidly from the tank,
- d) 80% of assimilated nitrogen is excreted, and
- e) all of the TAN is excreted during  $t$  hours.

The numeric coefficient 0.102 represents the product of values suggested by assumptions 1 through 4 (as decimal fractions) in the estimation of the TAN produced from the metabolic activity of fish (i.e.,  $0.16 \times 0.8 \times 0.8 = 0.102$ ).

The mass flow rate of TAN to RBC, or ammonia loading ( $L_{TAN}$ , mass N time<sup>-1</sup>) was determined from known data ( $Q_f$ ) and experimentally-determined ( $C_{TANf}$ ) using the equation:

$$L_{TAN} = Q_f \times C_{TANf} \quad (4.17)$$

where:

$C_{TANf}$  = TAN concentration of RBC influent.

The ammonia removal rate ( $R_{TAN}$ , mass time<sup>-1</sup>) was determined using the equation:

$$R_{TAN} = (C_{TANf} - C_{TANe}) \times Q_f \quad (4.18)$$

Equation:

$$(R_{TAN} \times 100) / P_{TAN} \quad (4.19)$$

gave the percentage of TAN that was removed by means other than the RBC.

The RBC nitrification performance plateau was expressed as an areal conversion rate ( $ACR$ ). It represented the amount of TAN oxidized by a unit of surface area in 24 hours (i.e.,  $ACR = \text{mass surface}^{-1} \text{ time}^{-1}$ ), and was determined using the equation:

$$ACR = R_{TAN} / S \quad (4.20)$$

where:

$S$  = surface area of RBC (acknowledged, 13336 m<sup>2</sup>).

Finally, the mass balance expressing the partitioning of  $P_{TAN}$  removal was expressed using the equation:

$$P_{TAN} = TAN_{pass + vol} + TAN_{RBC\ nitrification} + TAN_{exchange}. \quad (4.21)$$

where:

$TAN_{pass + vol}$  = TAN removed by passive nitrification into system and ammonia volatilization (mass/volume),

$TAN_{RBC\ nitrification}$  = TAN removed by nitrification into RBC biofilter (mass volume<sup>-1</sup>),

and

$TAN_{exchange}$  = TAN removed with the exchange water (mass volume<sup>-1</sup>).

$TAN_{nitrification}$  and  $TAN_{exchange}$  were determined experimentally, and  $TAN_{pass + vol}$  was determined by subtracting the summation of the other two values from  $P_{TAN}$ .

A similar approach was used to determine NO<sub>3</sub><sup>-</sup>-N partitioning, using equation:

$$P_{NO_3^- - N} = NO_3^- - N_{pass} + NO_3^- - N_{exch}. \quad (4.22)$$

where:

$NO_3^- - N_{pass}$  = NO<sub>3</sub><sup>-</sup>-N removed by passive denitrification into system (mass volume<sup>-1</sup>),

and

$NO_3^- - N_{exch}$  = NO<sub>3</sub><sup>-</sup>-N removed with exchange water (mass volume<sup>-1</sup>).

#### 4.8. Statistical analysis

Linear regressions were conducted to determine the relationship between daily TAN production ( $P_{TAN}$ ) and TAN removal efficiency per pass ( $E_a$ ), and between fish biomass and percent  $P_{TAN}$  transformed by passive denitrification in the four systems tested

Statistical analyses were conducted using Microsoft Excel.

## 4.9. Results

### 4.9.1. Nitrogen budget for production subsystem

The nitrogen budget for the PS was derived under mean conditions of 28.4°C, pH 7.14, and alkalinity 119.0 mg/L as CaCO<sub>3</sub>. For an annual production of 1300 metric tons of fish biomass in the PS, BRA administers 2210 metric tons of feeds. These amounts correspond to 6054.8 kg feed consumed day<sup>-1</sup> and 3561.6 kg fish gain day<sup>-1</sup>. Of the feed utilized, 95% (5752.0 kg) was nominally 36% protein and 5% (302.8 kg) 40% protein content. However, laboratory analyses showed that the actual protein contents of the two sorts of feed were somewhat lower, 35.0±0.2% and 39.8±0.2%, respectively. The estimated percentages of feed types and the laboratory-determined protein concentrations were used in equation 4.2 for determining  $N_{feed} = 56.38 \text{ g kg}^{-1}$  feed. By extrapolating  $N_{feed}$  to daily feed input, a  $TNI = 341.381 \text{ kg day}^{-1}$  was determined.

Laboratory analyses showed that the three classes of fish (order by size from small to large) had 18.04±0.16, 20.75±0.02, and 22.26±0.74% protein content. From these data  $N_{fish} = 33.83 \text{ g kg}^{-1}$  fish produced was determined (equation 4.3). Extrapolating to the daily biomass of fish produced, the total nitrogen assimilated in fish was 120.488 kg day<sup>-1</sup>.

Although loss of fish represented 3.5% of the total production by number, weighing of the dead fish indicated losses of 2, 1, and 0.5% from the respective size classes. This was the equivalent of 30.6 kg fish day<sup>-1</sup> or 1.03 kg total  $N_{mort} \text{ day}^{-1}$ , and represented 0.86% of the total nitrogen assimilated. Hence, 35.3% of nitrogen from feed was assimilated in fish flesh (34.4 harvested and 0.86% removed with mortalities), and 64.7% was unassimilated or excreted in different forms. In this latter term was included nitrogen in uneaten feed that was accounted for in the overall budget as  $N_{TON}$ .

The percentage of nitrogen excreted,  $L_N$ , determined from equation 4.4, was 62.0 g kg<sup>-1</sup> fish produced. Subsequently, the cumulative  $L_N$  (i.e., daily value for the entire PS) was 221.3 kg.

Analyses of the effluent wastewater (estimated at  $2017 \text{ m}^3 \text{ day}^{-1}$ ) indicated that it contained (on average)  $2.88 \text{ mg L}^{-1}$  TAN,  $1.09 \text{ mg L}^{-1} \text{ NO}_2^- \text{-N}$ ,  $49.3 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$ , and  $32.05 \text{ mg L}^{-1}$  TON. Extrapolated to the entire volume, the overall flows were  $5.8 \text{ kg } N_{TAN} \text{ day}^{-1}$  (1.70% *TNI*),  $2.2 \text{ kg } N_{NO_2} \text{ day}^{-1}$  (0.64% *TNI*),  $99.4 \text{ kg } N_{NO_3} \text{ day}^{-1}$  (29.1% *TNI*), and  $64.6 \text{ kg } N_{TON} \text{ day}^{-1}$  (18.9% *TNI*). Determination of  $N_{TON}$  allowed estimation of  $P_{TAN} = 25.81 \text{ g kg}^{-1}$  feed (equation 4.5). The recovered fraction of  $N_{DIN}$  resulted from the summation:

$$1.70\% N_{TAN} + 0.64\% N_{NO_2} + 29.13\% N_{NO_3} = 31.47\%$$

*TNR* was determined as a percentage of *TNI* from equation 4.6:

$$85.69\% \text{ TNR} = 34.43\% N_{fish} + 0.86\% N_{mort} + 1.70\% N_{TAN} + 0.64\% N_{NO_2} + 29.13\% N_{NO_3} + 18.93\% N_{TON}$$

The value of *TNUA* then was estimated to represent 14.3% of *TNI* (equation 4.7). Hence, the subsequent nitrogen mass balance was (equation 4.8):

$$341.381 \text{ kg } TNI \text{ day}^{-1} = 292.529 \text{ kg } TNR \text{ day}^{-1} + 48.852 \text{ kg } TNUA \text{ day}^{-1}$$

The relatively low percentage of *TNUA* was probably due to nitrogen lost as  $N_{denit}$  and as  $N_{TAN \text{ vol}}$ . However, passive denitrification was likely the primary cause, considering that the water was passing through the sedimentation basin numerous times. In this unit, a sediment blanket and the associated thick biofilm that formed in the multitube clarifier surface created conditions favorable for denitrification. Figure 4.2 shows the nitrogen mass balance based on the mass fraction nitrogen composition of all measured budget elements relative to nitrogen in the feed input into the PS.

#### 4.9.2. Nitrogen budget for broodstock subsystem

As described in Section 4.7,  $N_{feed}$  and  $N_{fish}$  were assumed to be known from PS determinations, with values of  $56.38 \text{ g kg}^{-1}$  feed and  $33.83 \text{ g kg}^{-1}$  fish produced,

respectively. Before water exchange, average alkalinity was  $77 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ , pH 7.58, and temperature  $28.1^\circ\text{C}$ . After exchange, average alkalinity was  $84 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ , pH 7.34, and temperature  $28.7^\circ\text{C}$ . According to BRA records, the fish biomass was 325 kg, feed conversion rate was  $0.8 \text{ kg feed kg}^{-1}$  fish growth, and feed ration was  $6 \text{ kg day}^{-1}$  (i.e., 1.5 kg per basin). The gain in fish biomass (i.e., growth) was  $7.5 \text{ kg day}^{-1}$ , determined by using equation 4.10. This high *CF* indicated that artificial feed could not be the only source of nitrogen to the BS, suggesting that the algal biomass that developed from the broodstock-released nutrients and from nutrients introduced with water from the fingerling subsystems had a role in sustaining the growth of fish. Also, the feed had low moisture and the fish flesh high moisture, contributing to high feed conversion ratio. However, nitrogen input from the artificial feed represented  $0.34 \text{ kg day}^{-1}$ , and this value was considered as *TNI* for the nitrogen budget. This assumption allowed determination of the fraction of nitrogen that entered the subsystem by other means. The total nitrogen assimilated in fish was  $0.27 \text{ kg day}^{-1}$ , considering a fish crude protein content of 22.3%. Under these circumstances, 79.5% of nitrogen from the feed appears to have been assimilated by fish, and only 20.5% was excreted. This high nitrogen uptake by fish, coupled with the small value determined for  $L_N$  ( $9.18 \text{ g nitrogen kg}^{-1}$  fish produced) (equation 4.4), suggested that inorganic nitrogen passed rapidly into another pools, first probably into  $N_{TON}$  by algal uptake, and then further into fish by algae consumption. Consequently, from the excreted nitrogen, 5.2 g (1.55% *TNI*) was recovered as  $N_{TAN}$ , 1.9 g (0.55% *TNI*) as  $N_{NO_3}$ , none as  $N_{NO_2}$ , and 131.7 g (39.2% *TNI*) as  $N_{TON}$ . The  $P_{TAN}$  value was negative ( $-10.47 \text{ g kg}^{-1}$  feed) (equation 4.5), which supports the algal uptake hypothesis. Under these circumstances, it was not surprising that *TNR* was 405.8 g, which is larger than *TNI* by 20.8% (equation 4.6). Assuming that nitrogen lost by passive denitrification and by ammonia volatilization were negligible due to the high DO and to the low concentration of these compounds in water, the excess nitrogen found in the subsystem was assumed to have entered from the fingerling subsystems (i.e., to have been introduced with water for replacing evaporation), and assimilated as  $N_{TON}$ . Consequently, the excess nitrogen (i.e., the amount that exceeded *TNI*) was subtracted from  $N_{TON}$  when the data were used for estimation of the overall BRA nitrogen budget, in order to avoid double accounting.

#### 4.9.3. Nitrogen budget for hatchery subsystem

The growth cycle tested for the HS lasted for 16 days. During that period of time 130 kg of feed (43.33±0.08% crude protein content determined by laboratory analyses) were used, and 26 kg of fish (18.04±0.16% crude protein content) were produced. Hence, the *CF* was 5 kg feed kg<sup>-1</sup> fish biomass produced. Daily averages for these parameters were determined directly by division by the number of days. Hence, for each 8.125 kg of feed distributed 1.62 kg of fish was produced.  $N_{feed}$  was 69.3 g kg<sup>-1</sup> feed (equation 4.2), which yielded a *TNI* of 563.3 g day<sup>-1</sup>.  $N_{fish}$  was 28.9 g kg<sup>-1</sup> fish produced (equation 4.3), representing a total of 46.9 g day<sup>-1</sup> (8.33% *TNI*).

From the nitrogen assimilated as  $N_{fish}$ , 2.3 g (5%) was lost with mortalities.  $L_N$  was 317.8 g kg<sup>-1</sup> fish produced (equation 4.4). On a daily basis, from the non-assimilated nitrogen, 151.4 g (26.9% *TNI*) was recovered as  $N_{TAN}$ , 22.3 g (4.0% *TNI*) as  $N_{NO_2}$ , 95.0 g (16.9% *TNI*) as  $N_{NO_3}$ , and 183.5 g (32.6% *TNI*) as  $N_{TON}$ . These parameters were determined by using numerical integration (equation 4.11). Before water exchange, average alkalinity was 77 mg L<sup>-1</sup> as CaCO<sub>3</sub>, pH was 7.58, and temperature was 28.1°C. After exchange, average values were 84 mg L<sup>-1</sup> alkalinity as CaCO<sub>3</sub>, pH 7.34, and temperature 28.7°C.

Based on *TNI*,  $N_{fish}$  and  $N_{TON}$  values, a  $P_{TAN} = 40.97$  g kg<sup>-1</sup> feed was determined (equation 4.5). The recovered fraction of  $N_{DIN}$  was estimated from the summation:

$$26.88\% N_{TAN} + 3.95\% N_{NO_2} + 16.85\% N_{NO_3} = 47.68\%$$

Then, *TNR* was determined as percent *TNI* from equation 4.6:

$$88.58\% TNR = 7.91\% N_{fish} + 0.42\% N_{mort} + 26.88\% N_{TAN} + 3.95\% N_{NO_2} + 16.85\% N_{NO_3} + 32.57\% N_{TON}$$

The difference (11.4% *TNI*) represented *TNUA*, which was determined by using equation 4.7. Finally, by using equation 4.8, the general mass balance of nitrogen in the HS was determined:

$$563.29\text{g } TNI \text{ day}^{-1} = 499.01\text{g } \text{day}^{-1} \text{ } TNR + 64.28\text{g } \text{day}^{-1} \text{ } TNUA$$

The percentage of *TNUA* was probably the nitrogen lost as  $N_{TAN\ vol}$ , or was due to measurement errors, as in the case of  $N_{TON}$  determination, where fast sedimentation of uneaten feed could have distorted the results. Figure 4.3 shows a graphic representation of the nitrogen mass balance based on the mass fraction nitrogen composition of all measured budget elements relative to nitrogen in the feed input to the HS.

#### 4.9.4. Nitrogen budget for the first fingerling subsystem

An nitrogen budget was derived for FS<sub>1</sub> from samples collected over a 42-day period. During that interval, an initial batch of 26 kg of fish grew to 910 kg (containing 18.04±0.16% crude protein), and 1945 kg of feed were distributed (containing 43.3% crude protein). Subsequently, *CF* represented 2.2 kg feed kg<sup>-1</sup> fish biomass produced.  $N_{feed}$  was 69.3 g kg<sup>-1</sup> feed (equation 4.2), and the daily average feed input was 46.3 kg, equivalent to 3209.9 g as *TNI*.  $N_{fish}$  was 28.9 g kg<sup>-1</sup> fish (equation 4.3), totaling 606.1 g day<sup>-1</sup> (18.9% *TNI*) from 21.05 kg<sup>-1</sup> fish produced.

Of the total nitrogen assimilated, 3% was removed by mortalities, which represented 18.18 g nitrogen day<sup>-1</sup>.  $L_N$  was 124.0 g kg<sup>-1</sup> fish produced (equation 4.4). By using numerical integration (equation 4.11), it was determined that from the daily non-assimilated nitrogen, 430.7 g (13.4% *TNI*) was recovered as  $N_{TAN}$ , 37.4 g (1.2% *TNI*) as  $N_{NO_2}$ , 282.9 g (8.8% *TNI*) as  $N_{NO_3}$ , and 1003.2 g (31.25% *TNI*) as  $N_{TON}$ . Before water exchange, the average alkalinity was 76 mg L<sup>-1</sup> as CaCO<sub>3</sub>, pH was 7.16, and temperature was 28.4°C. After exchange, average values were 90 mg L<sup>-1</sup> alkalinity as CaCO<sub>3</sub>, pH 7.11, and temperature 28.8°C.

$P_{TAN}$  was 34.56g kg<sup>-1</sup> feed (equation 4.5), and the recovered fraction of  $N_{DIN}$  was estimated from the summation:

$$13.41\% \text{ } N_{TAN} + 1.16\% \text{ } N_{NO_2} + 8.81\% \text{ } N_{NO_3} = 23.38\%$$

*TNR* was determined as a percentage of *TNI* from equation 4.6:

$$73.51\% \text{ TNR} = 18.31\% N_{fish} + 0.57\% N_{mort} + 13.41\% N_{TAN} + 1.16\% N_{NO_2} + 8.81\% N_{NO_3} + 31.25\% N_{TON}$$

*TNUA* was determined from equation 4.7, representing 26.5% of *TNI*. Using equation 4.8, the general mass balance of nitrogen in FS<sub>1</sub> was determined:

$$3209.89 \text{ g } \textit{TNI} \text{ day}^{-1} = 2360.28 \text{ g day}^{-1} \textit{ TNR} + 849.61 \text{ g day}^{-1} \textit{ TNUA}$$

The largest part of *TNUA* was probably the *N<sub>TON</sub>* fraction in the forms of uneaten feed, algae and feces trapped by the sand filter during recirculation. Loss of nitrogen by passive denitrification is unlikely, due to frequent water exchange and intense oxygenation. Ammonia volatilization could be a factor, although it was reported to represent less than 0.25% of the total nitrogen lost in RAS (Thoman et al., 2001). For these reasons, the *TNUA* was accounted as *N<sub>TON</sub>* for FS<sub>1</sub> for derivation of the general nitrogen budget over the facility, rather than assuming that it was lost as volatile gas forms. Consequently, *TNR* equaled *TNI*. Figure 4.4 shows a graphic representation of the nitrogen mass balance based on the mass fraction nitrogen composition of all measured budget elements relative to nitrogen in the feed input to the FS<sub>1</sub>.

#### 4.9.5. Nitrogen budget for the second fingerling subsystem

To derive the nitrogen budget over FS<sub>2</sub>, data were collected at seven day intervals over a cycle of 28 days. Initially, the subsystem received 910 kg of fish (i.e., the ending production from FS<sub>1</sub>). After 28 days, the total weight of fish was 3624 kg, representing a net yield of 2714 kg fish biomass, or 96.9 kg day<sup>-1</sup>. The crude protein content of fish was 18.04±0.16%. The feed delivered amounted to 5157 kg (39.8% crude protein content), which represented a *CF* of 1.9 kg feed kg<sup>-1</sup> fish produced. At an *N<sub>feed</sub>* of 63.7 g kg<sup>-1</sup> feed (equation 4.2), *TNI* was 11729.9 g, considering the daily average feed input of 184.2 kg. *N<sub>fish</sub>* was 2797.8 g day<sup>-1</sup> (23.9% *TNI*) (equation 4.3).

The mortalities were estimated to be only 0.3% of the fish biomass produced, representing 8.4 g nitrogen day<sup>-1</sup> (0.07% *TNI*). *L<sub>N</sub>* was found to be 92.2 g kg<sup>-1</sup> fish

produced (equation 4.4). From the daily amount of non-assimilated nitrogen, 1279.2 g (10.9% *TNI*) was recovered as  $N_{TAN}$ , 46.9 g (0.4% *TNI*) as  $N_{NO_2}$ , 777.8 g (6.6% *TNI*) as  $N_{NO_3}$ , and 1519.8 g (13.0% *TNI*) as  $N_{TON}$  (equation 4.11). Before water exchange, average alkalinity was 87 mg L<sup>-1</sup> as CaCO<sub>3</sub>, pH was 7.17, and temperature was 28.2°C. After exchange, these parameters had values of 97 mg L<sup>-1</sup> alkalinity as CaCO<sub>3</sub>, pH 7.10, and temperature 28.7°C.

$P_{TAN}$  was found to be 40.2 g kg<sup>-1</sup> feed (equation 4.5). The recovered fraction of  $N_{DIN}$  was:

$$10.91\% N_{TAN} + 0.40\% N_{NO_2} + 6.63\% N_{NO_3} = 17.94\%$$

*TNR* was determined as a percentage of *TNI* using equation 4.6:

$$54.75\% TNR = 23.78\% N_{fish} + 0.07\% N_{mort} + 10.91\% N_{TAN} + 0.40\% N_{NO_2} + 6.63\% N_{NO_3} + 12.96\% N_{TON}$$

The difference of 45.3% *TNI* (equation 4.7) represented *TNUA*. The general mass balance of nitrogen in FS<sub>2</sub> was determined by using equation 4.8:

$$11729.86 \text{ g } TNI \text{ day}^{-1} = 6421.54 \text{ g day}^{-1} TNR + 5308.32 \text{ g day}^{-1} TNUA$$

The results indicate that a significant amount of nitrogen remained unaccounted for. A plausible explanation could be that large amounts of feed remained unconsumed by fish, and settled easily at the bottom of the basins. Technically, this residual was removed by vacuuming during water exchange, and although this effluent could be sampled, it had large fluctuations of particle concentration over short periods of time, which made accurate evaluation very difficult. Additionally, it was not possible to estimate the volume of water removed by the vacuum. Hence, it only could be assumed that the largest part of the missing nitrogen belonged to the  $N_{TON}$  pool. The assumption is based on the high DO found in water, making denitrification improbable. Also, ammonia volatilization was not a significant way of losing nitrogen from the subsystem because of

the high dilution. For these reasons, the  $TNUA$  was accounted for as  $N_{TON}$  in  $FS_2$  for derivation of the general nitrogen budget over the facility, considering that  $TNI$  equals  $TNR$  (i.e., similarly to the case of  $FS_1$ ). Figure 4.5 presents the nitrogen mass balance based on the mass fraction nitrogen composition of all measured budget elements relative to nitrogen in the feed input into the  $FS_2$ .

#### 4.9.6. General nitrogen budget

The feed distributed at BRA amounted to  $6300 \text{ kg day}^{-1}$ . Of this amount, 96.2% had a crude protein content of  $35.0 \pm 0.2\%$ , and 3.8% had higher concentrations. Overall,  $TNI$  was  $357.2 \text{ kg day}^{-1}$ , of which  $123.1 \text{ kg day}^{-1}$  was recovered as  $N_{fish}$  by a fish biomass production of  $3688.7 \text{ kg day}^{-1}$ . Of this amount of biomass, 96.6% was generated in the PS, and 3.4% in other subsystems. Table 4.1 shows the daily nitrogen budget derived over each subsystem and as a total over the BRA facility, based on the mass fraction nitrogen composition of all measured budget elements. Additionally, the percentage distributions of  $TNI$  and  $N_{fish}$  (as the main parameter of interest response) among the subsystems are presented.

Data generated by equation 4.6 (i.e.,  $TNR$  determined as a percentage of  $TNI$ ) for the cumulative data are presented graphically in Figure 4.6. Results showed that most of the recovered nitrogen was  $N_{fish}$  (34.5%  $TNI$ ), followed by  $N_{NO_3}$  (28.2%  $TNI$ ), and by  $N_{TON}$  (20.6%  $TNI$ ).  $TNUA$  represented 13.7% of  $TNI$ . The overall BRA mass balance of nitrogen then was determined using equation 4.8:

$$357.22 \text{ kg } TNI \text{ day}^{-1} = 308.30 \text{ kg day}^{-1} TNR + 48.92 \text{ kg day}^{-1} TNUA$$

Determinations of the fate of nitrogen throughout PS conducted in the next section showed that most of the  $TNUA$  was lost by passive denitrification inside the RAS.

### 4.10. Evaluation of carrying capacity, RBC design, TAN and $NO_3^-$ -N removal

#### 4.10.1. TAN

The use of the model indicated that a RAS from BRA could support transformation of a maximum concentration of  $3.15 \text{ mg TAN L}^{-1}$  (equation 4.13). This value table, corresponded to  $0.025 \text{ mg L}^{-1}$  maximum allowable unionized ammonia ( $A_{TAN}$ ) under conditions of  $\text{pH} \approx 7.0$  and temperature  $\approx 30^\circ\text{C}$  (Huguenin and Colt 1989) - the average values of these parameters determined over the four tested RASs were  $\text{pH} 7.09$  and temperature  $27.8^\circ\text{C}$ . Additionally, alkalinity was maintained at around  $88 \text{ mg L}^{-1}$  as  $\text{CaCO}_3$ , and DO at  $5.70 \text{ mg L}^{-1}$ . At this maximum allowable concentration of TAN, a system should be able to receive an average of  $269.8 \text{ kg feed day}^{-1}$  ( $FR_{max TAN}$ , determined using equation 4.13), which supports an average fish biomass ( $SBM_{max TAN}$ ) of  $10287.4 \text{ kg fish system}^{-1}$  (equation 4.15). Data showing the predicted values of these parameters for each selected RAS are presented in Table 4.3. Comparing these data to those known for the respective basins at the time of experiment (Table 4.2), resulted that system loadings were between 56.7 and 91.5% of the maximum estimated (Table 4.3). These proportions also apply to estimated maximum feeding rates. Average RAS concentrations of TAN determined for each fish tank, biofilter influent and effluent are presented in Table 4.3. Over the four selected tanks, TAN removal efficiency per pass ( $E_a$ ) was on average 54.4% (equation 4.14). The rate of TAN production ( $P_{TAN}$ ) was determined on a daily basis using equation 4.16 (Table 4.3).  $P_{TAN}$  per kg of feed consumed was then determined by dividing these values by the daily amount of feed introduced into a system, i.e.,  $40.6 \text{ g kg feed}^{-1}$  for the feed with 40% crude protein content, and  $36.7 \text{ g kg feed}^{-1}$  for the feed with 36% crude protein content. A positive, linear relationship was found between  $P_{TAN}$  (which also was proportional to the feeding rate) and  $E_a$  (slope = 0.0013,  $r^2 = 0.72$ ) (Figure 4.7). This analysis suggests that the RBCs were able to remove efficiently various amounts of ammonia, and that none of the RBCs in the recirculation systems tested were working at their maximum capacity.

The mass flow rate of TAN to an RBC ( $L_{TAN}$ ) had an average of  $467.7 \text{ g hr}^{-1}$  (equation 4.17), which was removed at an average rate ( $R_{TAN}$ ) of  $255.2 \text{ g hr}^{-1}$  (equation 4.18). Details (i.e., per-system values) are presented in Table 4.3. The ratio between  $R_{TAN}$  and  $P_{TAN}$  (equation 4.19) showed that RBCs removed an average of 84.0% of  $P_{TAN}$  from the selected systems. From the difference, 1.09% was recovered from the exchanged water and 14.96% remained unaccounted for, probably being transformed in  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -

N by passive nitrification, or being lost by ammonia volatilization. From Table 4.3, it can be noted that the fish biomass in the system was positively correlated with the percentage of  $P_{TAN}$  transformed by passive nitrification (slope = 0.0015,  $r^2 = 0.69$ ). Although the correlation was not very strong (Figure 4.8), it suggests that systems with higher biomass had lower water quality (e.g., larger microbial populations, including nitrifiers) that promoted in situ biotransformation of nutrients.

In terms of areal conversion rate (equation 4.20), the tests showed that RBCs removed between 378.4 and 534.2 mg TAN m<sup>-2</sup> day<sup>-1</sup> (442.5 mg TAN m<sup>-2</sup> day<sup>-1</sup> in average)(Table 4.3). On the other hand, the average  $ACR$  in conditions of maximum system biomass was estimated to be 634.0 mg TAN m<sup>-2</sup> day<sup>-1</sup>. In terms of percentages, the difference between existing and predicted  $ACR$  is consistent with that between the existing and predicted maximum fish biomass.  $ACR$  also increased with the increase of  $P_{TAN}$ .

Finally, the data acquired (Table 4.4) allowed derivation of a daily balance (mass and percent) expressing the partitioning of  $P_{TAN}$  removal from each RAS, and estimating averages among them (equation 4.21). On average among systems, 84.0% TAN was removed by RBCs, 14.9% by passive nitrification and ammonia volatilization, and only 1.1% was removed by the periodic water exchange.

#### 4.10.2. NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N

For the same RASs, tests were conducted in order to determine the fate of NO<sub>3</sub><sup>-</sup>-N following its generation from TAN via nitrification. The stoichiometry states that any nitrogenous compound should contain the same amount of nitrogen, when measured in nitrogen units. Hence,  $P_{NO_3^- - N}$  was considered approximately equal with  $P_{TAN}$ , under the assumption that TAN lost from the systems by water exchange and volatilization were negligible. The available data on  $P_{NO_3^- - N}$ , water exchange rates, and NO<sub>3</sub><sup>-</sup>-N concentrations before and after water exchange allowed determination of the total mass of NO<sub>3</sub><sup>-</sup>-N in the RASs at these instances, and the amounts (or proportions) of NO<sub>3</sub><sup>-</sup>-N lost by water exchange and passive denitrification (equation 4.22). That is, a daily mass balance expressing the partitioning of  $P_{NO_3^- - N}$  removal from each RAS was derived. The results are presented in Table 4.5, including the averages among the systems. The results indicated that despite a large production of NO<sub>3</sub><sup>-</sup>-N, accumulation was slow, in the range

of 9.1 – 17.2 mg L<sup>-1</sup> in each RAS over a 24-hour period. Under these circumstances, it was not surprising to find that on average, only 44.1% of the NO<sub>3</sub><sup>-</sup>-N was removed by water exchange, and the difference of 55.9% was lost due to passive denitrification. The former value represents the effluent loading, which could be the subject for biological treatment if water reuse is desired.

Data on NO<sub>2</sub><sup>-</sup>-N indicated that this intermediate compound always remained at concentrations lower than 0.3 mg L<sup>-1</sup> in the fish tanks. Its concentration appeared to increase slightly as the water passed through the sedimentation basin, but it decreased again to concentrations lower than those from the fish tanks after contact with the RBC, creating an equilibrium concentration. Because NO<sub>2</sub><sup>-</sup>-N concentrations were in general stable and at levels that were not considered a threat for the fish population, further determinations for NO<sub>2</sub><sup>-</sup>-N were considered extraneous for this study.

#### 4.11. Discussion

The results showed that the vast majority of nitrogen at BRA flows through the PS (i.e., 96.0% of *TNI* and 97.0% of nitrogen assimilated as *N<sub>fish</sub>*). The proportion of *TNI* assimilated by fish in this subsystem (34.4%) indicates excellent utilization of nitrogen for purpose of supporting fish growth relative to rates reported by other authors. For example, Suresh and Kwei Lin (1992) found that less than 20% of nitrogen was utilized by tilapia, using feed with 22% crude protein content and much lower densities of fish than those in the BRA systems. Although they found a decrease of nitrogen assimilation with increasing fish density, they also cited several authors that reported an inverse situation (Refstie, 1977; Rakcoy and Allison, 1981; Vijayan and Leatherland, 1988). By using feed with 34% crude protein content, Siddiqui and Al-Harbi (1999) reported 21.4% nitrogen assimilation by red tilapia. In a pond-based marine system growing *Sparus aurata* and *Mugil sp.*, Krom et al. (1985) found nitrogen assimilation of 20 – 40% from feeds with various nitrogen contents. Porter et al. (1987) reported 30% nitrogen assimilation in flow-through systems growing *Sparus aurata*. The larger percent of nitrogen assimilation found in this study could be due to a better quality of feeds, i.e., to higher protein concentration and better balance of the amino acids. Also, most of the

studies cited reported greater mortalities, which could diminish the total nitrogen accumulated in fish.

The small amounts of nitrogen recovered as TAN and  $\text{NO}_2^-$ -N was likely due to the systems' RBCs, which oxidized them to  $\text{NO}_3^-$ -N. Nevertheless, this is a general characteristic of RASs that include online aerobic biofiltration, which also explains the large amount of nitrogen recovered as  $\text{NO}_3^-$ -N in this study. Most of the nitrogen recovered as  $N_{TON}$  (18.93%) was probably due to feces, taking into account the observation that the feed was consumed by fish almost instantly at distribution, and only dust could escape as wasted feed. Assuming that some of the organic nitrogen bounded in feces dissolved upon contact with water, the results from this study, which took into account the nitrogen from the entire organic pool, are in agreement with those of Porter et al. (1987) who found 10% faecal nitrogen, and Thoman et al. (2001) who recovered 14% nitrogen from the suspended solids.

From the nitrogen lost as  $TNUA$  (14.31%), removal of  $\text{N}_2$  gas through passive denitrification is the most reasonable explanation. Although this may appear surprising (considering the relatively high DO in different units of the RASs), the conditions for denitrification can occur in some sections of the systems. Brandes and Devol (1997) indicated that development of anoxic microsites in the sediment produces likely sites for denitrification in recirculating aquaculture systems. In BRA systems, these microsites could be zones in fish tanks where particles may have accumulated. With greater certainty, these zones arose in the sedimentation basin, where a blanket of sediments developed for 19 – 36 hours before removal. In fact, large amounts of gas were collected from beneath the surface of water in the sedimentation basin surface during short periods of time. Unfortunately, they were contaminated with stripped oxygen from the water and an evaluation of biologically-generated nitrogen production was not possible. Thoman et al. (2001) determined that ammonia volatilization did not represent more than 0.25% of the unaccounted nitrogen, and that the vast majority of the unaccounted nitrogen could be lost by passive denitrification.

For the rest of the subsystems, derivations of nitrogen budgets were more difficult, especially when the fate of unaccounted nitrogen was evaluated. The difficulties were due to special modes of operation and management practices, as discussed above in

Section 4.7. Among the main reasons, large amounts of feeds remained unconsumed in subsystems raising juvenile size-classes. Consequently, large proportions of these feeds settled and sampling was difficult, leaving a significant proportion of nitrogen unaccounted for in the balance. For example, in the HS,  $L_N$  was as high as  $317.8 \text{ g kg}^{-1}$  fish produced. At the opposite extreme was the BS, where measurable  $L_N$  was only  $9.18 \text{ g kg}^{-1}$  fish produced, due to an active nitrogen uptake by algae. Because of these particularities, some assumptions were made based on such observations. The assumptions helped to eliminate the inconvenience of not getting all data from direct measurements, and provided useful for estimating the general nitrogen budget over the entire facility. However, as noted at the beginning of this section, these subsystems together accounted for slightly over 4% of the nitrogen flow, and lack of accuracy did not dramatically affect the overall nitrogen balance.

The production of TAN ( $25.1 \text{ g kg}^{-1}$  to  $40.97 \text{ g kg}^{-1}$  fish produced) generally increased with the size of fish in the subsystem and with the amount of feed consumed per day as a percentage of body weight. Also,  $P_{TAN}$  variation in this study appeared to increase with the feed nitrogen content. As in the case of other parameters,  $P_{TAN}$  determination was distorted for BS, due to exogenous dissolved nitrogen introduced regularly with the replacement water, and to algal uptake of these forms. However, the results were in agreement with those of Wheaton et al. (1988), who reported a  $P_{TAN}$  of  $28.9 \text{ g kg}^{-1}$  fish for striped bass grown to market size on feeds similar to those used at BRA for the PS and BS.

Overall, the nitrogen budgets supplied information that allows a better estimation of nitrogen flow through the subsystems, identifying and quantifying each nitrogen pool throughout the facility. The estimation of nitrogen utilization by fish in this study showed that use of high-energy feeds at BRA is not only an attractive idea, but also worthwhile even when employed with fish having lower requirements for feed quality, such as tilapia. However, there was a feedback, because of the larger amount of nitrogenous compounds produced and released into water through other pools following digestion of these feeds rich in proteins.

By modeling, I determined how much additional biomass the existing RASs could support with respect to TAN, and how these systems would handle the additional

inorganic nitrogenous compounds generated under such conditions. The results indicated that despite the high fish densities practiced at BRA, the systems are not, in general, used at their maximum carrying capacity. Results showed that an average of 73% of the RAS productive potential is utilized in the PS, although the degree of utilization approached 92% in RASs holding fish close to harvest size. Hence, there exists a much lower degree of occupancy in the systems holding fish of smaller size for long periods of time. This inference suggests that by improving management practices (such as better partition of fish biomass among systems and more frequent grading), net production could be increased in existing space. This possibility is supported by the excellent average removal efficiency found for RBCs (54.4%) at a recirculation rate of almost one pass per hour and by an average ACR of  $442.5 \text{ mg TAN m}^{-2} \text{ day}^{-1}$ , which maintains an average TAN of  $2.06 \text{ mg L}^{-1}$  in fish tanks. According to Rogers and Klemeston (1985), up to  $2830 \text{ mg TAN m}^{-2} \text{ day}^{-1}$  could be removed by a RBC used in aquaculture, which indicates that the biofilters could function successfully in conditions of  $3.15 \text{ mg L}^{-1}$  TAN and/or  $634 \text{ mg TAN m}^{-2} \text{ day}^{-1}$  ACR predicted in this study. Additionally, simple calculations indicated that under conditions of returning water treated by a treatment train similar to that described in Chapter 3 (i.e., with  $1.6 \text{ mg L}^{-1}$  TAN), only 0.84% of the  $P_{TAN}$  will be reintroduced to the RASs. Hence, I conclude that this additional loading will be easily removed by the RBCs, without significant increase of TAN parameter throughout the systems. Consequently, the return of this residual TAN should not pose a treat to the fish.

The passive denitrification by which  $\text{NO}_3^- \text{-N}$  was removed from the RASs was found to account for a large proportion of nitrogen removal. Besides the formation of anoxic microsites on the sediment discussed earlier, it appeared that the thick biofilm formed on the tanks' walls also could have contributed to  $\text{NO}_3^- \text{-N}$  removal, providing anoxic microsites. This hypothesis was supported by the case of Tank A12 monitored in this study, where the fish were replaced less than two weeks before the test began. Following the removal of the marketed crop and before introducing the young fish, the system underwent cleaning, which removed the biofilm from the walls. Consequently, the time for regrowth of biofilm on walls at a thickness that could allow denitrification was probably insufficient, because less than 35%  $P_{\text{NO}_3^- \text{-N}}$  was removed by passive denitrification from this system, as compared to the significantly higher proportions in

the other three systems. The phenomenon of in situ denitrification also was reported by other authors. For example, Bovendeur et al. (1987) found that 40 – 80% of TAN oxidized by denitrification then was reduced by denitrification in lamellae separator basin. Thoman et al. (2001) attributed 9 – 21% losses of systems' nitrogen to the same mechanism. The 56% removal of  $\text{NO}_3^-$ -N by passive denitrification found in this study represented an important, positive outcome, because it could reduce by more than half the investments necessary for effluent nitrogen removal should the effluent be treated and reused.

#### 4.12. References

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Table 4.1. Daily nitrogen budget derived for subsystems for the entire Blue Ridge Aquaculture facility (BRA) based on the mass fraction nitrogen composition of all measured budget elements, and percent distribution of  $TNI$  and  $N_{fish}$  (as response) among subsystems.

| N pool (units)            | Subsystem |          |         |                 |                 | Total BRA |
|---------------------------|-----------|----------|---------|-----------------|-----------------|-----------|
|                           | PS        | BS       | HS      | FS <sub>1</sub> | FS <sub>2</sub> |           |
| $TNI$ (kg)                | 341.38100 | 0.33600  | 0.56329 | 3.20989         | 11.72986        | 357.22000 |
| $N_{fish}$ (kg)           | 119.45400 | 0.26700  | 0.04456 | 0.58796         | 2.78940         | 123.14290 |
| $N_{mort}$ (kg)           | 1.03400   | -        | 0.00234 | 0.01818         | 0.00839         | 1.06291   |
| $N_{TAN}$ (kg)            | 5.80900   | 0.00520  | 0.15143 | 0.43072         | 1.27920         | 7.67555   |
| $N_{NO_2}$ (kg)           | 2.19800   | -        | 0.02225 | 0.03738         | 0.04691         | 2.30639   |
| $N_{NO_3}$ (kg)           | 99.43900  | 0.00185  | 0.09496 | 0.28288         | 0.77783         | 100.59470 |
| $N_{TON}$ (kg)            | 64.64500  | 0.06183* | 0.18347 | 1.85277**       | 6.82813**       | 73.57120  |
| $TNUA$ (kg)               | 48.85200  | -        | 0.06428 | -               | -               | 48.91628  |
| $TNI$ /subsystem (%)      | 95.57     | 0.09     | 0.16    | 0.90            | 3.28            | 100.00    |
| $N_{fish}$ /subsystem (%) | 97.00     | 0.22     | 0.04    | 0.48            | 2.27            | 100.00    |

\* Nitrogen assumed to be transferred between subsystems, which was excluded from the BS budget.

\*\* Nitrogen unaccounted for but assumed to belong to  $N_{TON}$  pool, which was included into the respective subsystem's budget when was determined the overall budget.

Table 4.2. Characteristics of the systems selected for RAS design evaluation at BRA.

| Parameter                    | Units             | Tested RAS |         |        |         |         |
|------------------------------|-------------------|------------|---------|--------|---------|---------|
|                              |                   | A12        | A11     | B16    | A18     | Average |
| Water exchange rate          | % volume/day      | 11.5       | 21.3    | 14.8   | 18.4    | 16.5    |
| Flow rate through system (Q) | L/hr              | 1028.0     | 1903.7  | 1322.7 | 1645.8  | 1475.1  |
| Fish size                    | g/fish            | 43         | 192     | 245    | 424     | 226     |
| Fish biomass                 | Kg                | 2381.0     | 10473.0 | 6568.5 | 12002.0 | 7856.1  |
| Feeding rate (FR)            | Kg/day            | 136.0      | 262.0   | 174.0  | 222.3   | 198.6   |
| Feed protein content (FP)    | %                 | 40         | 36      | 36     | 36      | 37      |
| % BW fed                     | Kg feed/Kg fish-d | 5.72       | 2.50    | 2.65   | 1.85    | 3.18    |

Table 4.3. Experimentally determined and predicted parameters showing results of model estimation of maximum system carrying capacity with regard to TAN and RBC design for tested RASs.

| Parameter                                  | Units                    | Tested RAS |         |        |         |         |
|--|--------------------------|------------|---------|--------|---------|---------|
|  |                          | A12        | A11     | B16    | A18     | Average |
| Max. feed rate ( $FR_{maxTAN}$ )           | Kg/day                   | 240.4      | 286.1   | 261.6  | 290.9   | 269.8   |
| Max. system biomass ( $SBM_{maxTAN}$ )     | Kg                       | 4202.5     | 11443.0 | 9871.0 | 15633.0 | 10287.4 |
| Actual BW as % from $SBM_{maxTAN}$         | %                        | 56.66      | 91.52   | 66.54  | 76.77   | 72.87   |
| TAN tank concentration                     | mg/L                     | 1.77       | 2.32    | 2.04   | 2.10    | 2.06    |
| TAN concentr. in RBC influent ( $CTAN_f$ ) | mg/L                     | 1.77       | 2.32    | 2.04   | 2.10    | 2.06    |
| TAN concentr. in RBC effluent ( $CTAN_e$ ) | mg/L                     | 0.84       | 1.01    | 0.99   | 0.90    | 0.94    |
| TAN removal efficiency per pass ( $E_a$ )  | %                        | 52.39      | 56.47   | 51.47  | 57.28   | 54.40   |
| $P_{TAN}$ /Kg feed                         | g                        | 40.6       | 36.7    | 36.7   | 36.7    | 37.7    |
| Daily TAN production ( $P_{TAN}$ )         | g/day                    | 5522.4     | 9626.4  | 6397.9 | 8161.9  | 7427.1  |
| Ammonia loading ( $L_{TAN}$ )              | g/hr                     | 402.19     | 526.87  | 463.28 | 478.50  | 467.71  |
| Ammonia removal rate ( $R_{TAN}$ )         | g/hr                     | 210.75     | 297.50  | 238.46 | 274.11  | 255.20  |
| Areal Conversion Rate ( $ACR$ )            | mg TAN/m <sup>2</sup> -d | 378.4      | 534.2   | 428.2  | 429.2   | 442.5   |
| $ACR$ at $SBW_{maxTAN}$                    | mg TAN/m <sup>2</sup> -d | 667.8      | 583.7   | 643.4  | 641.1   | 634     |
| Mass TAN introduced by exchange            | g/day                    | 39.47      | 73.10   | 50.79  | 90.93   | 63.57   |
| $P_{TAN}$ introduced w/ water exchange     | %/day                    | 0.71       | 0.76    | 0.79   | 1.11    | 0.84    |
| *Total TAN removed by water exchange       | %/day                    | 0.08       | 0.34    | 0.22   | 0.35    | 0.25    |

\*Daily TAN percentage removal by water exchange for the instance that water treated by a strategy similar to that used for the pilot station tested in Chapter 3 would be used for exchange (i.e., with 1.60 mg L<sup>-1</sup> TAN).

Table 4.4.  $P_{TAN}$  removal partitioning (mass and percent) for each studied system.

| System  | <sup>1</sup> $P_{TAN}$ |     | <sup>2</sup> $TAN_{pass + vol}$ |       | <sup>3</sup> $TAN_{RBC\ nitrification}$ |       | <sup>4</sup> $TAN_{exchange}$ |      |
|---------|------------------------|-----|---------------------------------|-------|---|-------|-------------------------------|------|
|         | g                      | %   | g                               | %     | g                                       | %     | g                             | %    |
| A12     | 5522.4                 | 100 | 421.30                          | 7.63  | 5057.41                                 | 91.58 | 43.69                         | 0.79 |
| A11     | 9626.4                 | 100 | 2380.50                         | 24.73 | 7139.90                                 | 74.17 | 106.00                        | 1.10 |
| B16     | 6397.9                 | 100 | 610.22                          | 9.54  | 5722.92                                 | 89.45 | 64.76                         | 1.01 |
| A18     | 8161.9                 | 100 | 1463.66                         | 17.93 | 6578.49                                 | 80.60 | 119.75                        | 1.47 |
| Average | 7427.2                 | 100 | 1108.51                         | 14.93 | 6235.09                                 | 83.95 | 83.55                         | 1.12 |

<sup>1</sup>TAN production over a 24-hour period.

<sup>2</sup>TAN removed by passive nitrification and by ammonia volatilization.

<sup>3</sup>TAN removed by nitrification in RBC.

<sup>4</sup>TAN removed with the exchanged water.

Table 4.5. Water exchange rates,  $P_{NO_3^- - N}$  and  $NO_3^- - N$  mass and concentrations before and after water removal, and balances showing  $P_{NO_3^- - N}$  partitioning (daily mass and percent) for each system.

| Parameter  | Units        | Tested RAS |          |          |          |          |
|--|--------------|------------|----------|----------|----------|----------|
|  |              | A12        | A11      | B16      | A18      | Average  |
| Water exchange rate                                | % volume/day | 11.5       | 21.3     | 14.8     | 18.4     | 16.5     |
| Daily $NO_3^- - N$ production ( $P_{NO_3^- - N}$ ) | g            | 5522.4     | 9626.4   | 6397.9   | 8161.9   | 7427.9   |
| $NO_3^- - N$ conc. before exchange                 | mg/L         | 57.3       | 57.3     | 50.9     | 49.1     | 53.6     |
| System mass $NO_3^- - N$ before exchange           | g            | 12290.85   | 12290.85 | 10918.05 | 10531.95 | 11507.92 |
| $NO_3^- - N$ conc. after exchange                  | mg/L         | 40.5       | 40.1     | 38.9     | 40.0     | 39.9     |
| System mass $NO_3^- - N$ after exchange            | g            | 8687.25    | 8601.45  | 8344.05  | 7872.15  | 8376.22  |
| $NO_3^- - N$ and removed by exchange               | g/day        | 3603.6     | 3689.4   | 2574.0   | 2659.8   | 3132.45  |
| $P_{NO_3^- - N}$ removed by exchange               | %/day        | 65.25      | 38.33    | 40.23    | 32.58    | 44.10    |
| $NO_3^- - N$ lost by passive denitrification       | g/day        | 1918.8     | 5937.0   | 3823.9   | 5502.1   | 4295.45  |
| $P_{NO_3^- - N}$ lost by passive denitrification   | %/day        | 34.75      | 61.67    | 59.77    | 67.42    | 55.90    |

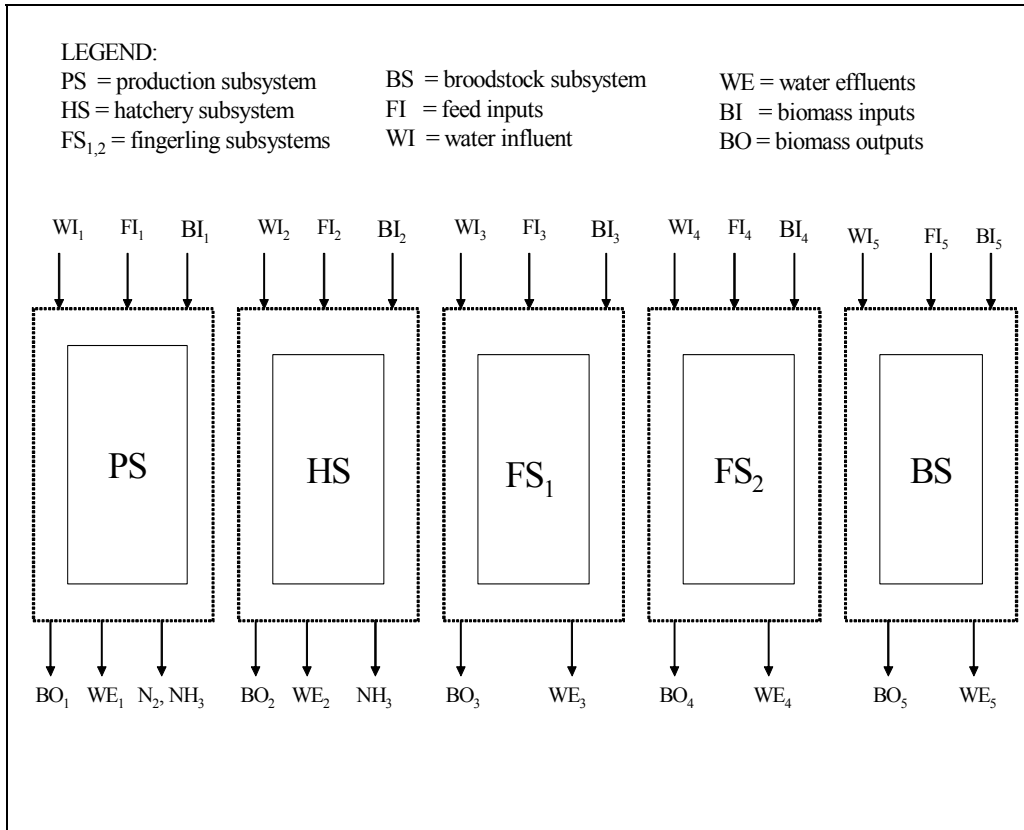


Figure 4.1. Subsystem delimitation, and inputs and outputs for the nitrogen budget at Blue Ridge Aquaculture. (----- denotes subsystem delimitation lines).

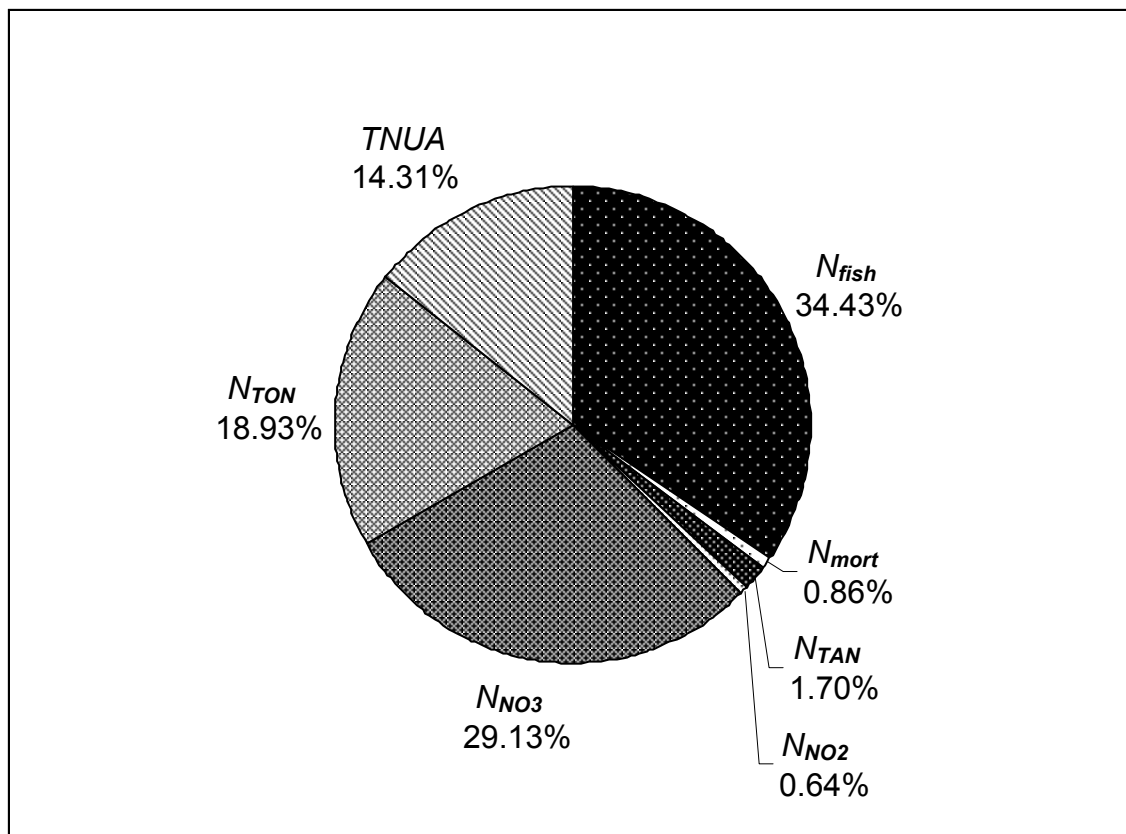


Figure 4.2. Nitrogen mass balance for the production subsystem (PS), based on the mass fraction nitrogen composition of all measured budget elements relative to feed input to the system.

$N_{fish}$  = nitrogen recovered from fish (i.e., nitrogen assimilated);  $N_{mort}$  = nitrogen recovered from dead fish (i.e., nitrogen assimilated by fish, but lost with mortalities);  $N_{TAN}$  = nitrogen recovered as TAN;  $N_{NO_2}$  = nitrogen recovered as  $NO_2^-$ -N;  $N_{NO_3}$  = nitrogen recovered as  $NO_3^-$ -N;  $N_{TON}$  = nitrogen recovered as total organic nitrogen;  $TNUA$  = total nitrogen unaccounted for.

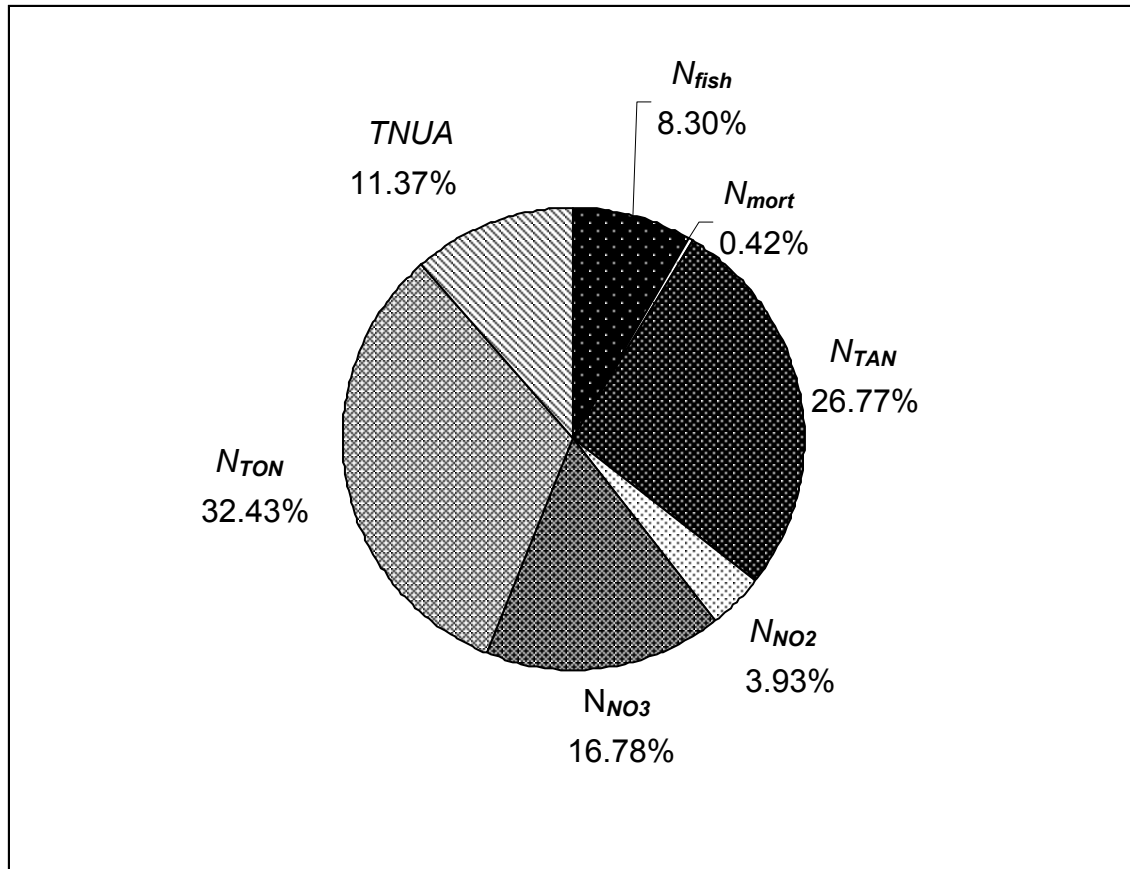


Figure 4.3. Nitrogen mass balance for the hatchery subsystem (HS), based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system.

$N_{fish}$  = nitrogen recovered from fish (i.e., nitrogen assimilated);  $N_{mort}$  = nitrogen recovered from dead fish (i.e., nitrogen assimilated by fish, but lost with mortalities);  $N_{TAN}$  = nitrogen recovered as TAN;  $N_{NO_2}$  = nitrogen recovered as  $NO_2^-$ -N;  $N_{NO_3}$  = nitrogen recovered as  $NO_3^-$ -N;  $N_{TON}$  = nitrogen recovered as total organic nitrogen;  $TNUA$  = total nitrogen unaccounted for.

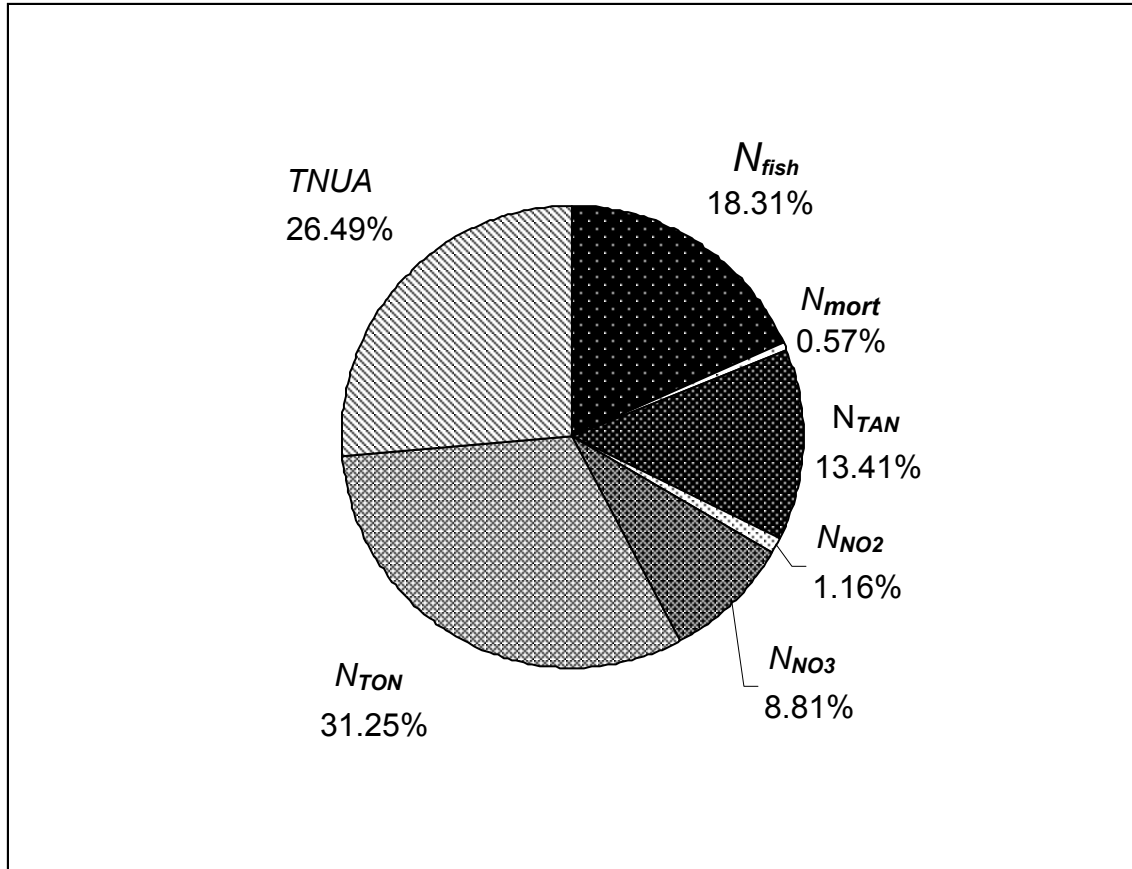


Figure 4.4. Nitrogen mass balance for the first greenhouse subsystem ( $FS_1$ ), based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system.

$N_{fish}$  = nitrogen recovered from fish (i.e., nitrogen assimilated);  $N_{mort}$  = nitrogen recovered from dead fish (i.e., nitrogen assimilated by fish, but lost with mortalities);  $N_{TAN}$  = nitrogen recovered as TAN;  $N_{NO_2}$  = nitrogen recovered as  $NO_2^-$ -N;  $N_{NO_3}$  = nitrogen recovered as  $NO_3^-$ -N;  $N_{TON}$  = nitrogen recovered as total organic nitrogen;  $TNUA$  = total nitrogen unaccounted for.

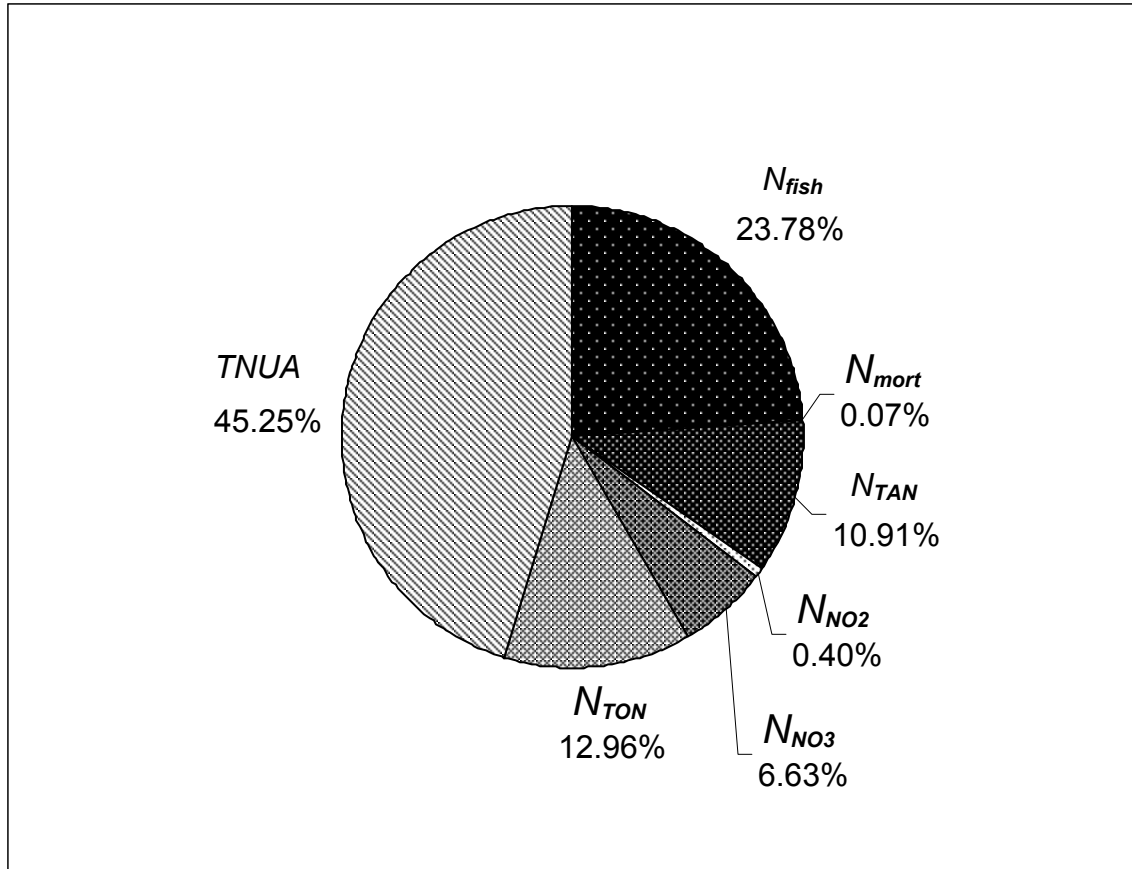


Figure 4.5. Nitrogen mass balance for the second greenhouse subsystem ( $FS_2$ ), based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system.

$N_{fish}$  = nitrogen recovered from fish (i.e., nitrogen assimilated);  $N_{mort}$  = nitrogen recovered from dead fish (i.e., nitrogen assimilated by fish, but lost with mortalities);  $N_{TAN}$  = nitrogen recovered as TAN;  $N_{NO_2}$  = nitrogen recovered as  $NO_2^-$ -N;  $N_{NO_3}$  = nitrogen recovered as  $NO_3^-$ -N;  $N_{TON}$  = nitrogen recovered as total organic nitrogen;  $TNUA$  = total nitrogen unaccounted for.

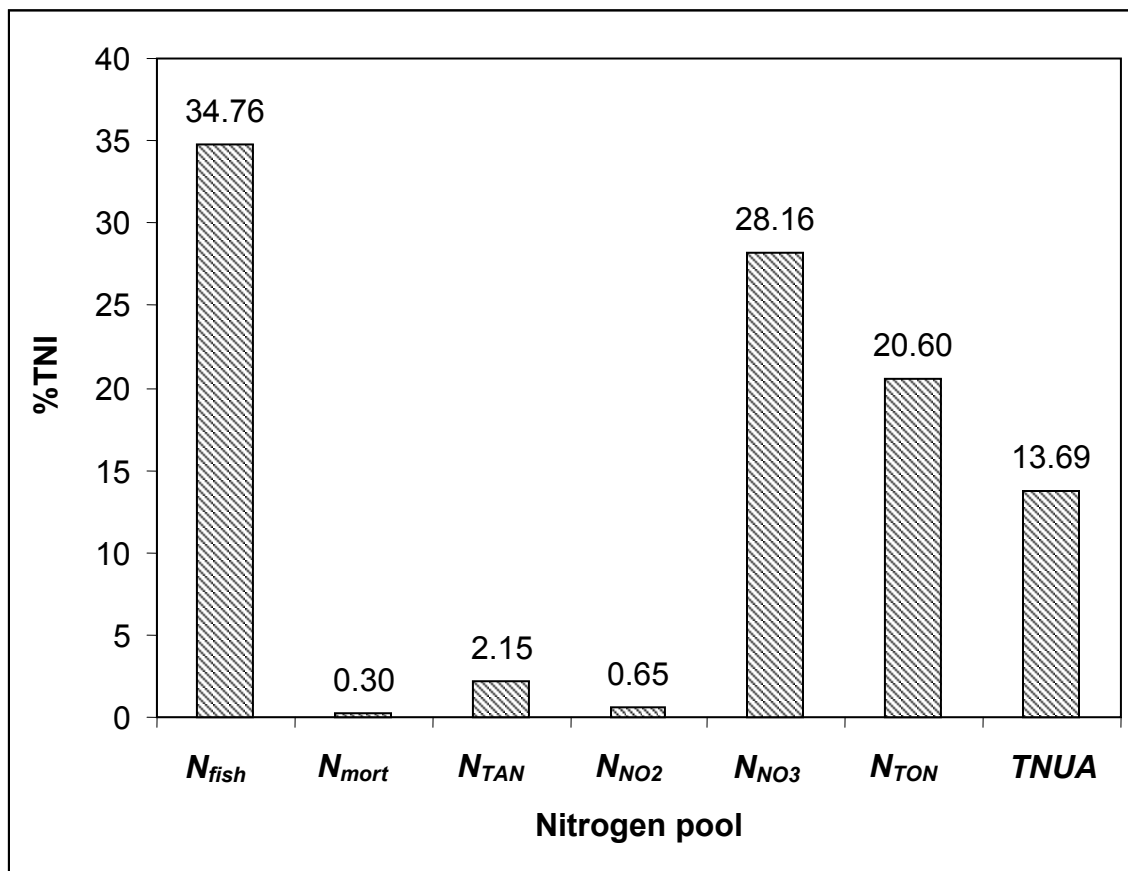


Figure 4.6. Overall nitrogen mass balance for Blue Ridge Aquaculture, based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system.

$N_{fish}$  = nitrogen recovered from fish (i.e., nitrogen assimilated);  $N_{mort}$  = nitrogen recovered from dead fish (i.e., nitrogen assimilated by fish, but lost with mortalities);  $N_{TAN}$  = nitrogen recovered as TAN;  $N_{NO_2}$  = nitrogen recovered as  $NO_2^-$ -N;  $N_{NO_3}$  = nitrogen recovered as  $NO_3^-$ -N;  $N_{TON}$  = nitrogen recovered as total organic nitrogen;  $TNUA$  = total nitrogen unaccounted for.

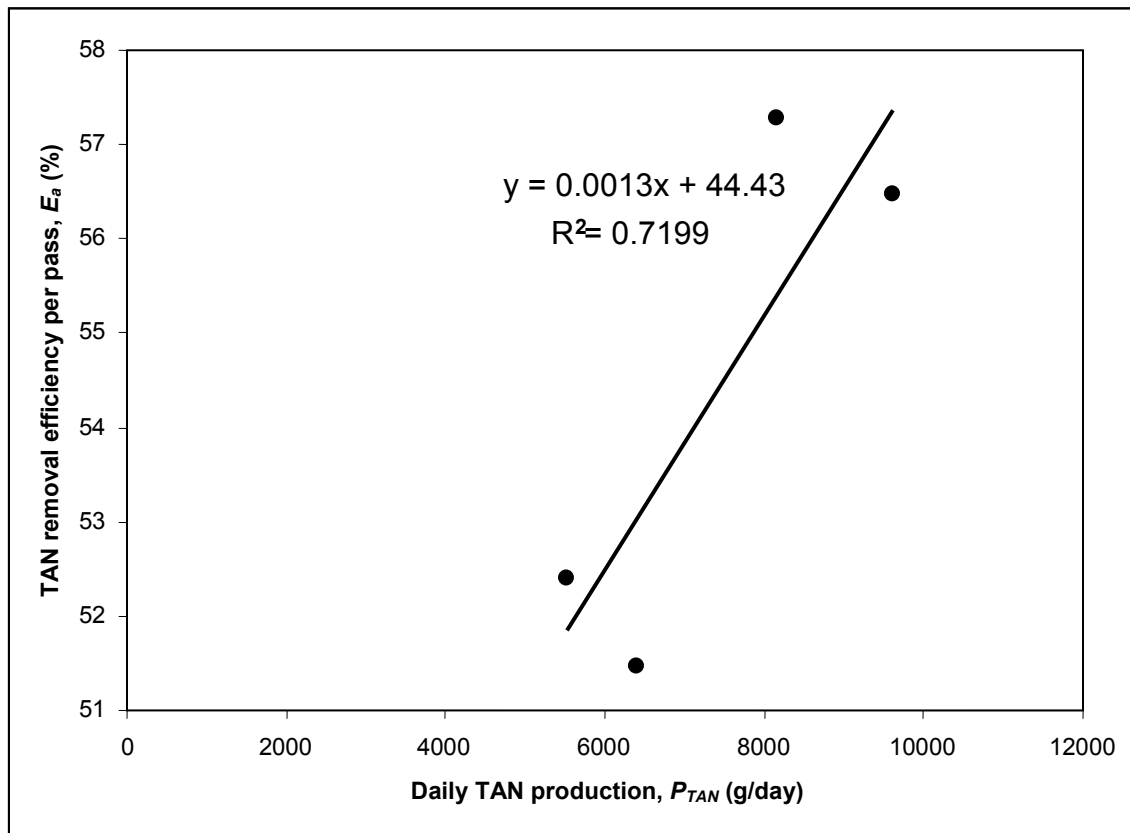


Figure 4.7. Relationship between the daily TAN production ( $P_{TAN}$ ) and TAN removal efficiency per pass ( $E_a$ ) in the four systems tested.

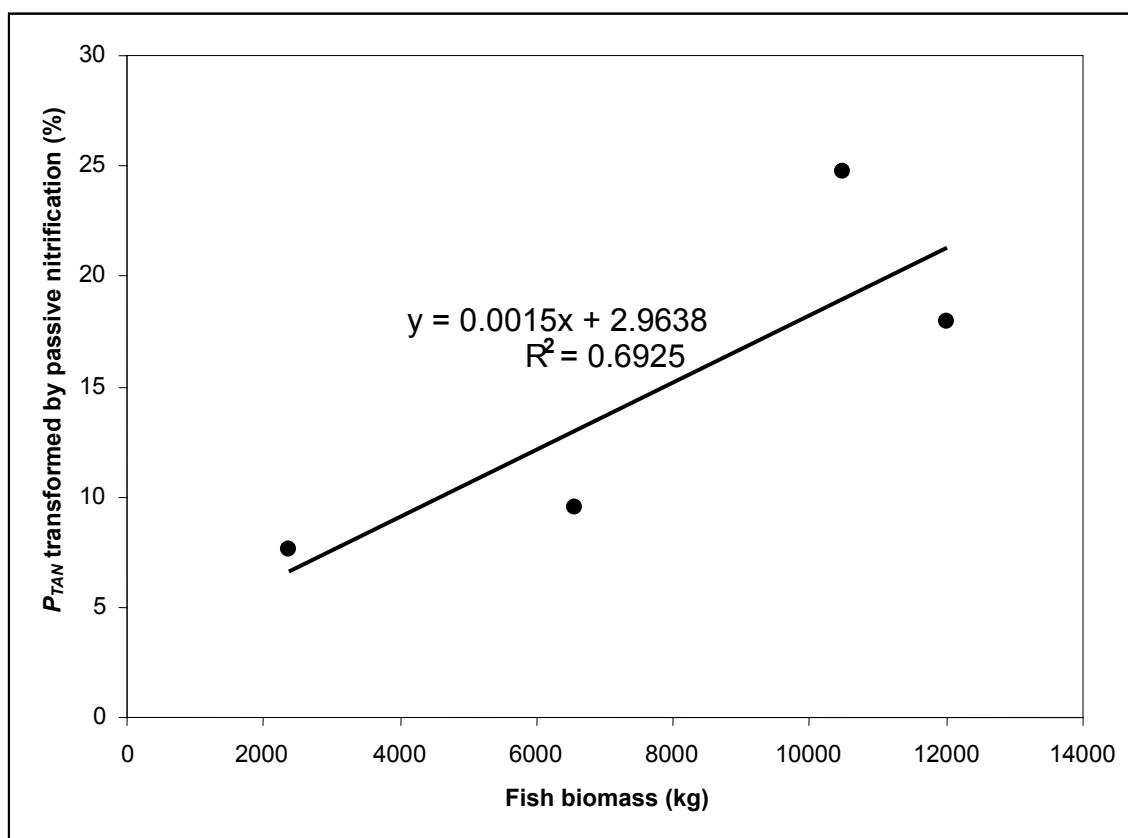


Figure 4.8. Relationship between the fish biomass and percent  $P_{TAN}$  transformed by passive nitrification in the four systems tested.

## Chapter 5. SUMMARY OF FINDINGS AND RECOMMENDATIONS

### 5.1. Ozonation

The ozone treatability study demonstrated the oxidizing power of ozone and the advantages of its application for treatment of BRA's aquaculture wastewater. In this study, data showed that more ozone reacted when the initial load of pollutant was higher. Higher reaction rates under such conditions appeared to be triggered by greater ozone absorption and consumption rates. As reaction progressed, the reaction rate decreased due to pollutant depletion and to formation of compounds more refractory to ozonation. However, results of this study showed a tradeoff: if ozone is injected at a constant dosage, better ozone utilization could be achieved at the expense of incomplete or poor pollutant removal, while a high degree of pollutant removal could be achieved only at the expense of wasting large amounts of ozone. Because an acceptable trade-off between these two outcomes is difficult to achieve in a batch-type reactor at constant ozone feed, especially when a high degree of removal is desired, an alternative solution should be considered. That is, lowering the ozone feed rate  $F(O_3)$ , as pollutant removal progresses. A similar effect (i.e., increasing ozone efficiency) could be achieved in flow-through (i.e., steady state) conditions by increasing the number of stages (e.g., serial reactors). During passage through subsequent ozonation stages, compounds will be partially oxidized, and in the next stage, ozone dosage would be reduced accordingly.

Foam formation and removal contributed greatly to organic pollutant removal. Because of foam-mediated removal, the degree of COD removal  $\eta(\text{COD})$  reached the maximum limit of feasibility (known to be around 70% removal of COD) in less than 30 minutes at a dosage of 6.9 g  $O_3/35$  L wastewater. This meant up to three times faster removal than if all removed organics were mineralized. Accordingly, much less ozone was necessary to achieve this performance. Consequently, the ozone yield coefficient  $Y(O_3/\text{COD}_t)$  was between 0.3 and 2g, lower than the expected  $Y(O_3/\text{COD})$  of 3 g even toward the end of tests after easily-oxidized organics were removed. However, because the ozone reaction and pollutant removal rates decreased significantly after 9 – 15 minutes of ozonation, the treatment should not exceed this interval of time. After the

concentrations of organic pollutants decrease and mostly ozone-resistant molecules remain, the ozone will tend to react with alkalinity ions, because of a higher probability of  $\text{OH}^\circ$  radical formation. Hence, the alkalinity-scavenging effect of ozone is another reason why ozonation is not feasible if applied for long time.

Considering the advantage of removing foam from the ozonation process, I recommend that any treatment strategy that uses ozone should consider this opportunity. Because the reactor used in this study was not specifically designed to remove foam, my inference is that a device with a design facilitating foam collection will further improve removal of organics. Also, it is possible to obtain more concentrated foam condensate, which will require smaller storage/transportation volumes. The high biodegradability of the condensed foam (over 50% of COD) found in this study indicates that foam could eventually be used to obtain volatile fatty acids by fermentation, which will provide savings on methanol. Overall, treatment of BRA effluent benefited from its strong surfactant characteristics when subjected to ozonation, because foam removal increased significantly the proportion of solids and COD removal, and reduced the ozone yield coefficient. The study also indicated a significant reduction of solids, turbidity and TKN following ozonation, an increase in remnant organic biodegradability. Despite partial mineralization, an intense solubilization of organics was recorded, expressed as little or no variation in DOC. This indicated the formation of dissolved, organic byproducts, which were refractory to ozone attack. TAN was also a byproduct of ozonation, increasing by 13.6 – 45.5% during ozonation. These two last observations indicate that ozone is not a solution for removal of all pollutants from BRA effluent, but rather a major contributor to the decontamination process. Hence, ozonation should be accompanied by other methods of treatment (i.e., biological oxidation), if reuse of the waste stream is intended.

## **5.2. Pilot station**

Work with the pilot station showed that the treatment strategy employed could support effective recovery and recycling of BRA effluent, and findings would be useful for design of a scaled-up system. The sequential treatment process indicated that although settleable solids, colloids, dissolved organic substances and nitrogenous compounds could

be eliminated to an acceptable degree, refinements of the methods are still needed, and practically attainable. First, the subsequent treatment units will benefit from better solids separation at the beginning of the treatment train. The experience with the pilot station indicated that it is important to provide effective solids removal by sedimentation, because it will be harder and more expensive to remove solids from subsequent units. That is, despite an overall 99% removal of solids, sedimentation basin (SB) performance of around 70% removal was not considered satisfactory. The improvement of SB removal could be achieved by a better effluent release management (i.e., more uniform volumetric discharge), but also by a more efficient sludge removal mechanism that will prevent solids floatation. Care also should be taken to pass the discharged effluent as few times as possible (preferably none) through high-speed pumps before reaching the sedimentation basin, in order to prevent break-up of solids into colloidal particles. TSS removal of 85% in the SB should be an objective for the full-scale unit, considering current technology used in primary wastewater treatment. Otherwise, for the case of BRA, a higher solids concentration will interfere with the operation of the subsequent units, negatively affecting their performance. For example, high solids concentrations could clog the denitrification (fluidized sand) filters (DR), and could divert ozone from reacting more efficiently with fine colloidal and dissolved organics in the ozonation reactor (OR).

The DR biofilter performed well on  $\text{NO}_3^-$ -N removal (95% removal or higher under conditions of large diurnal fluctuations), but the uncoordinated addition of methanol with the  $\text{NO}_3^-$ -N concentration created conditions for surplus organic loadings on the OR, which affected its performance. This problem could be eliminated by an automated synchronization between the nitrate concentration and the dose of methanol provided to the system. Due to partial recycling of the DR effluent, lower denitrification rates were achieved, resulting in a higher biological yield ( $Y$ ) of solids. In my pilot station, the need for recycling was imposed by the need of having a minimum velocity for sand fluidization. More DRs operated in parallel could decrease the total cross section area, and eliminate the need for recycling. The immediate benefit of such a design would be a decrease in solids production by this process, due to increased  $\text{NO}_3^-$ -N in DR influent (i.e., a concentration closer to saturation). In this study, maximum nitrogen removal performance ( $23.4 \text{ kg NO}_3^-$ -N  $\text{m}^{-3} \text{ day}^{-1}$ ) of the DR was better than what is generally

expected from this technology for domestic wastewater treatment, and the elimination of recycling could make it even higher. Although it was not the subject of this study to determine the causes of such high performance, I hypothesize that the high temperature of operation (29°C) and the generally high concentrations of  $\text{NO}_3^-$ -N contributed to it.

Within the range of doses used in this study (36.6 – 82.5 mg  $\text{O}_3$ /L water), the removal of COD, for example, was correlated with ozone dose. The study also showed that BRA effluent is rich in organics that react easily with ozone, confirming the findings of the ozone treatability study. Nevertheless, foam formation again contributed to greater removal of solids and COD. For example, from a maximum COD removal of 40.7% at 82.5 mg  $\text{O}_3$ /L water during a 9-minute period of ozonation, 64.4% of COD was removed by foam and the balance was mineralized. Under the same conditions, 83.7% of the injected ozone reacted, TSS was reduced by 77.7%, turbidity by 65.0%, TKN by 54.3%, and the water biodegradability index increased by 20.6%. Additionally, a larger dose of ozone showed beneficial effects on the performance of trickling filter (TF) and chemical flocculation (CF) units. However, the potential problems identified in the treatability study were confirmed, such that DOC and TAN increased during ozonation. The study of the ozonation process indicated that the ozone reaction rate (83.7%) was acceptable, and that a dose of 100 mg  $\text{O}_3$ /L water could be employed in a scaled-up system. To overcome the problem of wasting ozone, improvements could be operated by using smaller diffused bubbles that will improve ozone transfer and reaction efficiencies.

The TF was designed for supplemental reduction of dissolved organics and for ammonia removal. Reducing COD by 28.9-34.4%,  $\text{cBOD}_5$  by 30.0-48.6%, and DOC by 26.8-33.9%, it is clear that the ozonation effluent has a good biodegradability, and hence, water quality could be improved further by post-ozonation biological treatment. However, although TAN was reduced by up to 64%, the concentration in the final effluent still remained at an overall average of 1.6 mg/L. Incomplete TAN removal was probably due to a great organic loading on the TF, but also due to high concentrations of TAN in OR effluent. Despite that, the results from the nitrogen budget and RBC evaluation indicated that the return of exchange water containing that TAN will not pose a great problem and will not be a threat to the fish. To improve the biological post-ozonation treatment, a TF should receive an effluent containing a lower load of organics

that will facilitate a better nitrification, or else a different type of biofilter with greater performance in organic/ammonia removal should be employed (e.g., fluidized or bead filters).

The CF was employed in this study as a means to test the suitability of the final effluent to this process. Results indicated that it is capable of greatly improving final effluent quality and should be considered in a full-scaled operational system. The main effect of CF was to reduce turbidity, solids (and organics associated with their volatile fraction), and also had a positive effect on pH, reducing it to around 7.6, suitable for fish.

Despite comprehensive coverage of many water quality parameters in this study, potential problems with the recovered effluent require further investigation. One of these problems address dissolved minerals and salts. The strategy employed in this study was not expected to affect them much, with the exception of ions constituting alkalinity and hardness. Hence, once a system becomes operational, a rapid increase in concentration of some of these compounds should be expected. They enter the system with feed and by specific management practices. Another potential problem could be posed by the accumulation of organics refractory to ozone and with a low biological oxidation rate. The accumulation of each of these pollutants or a combination of them could become toxic to fish. There is little information suggesting maximum allowable concentrations for fish, less for combinations of stressors, and even less specific information for tilapia. Considering this situation, the suggestion would be to control the levels of these constituents by periodically exchanging water to dilute the concentrations experienced by the fish. The volumes required and the frequency of exchanges will be determined by adaptive management once the system becomes operational. Finally, a scaled-up system will produce some concentrated effluents such as sludge from the SB, foam from OR and chemically bonded sludge from CF units. The fate and possible utilization of these residuals also should be considered on the design of a full-scale operating system. The information provided by this study should be useful to the design of a scaled-up system.

### 5.3. Nitrogen budget

The study on the nitrogen budget indicated that the current practice of feeding tilapia at BRA with high protein content feeds is worthwhile, with fish assimilating 34.5% of the nitrogen. Although this also implies higher amounts of ammonia excreted, the existing biofilters appear able to remove it, operating at an average areal conversion rate (ACR) of  $442.5 \text{ mg m}^{-2} \text{ day}^{-1}$ . Additionally, it was determined that if effluent of a quality similar to that obtained from the operation of the pilot station were used as replacement water at exchange, it will bring back into the systems less than 1% of the daily TAN production. This influent should also be removed by the biofilter, even if the systems are operated at their maximum carrying capacity. It was estimated that the current average systems' occupancy is only around 73%. Hence, better management, such as synchronizing shipping with repopulation and frequent grading could increase the production in the existing infrastructure. Of TAN production, 84% was removed by biofilters and the balance by passive nitrification throughout the systems. However,  $\text{NO}_3^-$ -N accumulated more slowly than expected in the systems, because 56% was removed by passive denitrification and only 44% with the water exchange. The determination of  $\text{NO}_3^-$ -N fate represented an important, positive outcome from this study, indicating that the needed investment in treatment could be reduced by more than half with regard to this pollutant, were the effluent be treated and reused.

APPENDIX A. Data tables.

Table A1. Data plotted for Figure 2.3a.

| Time (min) | O <sub>3</sub> inj. (D <sub>6.9 g</sub> ) | O <sub>3</sub> react. (D <sub>6.9 g</sub> ) | O <sub>3</sub> wast. (D <sub>6.9 g</sub> ) | O <sub>3</sub> inj. (D <sub>4.8 g</sub> ) | O <sub>3</sub> react. (D <sub>4.8 g</sub> ) | O <sub>3</sub> wast. (D <sub>4.8 g</sub> ) | O <sub>3</sub> inj. (D <sub>2.4 g</sub> ) | O <sub>3</sub> react. (D <sub>2.4 g</sub> ) | O <sub>3</sub> wast. (D <sub>2.4 g</sub> ) |
|------------|---|---|--|---|---|--|---|---|--|
| 0          | 0.00                                      | 0.00  | 0.00                                       | 0.00                                      | 0.00  | 0.00                                       | 0.00                                      | 0.00  | 0.00                                       |
| 3          | 0.69                                      | 0.68  | 0.01                                       | 0.48                                      | 0.48  | 0.00                                       | 0.24                                      | 0.24  | 0.00                                       |
| 6          | 1.38                                      | 1.35  | 0.03                                       | 0.96                                      | 0.94  | 0.02                                       | 0.48                                      | 0.47  | 0.01                                       |
| 9          | 2.07                                      | 1.96  | 0.11                                       | 1.44                                      | 1.37  | 0.07                                       | 0.72                                      | 0.68  | 0.04                                       |
| 15         | 3.45                                      | 3.04  | 0.41                                       | 2.40                                      | 2.15  | 0.25                                       | 1.20                                      | 1.05  | 0.15                                       |
| 20         | 4.60                                      | 3.78  | 0.82                                       | 3.20                                      | 2.73  | 0.47                                       | 1.60                                      | 1.31  | 0.29                                       |
| 30         | 6.90                                      | 4.57  | 2.33                                       | 4.80                                      | 3.36  | 1.44                                       | 2.40                                      | 1.73  | 0.67                                       |

Table A2. Data plotted for Figure 2.3b.

| Time (min) | O <sub>3</sub> inj. (D <sub>6.9 g</sub> ) | O <sub>3</sub> react. (D <sub>6.9 g</sub> ) | O <sub>3</sub> wast. (D <sub>6.9 g</sub> ) | O <sub>3</sub> inj. (D <sub>4.8 g</sub> ) | O <sub>3</sub> react. (D <sub>4.8 g</sub> ) | O <sub>3</sub> wast. (D <sub>4.8 g</sub> ) | O <sub>3</sub> inj. (D <sub>2.4 g</sub> ) | O <sub>3</sub> react. (D <sub>2.4 g</sub> ) | O <sub>3</sub> wast. (D <sub>2.4 g</sub> ) |
|------------|---|---|--|---|---|--|---|---|--|
| 0          | 0.00                                      | 0.00  | 0.00                                       | 0.00                                      | 0.00  | 0.00                                       | 0.00                                      | 0.00  | 0.00                                       |
| 3          | 0.69                                      | 0.68  | 0.01                                       | 0.48                                      | 0.47  | 0.01                                       | 0.24                                      | 0.24  | 0.00                                       |
| 6          | 1.38                                      | 1.35  | 0.03                                       | 0.96                                      | 0.94  | 0.02                                       | 0.48                                      | 0.47  | 0.01                                       |
| 9          | 2.07                                      | 1.95  | 0.12                                       | 1.44                                      | 1.36  | 0.08                                       | 0.72                                      | 0.69  | 0.03                                       |
| 15         | 3.45                                      | 2.98  | 0.47                                       | 2.40                                      | 2.12  | 0.28                                       | 1.20                                      | 1.11  | 0.09                                       |
| 20         | 4.60                                      | 3.67  | 0.93                                       | 3.20                                      | 2.68  | 0.52                                       | 1.60                                      | 1.44  | 0.16                                       |
| 30         | 6.90                                      | 4.36  | 2.54                                       | 4.80                                      | 3.37  | 1.43                                       | 2.40                                      | 2.04  | 0.36                                       |

Table A3. Data plotted for Figure 2.4a.

| Time (min) | F(O3) D <sub>6.9 g</sub> | r(O3) D <sub>6.9 g</sub> | rA(O3) D <sub>6.9 g</sub> | F(O3) D <sub>4.8 g</sub> | r(O3) D <sub>4.8 g</sub> | rA(O3) D <sub>4.8 g</sub> |
|------------|--------------------------|--------------------------|---------------------------|--------------------------|--------------------------|---------------------------|
| 0          | 0.110                    | -                        | -                         | 0.076                    | -                        | -                         |
| 3          | 0.117                    | 0.116                    | 0.116                     | 0.081                    | 0.080                    | 0.080                     |
| 6          | 0.123                    | 0.121                    | 0.121                     | 0.085                    | 0.084                    | 0.084                     |
| 9          | 0.128                    | 0.118                    | 0.118                     | 0.088                    | 0.082                    | 0.082                     |
| 15         | 0.131                    | 0.109                    | 0.109                     | 0.090                    | 0.076                    | 0.076                     |
| 20         | 0.133                    | 0.093                    | 0.095                     | 0.091                    | 0.070                    | 0.070                     |
| 30         | 0.134                    | 0.063                    | 0.067                     | 0.092                    | 0.050                    | 0.052                     |

| Time (min) | F(O3) D <sub>2.4 g</sub> | r(O3) D <sub>2.4 g</sub> | rA(O3) D <sub>2.4 g</sub> |
|------------|--------------------------|--------------------------|---------------------------|
| 0          | 0.038                    | -                        | -                         |
| 3          | 0.040                    | 0.039                    | 0.039                     |
| 6          | 0.041                    | 0.040                    | 0.040                     |
| 9          | 0.042                    | 0.039                    | 0.039                     |
| 15         | 0.043                    | 0.036                    | 0.036                     |
| 20         | 0.044                    | 0.031                    | 0.032                     |
| 30         | 0.045                    | 0.027                    | 0.027                     |

Table A4. Data plotted for Figure 2.4b.

| Time (min) | F(O3)<br><i>D</i> <sub>6.9 g</sub> | r(O3)<br><i>D</i> <sub>6.9 g</sub> | rA(O3)<br><i>D</i> <sub>6.9 g</sub> | F(O3)<br><i>D</i> <sub>4.8 g</sub> | r(O3)<br><i>D</i> <sub>4.8 g</sub> | rA(O3)<br><i>D</i> <sub>4.8 g</sub> | F(O3)<br><i>D</i> <sub>2.4 g</sub> | r(O3)<br><i>D</i> <sub>2.4 g</sub> | rA(O3)<br><i>D</i> <sub>2.4 g</sub> |
|------------|------------------------------------|------------------------------------|-------------------------------------|------------------------------------|------------------------------------|-------------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| 0          | 0.110                              | -                                  | -                                   | 0.076                              | -                                  | -                                   | 0.038                              | -                                  | -                                   |
| 3          | 0.116                              | 0.114                              | 0.114                               | 0.080                              | 0.079                              | 0.079                               | 0.040                              | 0.040                              | 0.040                               |
| 6          | 0.122                              | 0.119                              | 0.119                               | 0.084                              | 0.082                              | 0.082                               | 0.041                              | 0.041                              | 0.041                               |
| 9          | 0.126                              | 0.115                              | 0.116                               | 0.087                              | 0.080                              | 0.080                               | 0.043                              | 0.040                              | 0.040                               |
| 15         | 0.129                              | 0.102                              | 0.104                               | 0.089                              | 0.074                              | 0.074                               | 0.044                              | 0.039                              | 0.039                               |
| 20         | 0.131                              | 0.084                              | 0.089                               | 0.090                              | 0.066                              | 0.067                               | 0.044                              | 0.038                              | 0.038                               |
| 30         | 0.133                              | 0.057                              | 0.060                               | 0.091                              | 0.050                              | 0.051                               | 0.045                              | 0.035                              | 0.035                               |

Table A5. Data plotted for Figure 2.5.

| Time (min) | $\eta$ (O3)<br><i>D</i> <sub>6.9 g</sub> | $\eta$ (O3)<br><i>D</i> <sub>4.8 g</sub> | $\eta$ (O3)<br><i>D</i> <sub>2.4 g</sub> | $\eta$ (O3)<br><i>D</i> <sub>6.9 g</sub> * | $\eta$ (O3)<br><i>D</i> <sub>4.8 g</sub> * | $\eta$ (O3)<br><i>D</i> <sub>2.4 g</sub> * |
|------------|--|--|--|--|--|--|
| 0          | 100.00                                   | 100.00                                   | 100.00                                   | 100.00                                     | 100.00                                     | 100.00                                     |
| 3          | 98.48                                    | 99.00                                    | 98.50                                    | 98.43                                      | 98.69                                      | 99.50                                      |
| 6          | 98.04                                    | 98.41                                    | 97.06                                    | 97.89                                      | 97.88                                      | 98.56                                      |
| 9          | 92.50                                    | 93.03                                    | 91.81                                    | 91.85                                      | 91.91                                      | 94.25                                      |
| 15         | 83.07                                    | 84.91                                    | 82.44                                    | 80.74                                      | 83.44                                      | 89.06                                      |
| 20         | 71.59                                    | 76.88                                    | 72.06                                    | 67.83                                      | 74.91                                      | 85.06                                      |
| 30         | 49.50                                    | 55.94                                    | 59.88                                    | 45.26                                      | 56.41                                      | 78.44                                      |

Table A6. Data plotted for Figure 2.6.

| Time | COD (mg/L) at<br><i>D</i> <sub>6.9 g</sub> * | COD (mg/L) at<br><i>D</i> <sub>4.8 g</sub> * | COD (mg/L) at<br><i>D</i> <sub>2.4 g</sub> * | DOC (mg/L) at<br><i>D</i> <sub>6.9 g</sub> | DOC (mg/L) at<br><i>D</i> <sub>4.8 g</sub> | DOC (mg/L) at<br><i>D</i> <sub>2.4 g</sub> |
|------|--|--|--|--|--|--|
| 0    | 307  | 307  | 307  | 30   | 30   | 30   |
| 3    | 262  | 272  | 274  | 50   | 42   | 40   |
| 6    | 219  | 232  | 241  | 57   | 57   | 42   |
| 9    | 173  | 197  | 212  | 58   | 51   | 43   |
| 15   | 133  | 165  | 165  | 64   | 53   | 43   |
| 20   | 108  | 135  | 150  | 58   | 55   | 41   |
| 30   | 84   | 109  | 140  | 53   | 48   | 39   |

Table A7. Data plotted for Figure 2.7a.

| Time (min) | $\eta(\text{COD})$ rmvd. $D_{6.9g}$ | $\eta(\text{COD})$ rmvd. $D_{4.8g}$ | $\eta(\text{COD})$ rmvd. $D_{2.4g}$ | $\eta(\text{COD})$ rmvd. $D_{6.9g}^*$ | $\eta(\text{COD})$ rmvd. $D_{4.8g}^*$ | $\eta(\text{COD})$ rmvd. $D_{2.4g}^*$ |
|------------|-------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| 0          | 0.00                                | 0.00                                | 0.00                                | 0.00                                  | 0.00                                  | 0.00                                  |
| 3          | 13.61                               | 12.87                               | 11.74                               | 14.66                                 | 11.40                                 | 10.75                                 |
| 6          | 28.33                               | 25.75                               | 23.47                               | 28.66                                 | 24.43                                 | 21.50                                 |
| 9          | 43.89                               | 37.72                               | 34.27                               | 43.65                                 | 35.83                                 | 30.94                                 |
| 15         | 56.11                               | 47.90                               | 44.60                               | 56.68                                 | 46.25                                 | 43.00                                 |
| 20         | 63.33                               | 54.79                               | 53.99                               | 64.82                                 | 56.03                                 | 51.14                                 |
| 30         | 68.89                               | 60.18                               | 67.61                               | 72.64                                 | 64.50                                 | 54.40                                 |

Table A8. Data plotted for Figure 2.7b.

| Time (min) | $\eta(\text{COD})$ ox. $D_{6.9g}$ | $\eta(\text{COD})$ ox. $D_{4.8g}$ | $\eta(\text{COD})$ ox. $D_{2.4g}$ | $\eta(\text{COD})$ ox. $D_{6.9g}^*$ | $\eta(\text{COD})$ ox. $D_{4.8g}^*$ | $\eta(\text{COD})$ ox. $D_{2.4g}^*$ |
|------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| 0          | 0.00                              | 0.00                              | 0.00                              | 0.00                                | 0.00                                | 0.00                                |
| 3          | 8.33                              | 7.62                              | 4.75                              | 9.19                                | 6.88                                | 4.23                                |
| 6          | 15.87                             | 14.85                             | 9.29                              | 17.75                               | 13.69                               | 8.03                                |
| 9          | 22.17                             | 20.76                             | 12.25                             | 25.24                               | 18.74                               | 11.21                               |
| 15         | 26.94                             | 25.32                             | 15.27                             | 31.02                               | 24.54                               | 16.93                               |
| 20         | 28.78                             | 27.73                             | 17.24                             | 33.49                               | 28.21                               | 18.67                               |
| 30         | 30.38                             | 29.40                             | 19.20                             | 35.28                               | 30.43                               | 20.63                               |

Table A9. Data plotted for Figure 2.8.

| Time (sec) | $r(M)$ $D_{6.9g}$ | $r(M)$ $D_{4.8g}$ | $r(M)$ $D_{2.4g}$ | $r(M)$ $D_{6.9g}^*$ | $r(M)$ $D_{4.8g}^*$ | $r(M)$ $D_{2.4g}^*$ |
|------------|-------------------|-------------------|-------------------|---------------------|---------------------|---------------------|
| 0          | 0.000             | 0.000             | 0.000             | 0.000               | 0.000               | 0.000               |
| 180        | 0.167             | 0.141             | 0.056             | 0.157               | 0.117               | 0.072               |
| 360        | 0.151             | 0.134             | 0.054             | 0.146               | 0.116               | 0.065               |
| 540        | 0.126             | 0.110             | 0.035             | 0.128               | 0.086               | 0.054               |
| 900        | 0.048             | 0.042             | 0.018             | 0.049               | 0.050               | 0.049               |
| 1200       | 0.022             | 0.027             | 0.014             | 0.025               | 0.038               | 0.018               |
| 1800       | 0.010             | 0.009             | 0.007             | 0.009               | 0.011               | 0.010               |

Table A10. Data plotted for Figure 2.9a.

| Time (min) | Y, $D_{6.9g}$ (O3/CODt) | Y, $D_{4.8g}$ (O3/CODt) | Y, $D_{2.4g}$ (O3/CODt) | Y, $D_{6.9g}^*$ (O3/CODt) | Y, $D_{4.8g}^*$ (O3/CODt) | Y, $D_{2.4g}^*$ (O3/CODt) |
|------------|-------------------------|-------------------------|-------------------------|---------------------------|---------------------------|---------------------------|
| 0          | 0.000                   | 0.000                   | 0.000                   | 0.000                     | 0.000                     | 0.000                     |
| 3          | 0.425                   | 0.336                   | 0.283                   | 0.457                     | 0.408                     | 0.217                     |
| 6          | 0.410                   | 0.351                   | 0.288                   | 0.498                     | 0.371                     | 0.222                     |
| 9          | 0.381                   | 0.369                   | 0.303                   | 0.450                     | 0.411                     | 0.249                     |
| 15         | 0.892                   | 0.807                   | 0.582                   | 0.921                     | 0.830                     | 0.381                     |
| 20         | 1.071                   | 0.908                   | 0.472                   | 1.009                     | 0.661                     | 0.453                     |
| 30         | 1.904                   | 1.674                   | 0.550                   | 1.415                     | 1.144                     | 2.084                     |

Table A11. Data plotted for Figure 2.9b.

| Time (min) | Y, $D_{6.9g}$ (O3/CODox) | Y, $D_{4.8g}$ (O3/CODox) | Y, $D_{2.4g}$ (O3/CODox) | Y, $D_{6.9g}^*$ (O3/CODox) | Y, $D_{4.8g}^*$ (O3/CODox) | Y, $D_{2.4g}^*$ (O3/CODox) |
|------------|--------------------------|--------------------------|--------------------------|----------------------------|----------------------------|----------------------------|
| 0          | 0.000                    | 0.000                    | 0.000                    | 0.000                      | 0.000                      | 0.000                      |
| 3          | 0.694                    | 0.569                    | 0.699                    | 0.730                      | 0.676                      | 0.551                      |
| 6          | 0.800                    | 0.625                    | 0.744                    | 0.815                      | 0.709                      | 0.629                      |
| 9          | 0.940                    | 0.747                    | 1.106                    | 0.900                      | 0.928                      | 0.740                      |
| 15         | 2.286                    | 1.800                    | 1.990                    | 2.079                      | 1.490                      | 0.802                      |
| 20         | 4.184                    | 2.600                    | 2.249                    | 3.328                      | 1.759                      | 2.130                      |
| 30         | 6.631                    | 5.397                    | 3.809                    | 6.167                      | 4.375                      | 3.450                      |

Table A12. Data plotted for Figure 2.10a.

| Time (min) | cBOD <sub>5</sub> /COD at $D_{6.9g}$ | cBOD <sub>5</sub> /COD at $D_{4.8g}$ | cBOD <sub>5</sub> /COD at $D_{2.4g}$ |
|------------|--------------------------------------|--------------------------------------|--------------------------------------|
| 0          | 0.278                                | 0.272                                | 0.282                                |
| 3          | 0.305                                | 0.296                                | 0.293                                |
| 6          | 0.337                                | 0.323                                | 0.307                                |
| 9          | 0.356                                | 0.341                                | 0.321                                |
| 15         | 0.367                                | 0.345                                | 0.330                                |
| 20         | 0.379                                | 0.351                                | 0.337                                |
| 30         | 0.393                                | 0.360                                | 0.362                                |

Table A13. Data plotted for Figure 2.10b.

| Time (min) | cBOD <sub>5</sub> /COD at $D_{6.9g}^*$ | cBOD <sub>5</sub> /COD at $D_{4.8g}^*$ | cBOD <sub>5</sub> /COD at $D_{2.4g}^*$ |
|------------|--|--|--|
| 0          | 0.287                                  | 0.287                                  | 0.287                                  |
| 3          | 0.321                                  | 0.313                                  | 0.314                                  |
| 6          | 0.350                                  | 0.341                                  | 0.332                                  |
| 9          | 0.393                                  | 0.355                                  | 0.340                                  |
| 15         | 0.414                                  | 0.388                                  | 0.376                                  |
| 20         | 0.426                                  | 0.393                                  | 0.387                                  |
| 30         | 0.440                                  | 0.422                                  | 0.407                                  |

Table A14. Data plotted for Figure 2.11.

| Time (min) | COD/DOC at $D_{6.9g}$ | COD/DOC at $D_{4.8g}$ | COD/DOC at $D_{2.4g}$ | COD/DOC at $D_{6.9g}^*$ | COD/DOC at $D_{4.8g}^*$ | COD/DOC at $D_{2.4g}^*$ |
|------------|-----------------------|-----------------------|-----------------------|-------------------------|-------------------------|-------------------------|
| 0          | 9.474                 | 9.824                 | 7.889                 | 10.233                  | 10.233                  | 10.233                  |
| 3          | 5.271                 | 5.291                 | 5.371                 | 5.240                   | 6.476                   | 6.850                   |
| 6          | 4.607                 | 4.769                 | 4.405                 | 3.842                   | 4.070                   | 5.738                   |
| 9          | 3.540                 | 3.714                 | 3.500                 | 2.983                   | 3.863                   | 4.930                   |
| 15         | 3.098                 | 3.222                 | 3.278                 | 2.078                   | 3.113                   | 3.837                   |
| 20         | 2.694                 | 2.961                 | 2.450                 | 1.862                   | 2.455                   | 3.659                   |
| 30         | 2.383                 | 3.244                 | 2.156                 | 1.585                   | 2.271                   | 3.590                   |

Table A15. Data plotted for Figure 2.12.

| Time (min) | TSS, $D_{6.9g}$ | TSS, $D_{4.8g}$ | TSS, $D_{2.4g}$ | TSS, $D_{6.9g}^*$ | TSS, $D_{4.8g}^*$ | TSS, $D_{2.4g}^*$ |
|------------|-----------------|-----------------|-----------------|-------------------|-------------------|-------------------|
| 0          | 229             | 210             | 142             | 218               | 218               | 218               |
| 3          | 192             | 176             | 120             | 180               | 189               | 194               |
| 6          | 160             | 151             | 105             | 145               | 150               | 159               |
| 9          | 127             | 129             | 88              | 108               | 112               | 121               |
| 15         | 97              | 104             | 73              | 90                | 95                | 106               |
| 20         | 81              | 86              | 60              | 69                | 78                | 91                |
| 30         | 65              | 70              | 43              | 50                | 65                | 82                |

Table A16. Data plotted for Figure 2.13.

| Time (min) | $D_{6.9g}$ (NTU) | $D_{4.8g}$ (NTU) | $D_{2.4g}$ (NTU) | $D_{6.9g}^*$ (NTU) | $D_{4.8g}^*$ (NTU) | $D_{2.4g}^*$ (NTU) |
|------------|------------------|------------------|------------------|--------------------|--------------------|--------------------|
| 0          | 70.2             | 66.1             | 52.0             | 62.4               | 62.4               | 62.4               |
| 3          | 58.1             | 53.5             | 41.2             | 48.8               | 50.2               | 50.4               |
| 6          | 44.9             | 42.8             | 33.7             | 38.6               | 37.2               | 43.1               |
| 9          | 34.8             | 34.8             | 26.8             | 30.2               | 31.6               | 34.6               |
| 15         | 24.2             | 24.6             | 20.2             | 22.4               | 24.1               | 25.5               |
| 20         | 17.9             | 19.4             | 14.6             | 16.8               | 18.2               | 21.0               |
| 30         | 10.4             | 12.9             | 8.5              | 9.0                | 12.2               | 13.9               |

Table A17. Data plotted in Figure 3.2.

| Sampling points                  | TSS | VSS | TSS SD | VSS SD |
|----------------------------------|-----|-----|--------|--------|
| Raw Influent                     | 682 | 559 | 118.2  | 96.7   |
| Sedimentation Basin Effluent     | 180 | 152 | 18.1   | 15.1   |
| Microscreen Effluent             | 124 | 103 | 21.9   | 19.8   |
| Denitrification Reactor Influent | 143 | 121 | 20.3   | 17.5   |
| Ozonation Reactor Influent       | 146 | 125 | 18.1   | 16.7   |
| Ozonation Reactor effluent       | 37  | 31  | 4.0    | 2.6    |
| Trickling Filter Influent        | 33  | 28  | 5.0    | 3.8    |
| Trickling Filter Effluent        | 33  | 27  | 3.7    | 2.9    |
| Chemical Flocculation Effluent   | 5   | 4   | 0.8    | 0.8    |

Table A18. Data plotted in Figure 3.4.

| Sampling points                  | NTU <sup>1</sup> | NTU <sup>2</sup> | SD <sup>1</sup> | SD <sup>2</sup> |
|----------------------------------|------------------|------------------|-----------------|-----------------|
| Raw Influent                     | 43               | 135              | 4.5             | 16.3            |
| Sedimentation Basin Effluent     | 29               | 72               | 2.2             | 13.3            |
| Microscreen Effluent             | 31               | 57               | 2.4             | 10.6            |
| Denitrification Reactor Influent | 25               | 62               | 4.4             | 11.0            |
| Ozonation Reactor Influent       | 35               | 63               | 3.9             | 25.2            |
| Ozonation Reactor effluent       | 12               | 17               | 0.8             | 1.8             |
| Condensed Foam effluent          | 175              | 148              | 12.8            | 17.7            |
| Trickling Filter Influent        | 11               | 17               | 0.5             | 4.0             |
| Trickling Filter Effluent        | 11               | 16               | 0.5             | 7.1             |
| Chemical Flocculation Effluent   | 2                | 4                | 0.1             | 1.2             |

<sup>1</sup> Indicates samples collected at 2:00 p.m.

<sup>2</sup> Indicates sample collected at 10:00 p.m. and 6:00 a.m.

Table A19. Data plotted in Figure 3.6.

| Sampling points                  | COD | DOC | COD SD | DOC SD |
|----------------------------------|-----|-----|--------|--------|
| Raw Influent                     | 734 | 11  | 160.8  | 0.0    |
| Sedimentation Basin Effluent     | 262 | 28  | 24.6   | 3.9    |
| Microscreen Effluent             | 217 | 0   | 22.3   | 0.0    |
| Denitrification Reactor Influent | 308 | 65  | 24.9   | 7.2    |
| Ozonation Reactor Influent       | 232 | 27  | 11.4   | 4.6    |
| Ozonation Reactor effluent       | 140 | 35  | 12.0   | 3.5    |
| Trickling Filter Influent        | 123 | 33  | 8.5    | 2.6    |
| Trickling Filter Effluent        | 90  | 24  | 6.8    | 2.5    |
| Chemical Flocculation Effluent   | 48  | 22  | 4.4    | 2.0    |

Table 20. Data plotted in Figure 3.9.

| Sampling points                  | COD | BOD <sub>5</sub> | DOC | COD SD | BOD5 SD | DOC SD |
|----------------------------------|-----|------------------|-----|--------|---------|--------|
| Raw Influent                     | 266 | 76               | 28  | 14.8   | 3.5     | 0.0    |
| Sedimentation Basin Effluent     | 208 | 59               | 25  | 14.1   | 5.7     | 2.3    |
| Microscreen Effluent             | 303 | 127              | 49  | 29.0   | 13.4    | 3.3    |
| Denitrification Reactor Influent | 231 | 59               | 20  | 31.8   | 7.8     | 0.6    |
| Ozonation Reactor Influent       | 135 | 43               | 36  | 32.5   | 7.8     | 2.2    |
| Ozonation Reactor effluent       | 970 | 459              | 91  | 119.5  | 62.9    | 6.3    |
| Trickling Filter Influent        | 129 | 42               | 36  | 31.8   | 8.5     | 1.9    |
| Trickling Filter Effluent        | 88  | 22               | 27  | 19.8   | 3.5     | 3.0    |
| Chemical Flocculation Effluent   | 43  | 9                | 24  | 4.9    | 1.4     | 2.1    |

Table A21. Data plotted in Figure 3.10.

| Stage | O3 dose | % DOC decrease |
|-------|---------|----------------|
| 1     | 36.6    | 19.1           |
| 2     | 55.0    | 36.0           |
| 3     | 82.5    | 30.1           |
| 4     | 55.0    | 37.7           |

Table A22. Data plotted in Figure 3.12.

| Stage/<br>Sample | NO <sub>3</sub> <sup>-</sup> -N<br>DR<br>influent | NO <sub>3</sub> <sup>-</sup> -N<br>Final<br>effluent | NO <sub>3</sub> <sup>-</sup> -N<br>DR<br>effluent | Stage/<br>Sample | NO <sub>3</sub> <sup>-</sup> -N<br>DR<br>influent | NO <sub>3</sub> <sup>-</sup> -N<br>Final<br>effluent | NO <sub>3</sub> <sup>-</sup> -N<br>DR<br>effluent |
|------------------|---|--|---|------------------|---|--|---|
| 1/1              | 13.4  | 4.3  | 0.0   | 3/1              | 13.4  | 1.4  | 0.0   |
| 1/2              | 62.4  | 3.6  | 2.0   | 3/2              | 62.4  | 2.8  | 0.0   |
| 1/3              | 44.0  | 2.5  | 1.0   | 3/3              | 44.0  | 1.4  | 0.8   |
| 1/1              | 11.0  | 3.0  | 0.2   | 3/1              | 11.0  | 2.5  | 0.0   |
| 1/2              | 59.5  | 2.7  | 1.0   | 3/2              | 59.5  | 2.1  | 1.6   |
| 1/3              | 44.6  | 1.9  | 0.1   | 3/3              | 44.6  | 3.1  | 1.5   |
| 1/1              | 15.5  | 1.1  | 0.0   | 3/1              | 15.5  | 1.9  | 0.0   |
| 1/2              | 48.5  | 1.8  | 0.6   | 3/2              | 48.5  | 1.2  | 1.2   |
| 1/3              | 55.0  | 2.6  | 0.5   | 3/3              | 55.0  | 1.3  | 0.8   |
| 2/1              | 14.0  | 0.8  | 0.0   | 4/1              | 14.0  | 1.8  | 0.2   |
| 2/2              | 52.4  | 3.0  | 0.4   | 4/2              | 52.4  | 1.9  | 1.0   |
| 2/3              | 51.6  | 6.0  | 0.0   | 4/3              | 51.6  | 3.2  | 2.1   |
| 2/1              | 6.8   | 1.6  | 0.0   | 4/1              | 6.8   | 2.2  | 0.0   |
| 2/2              | 53.6  | 1.6  | 0.4   | 4/2              | 53.6  | 2.8  | 1.2   |
| 2/3              | 40.0  | 1.0  | 0.1   | 4/3              | 40.0  | 3.1  | 0.2   |
| 2/1              | 11.4  | 3.6  | 0.0   | 4/1              | 11.4  | 3.1  | 0.1   |
| 2/2              | 42.4  | 2.8  | 0.8   | 4/2              | 42.4  | 0.6  | 1.0   |
| 2/3              | 48.7  | 2.8  | 1.1   | 4/3              | 48.7  | 3.6  | 0.6   |

Table A23. Data plotted for Figure 4.2.

| Budget element          | % <i>TNI</i> |
|-------------------------|--------------|
| <i>N<sub>fish</sub></i> | 34.43        |
| <i>N<sub>mort</sub></i> | 0.86         |
| <i>N<sub>TAN</sub></i>  | 1.70         |
| <i>N<sub>NO2</sub></i>  | 0.64         |
| <i>N<sub>NO3</sub></i>  | 29.13        |
| <i>N<sub>TON</sub></i>  | 18.93        |
| <i>TNUA</i>             | 14.31        |

Table A24. Data plotted for Figure 4.3.

| Budget element          | % <i>TNI</i> |
|-------------------------|--------------|
| <i>N<sub>fish</sub></i> | 8.33         |
| <i>N<sub>mort</sub></i> | 0.42         |
| <i>N<sub>TAN</sub></i>  | 26.88        |
| <i>N<sub>NO2</sub></i>  | 3.95         |
| <i>N<sub>NO3</sub></i>  | 16.85        |
| <i>N<sub>TON</sub></i>  | 32.57        |
| <i>TNUA</i>             | 11.42        |

Table A25. Data plotted for Figure 4.4.

| Budget element | % <i>TNI</i> |
|----------------|--------------|
| $N_{fish}$     | 18.31        |
| $N_{mort}$     | 0.57         |
| $N_{TAN}$      | 13.41        |
| $N_{NO2}$      | 1.16         |
| $N_{NO3}$      | 8.81         |
| $N_{TON}$      | 31.25        |
| <i>TNUA</i>    | 26.49        |

Table A26. Data plotted for Figure 4.5.

| Budget element | % <i>TNI</i> |
|----------------|--------------|
| $N_{fish}$     | 23.78        |
| $N_{mort}$     | 0.07         |
| $N_{TAN}$      | 10.91        |
| $N_{NO2}$      | 0.4          |
| $N_{NO3}$      | 6.63         |
| $N_{TON}$      | 12.96        |
| <i>TNUA</i>    | 45.25        |

Table A27. Data plotted for Figure 4.6.

| Budget element | % <i>TNI</i> |
|----------------|--------------|
| $N_{fish}$     | 34.47        |
| $N_{mort}$     | 0.30         |
| $N_{TAN}$      | 2.15         |
| $N_{NO2}$      | 0.65         |
| $N_{NO3}$      | 28.16        |
| $N_{TON}$      | 20.60        |
| <i>TNUA</i>    | 13.69        |

Table A28. Data plotted in Figure 4.7.

| Tank | $P_{TAN}$ | $E_a$ (%) |
|------|-----------|-----------|
| A12  | 5522.4    | 52.39     |
| A11  | 9626.4    | 56.47     |
| B16  | 6397.9    | 51.47     |
| A18  | 8161.9    | 57.28     |

Table A29. Data plotted in Figure 4.8.

| Tank | Fish biomass (Kg) | PTAN removed by passive nitrification (%) |
|------|-------------------|---|
| A12  | 2381.0            | 7.63                                      |
| A11  | 10473.0           | 24.73                                     |
| B16  | 6568.5            | 9.54                                      |
| A18  | 12002.0           | 17.93                                     |

Table A30. Raw data for parameters determined during studies on ozone treatability.

| Trial 1, Test 1               |            |            |                          |            |            |                 |
|-------------------------------|------------|------------|--------------------------|------------|------------|-----------------|
| Dosage (g/Σ time)             | Time (min) | Parameter  |                          |            |            |                 |
|                               |            | COD (mg/L) | cBOD <sub>5</sub> (mg/L) | DOC (mg/L) | TKN (mg/L) | Turbidity (NTU) |
| 0.00                          | 0          | 360±12     | 100±8                    | 38±3       | 31.9±2.2   | 70.2±2.5        |
| 0.69                          | 3          | 311±14     | 95±4                     | 59±3       | 22.2±1.6   | 58.1±2.0        |
| 1.38                          | 6          | 258±8      | 87±6                     | 56±4       | 17.3±1.2   | 44.9±2.1        |
| 2.07                          | 9          | 202±10     | 72±5                     | 57±2       | 12.5±1.0   | 34.8±1.5        |
| 3.45                          | 15         | 158±8      | 58±4                     | 51±2       | 7.3±0.6    | 24.2±1.2        |
| 4.60                          | 20         | 132±5      | 50±4                     | 49±3       | 4.7±0.8    | 17.9±1.0        |
| 6.90                          | 30         | 112±4      | 44±2                     | 47±2       | 2.9±0.5    | 10.4±0.6        |
| Total % rmvd. (-) / prod. (+) |            | -68.89     | -56.00                   | 23.68      | -90.91     | -85.19          |
| Trial 1, Test 2               |            |            |                          |            |            |                 |
| Dosage (g/Σ time)             | Time (min) | Parameter  |                          |            |            |                 |
|                               |            | COD (mg/L) | cBOD <sub>5</sub> (mg/L) | DOC (mg/L) | TKN (mg/L) | Turbidity (NTU) |
| 0.00                          | 0          | 334±6      | 91±6                     | 34±3       | 26.8±1.5   | 66.1±2.0        |
| 0.48                          | 3          | 291±10     | 86±5                     | 55±3       | 19.1±1.8   | 53.5±2.1        |
| 0.96                          | 6          | 248±9      | 80±5                     | 52±2       | 14.3±0.6   | 42.8±1.6        |
| 1.44                          | 9          | 208±6      | 71±2                     | 56±1       | 11.3±0.8   | 34.8±0.6        |
| 2.40                          | 15         | 174±4      | 60±5                     | 54±2       | 8.5±0.5    | 24.6±1.2        |
| 3.20                          | 20         | 151±5      | 53±4                     | 51±2       | 6.9±0.0    | 19.4±0.2        |
| 4.80                          | 30         | 133±6      | 48±4                     | 41±1       | 5.4±1.0    | 12.9±0.6        |
| Total % rmvd. (-) / prod. (+) |            | -60.18     | -47.25                   | 20.59      | -79.85     | -80.48          |
| Trial 1, Test 2               |            |            |                          |            |            |                 |
| Dosage (g/Σ time)             | Time (min) | Parameter  |                          |            |            |                 |
|                               |            | COD (mg/L) | cBOD <sub>5</sub> (mg/L) | DOC (mg/L) | TKN (mg/L) | Turbidity (NTU) |
| 0.00                          | 0          | 213±6      | 60±5                     | 27±2       | 19.9±1.6   | 52.0±1.8        |
| 0.24                          | 3          | 188±8      | 55±2                     | 35±2       | 14.2±1.1   | 41.2±1.2        |
| 0.48                          | 6          | 163±5      | 50±3                     | 37±3       | 10.6±0.8   | 33.7±1.4        |
| ±0.72                         | 9          | 140±6      | 45±3                     | 40±1       | 8.2±0.2    | 26.8±1.0        |
| 1.20                          | 15         | 118±4      | 39±2                     | 36±1       | 5.7±0.6    | 20.2±1.0        |
| 1.60                          | 20         | 98±4       | 33±3                     | 40±2       | 4.1±0.5    | 14.6±0.5        |
| 2.40                          | 30         | 69±4       | 25±2                     | 32±1       | 3.1±0.4    | 8.5±0.3         |
| Total % rmvd. (-) / prod. (+) |            | -67.61     | -58.33                   | 18.52      | -84.42     | -83.65          |

Table A30 continued.

| Trial 2, Test 1               |               |               |                             |               |               |                    |
|-------------------------------|---------------|---------------|-----------------------------|---------------|---------------|--------------------|
| Dosage<br>(g/ $\Sigma$ time)  | Time<br>(min) | Parameter     |                             |               |               |                    |
|                               |               | COD<br>(mg/L) | cBOD <sub>5</sub><br>(mg/L) | DOC<br>(mg/L) | TKN<br>(mg/L) | Turbidity<br>(NTU) |
| 0.00                          | 0             | 307±11        | 88±6                        | 30±2          | 25.7±1.3      | 62.4±1.0           |
| 0.69                          | 3             | 262±5         | 84±2                        | 50±2          | 18.6±1.0      | 48.8±1.7           |
| 1.38                          | 6             | 219±8         | 77±4                        | 57±3          | 13.4±1.1      | 38.6±1.1           |
| 2.07                          | 9             | 173±2         | 68±1                        | 58±1          | 9.8±0.2       | 30.2±0.6           |
| 3.45                          | 15            | 133±6         | 55±2                        | 64±0          | 6.1±0.5       | 22.4±0.2           |
| 4.60                          | 20            | 108±6         | 46±4                        | 58±2          | 2.4±0.1       | 16.8±0.4           |
| 6.90                          | 30            | 84±2          | 37±2                        | 53±1          | 1.6±0.4       | 9.0±0.1            |
| Total % rmvd. (-) / prod. (+) |               | -72.64        | -57.95                      | 76.67         | -93.77        | -85.58             |
| Trial 2, Test 2               |               |               |                             |               |               |                    |
| Dosage<br>(g/ $\Sigma$ time)  | Time<br>(min) | Parameter     |                             |               |               |                    |
|                               |               | COD<br>(mg/L) | cBOD <sub>5</sub><br>(mg/L) | DOC<br>(mg/L) | TKN<br>(mg/L) | Turbidity<br>(NTU) |
| 0.00                          | 0             | 307±11        | 88±6                        | 30±2          | 25.7±1.3      | 62.4±1.0           |
| 0.48                          | 3             | 272±10        | 85±4                        | 42±3          | 20.0±1.4      | 50.2±2.0           |
| 0.96                          | 6             | 232±6         | 79±2                        | 57±2          | 15.4±1.2      | 37.2±1.2           |
| 1.44                          | 9             | 197±8         | 70±1                        | 51±1          | 11.6±1.3      | 31.6±1.3           |
| 2.40                          | 15            | 165±8         | 61±3                        | 53±2          | 8.5±0.6       | 24.1±0.4           |
| 3.20                          | 20            | 135±2         | 53±1                        | 55±1          | 6.0±0.4       | 18.2±0.3           |
| 4.80                          | 30            | 109±4         | 46±2                        | 48±0          | 4.8±0.2       | 12.2±0.4           |
| Total % rmvd. (-) / prod. (+) |               | -64.50        | -47.73                      | 60.00         | -81.32        | -80.45             |
| Trial 2, Test 3               |               |               |                             |               |               |                    |
| Dosage<br>(g/ $\Sigma$ time)  | Time<br>(min) | Parameter     |                             |               |               |                    |
|                               |               | COD<br>(mg/L) | cBOD <sub>5</sub><br>(mg/L) | DOC<br>(mg/L) | TKN<br>(mg/L) | Turbidity<br>(NTU) |
| 0.00                          | 0             | 307±11        | 88±6                        | 30±1          | 25.7±1.3      | 62.4±1.0           |
| 0.24                          | 3             | 274±6         | 86±5                        | 40±2          | 20.9±0.9      | 50.4±1.6           |
| 0.48                          | 6             | 241±5         | 80±1                        | 42±0          | 16.5±1.4      | 43.1±1.2           |
| 0.72                          | 9             | 212±9         | 72±4                        | 43±1          | 13.1±1.0      | 34.6±0.6           |
| 1.20                          | 15            | 165±4         | 62±2                        | 43±1          | 10.8±0.4      | 25.5±0.8           |
| 1.60                          | 20            | 150±6         | 58±0                        | 41±2          | 8.7±0.6       | 21.0±0.2           |
| 2.40                          | 30            | 140±4         | 57±1                        | 39±1          | 7.2±0.6       | 13.9±0.3           |
| Total % rmvd. (-) / prod. (+) |               | -54.40        | -35.23                      | 30.00         | -71.98        | -77.72             |

Table A 30 continued.

| Trial 1, Test 1             |               |                  |           |                                 |                                 |            |          |
|-----------------------------|---------------|------------------|-----------|---------------------------------|---------------------------------|------------|----------|
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.34±0.01        | 3.12±0.10 | 1.04±0.04                       | 48.5±1.5                        | 114±4      | 146±4    |
| 0.69                        | 3             | 7.31±0.06        | 3.76±0.11 | 0.12±0.04                       | 50.0±1.2                        | 110±2      | 140±2    |
| 1.38                        | 6             | 7.25±0.02        | 4.10±0.06 | 0.00                            | 50.4±1.4                        | 108±4      | 133±4    |
| 2.07                        | 9             | 7.22±0.01        | 4.64±0.08 | 0.00                            | 50.4±0.6                        | 105±2      | 126±3    |
| 3.45                        | 15            | 7.17±0.03        | 4.88±0.05 | 0.00                            | 51.6±0.4                        | 97±3       | 121±3    |
| 4.60                        | 20            | 7.14±0.01        | 5.15±0.12 | 0.00                            | 54.0±0.3                        | 88±4       | 116±2    |
| 6.90                        | 30            | 7.11±0.04        | 5.61±0.04 | 0.00                            | 55.1±0.5                        | 80±2       | 110±0    |
| Total % rmvd. (-)/prod. (+) |               | -3.13            | 79.81     | -100.00                         | 13.61                           | -29.82     | -24.66   |
| Trial 1, Test 2             |               |                  |           |                                 |                                 |            |          |
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.30±0.05        | 2.80±0.07 | 0.83±0.04                       | 39.8±1.0                        | 122±2      | 142±4    |
| 0.48                        | 3             | 7.27±0.09        | 3.10±0.01 | 0.22±0.06                       | 40.0±0.4                        | 121±1      | 136±3    |
| 0.96                        | 6             | 7.24±0.04        | 3.36±0.02 | 0.00                            | 40.5±1.1                        | 118±0      | 130±4    |
| 1.44                        | 9             | 7.19±0.08        | 3.50±0.05 | 0.00                            | 41.7±1.0                        | 116±2      | 124±2    |
| 2.40                        | 15            | 7.15±0.04        | 3.68±0.04 | 0.00                            | 43.0±0.5                        | 111±3      | 120±1    |
| 3.20                        | 20            | 7.14±0.06        | 3.84±0.02 | 0.00                            | 43.9±0.2                        | 105±3      | 117±2    |
| 4.80                        | 30            | 7.10±0.02        | 4.15±0.04 | 0.00                            | 44.5±0.8                        | 99±2       | 114±2    |
| Total % rmvd. (-)/prod. (+) |               | -2.74            | 48.21     | -100.00                         | 11.81                           | -18.85     | -19.72   |
| Trial 1, Test 3             |               |                  |           |                                 |                                 |            |          |
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.12±0.05        | 3.68±0.02 | 1.20±0.02                       | 22.0±0.6                        | 106±2      | 136±2    |
| 0.24                        | 3             | 7.11±0.01        | 3.76±0.02 | 0.45±0.02                       | 22.8±0.8                        | 106±3      | 133±3    |
| 0.48                        | 6             | 7.08±0.06        | 3.86±0.01 | 0.10                            | 23.6±1.0                        | 104±1      | 129±2    |
| 0.72                        | 9             | 7.06±0.08        | 3.94±0.04 | 0.00                            | 24.6±0.6                        | 102±2      | 126±4    |
| 1.20                        | 15            | 7.06±0.02        | 4.06±0.02 | 0.00                            | 25.0±0.9                        | 97±2       | 122±3    |
| 1.60                        | 20            | 7.03±0.04        | 4.14±0.03 | 0.00                            | 25.7±0.4                        | 93±1       | 120±3    |
| 2.40                        | 30            | 7.04±0.02        | 4.26±0.03 | 0.00                            | 26.4±0.5                        | 89±2       | 117±3    |
| Total % rmvd. (-)/prod. (+) |               | -1.12            | 15.76     | -100.00                         | 20.00                           | -16.04     | -13.97   |

Table A 30 continued.

| Trial 2, Test 1             |               |                  |           |                                 |                                 |            |          |
|-----------------------------|---------------|------------------|-----------|---------------------------------|---------------------------------|------------|----------|
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.40±0.04        | 3.16±0.09 | 0.90±0.02                       | 54.2±1.1                        | 118±3      | 140±3    |
| 0.69                        | 3             | 7.36±0.04        | 3.52±0.05 | 0.15±0.00                       | 55.4±1.0                        | 117±4      | 134±3    |
| 1.38                        | 6             | 7.31±0.05        | 3.90±0.03 | 0.00                            | 56.2±0.9                        | 115±2      | 128±2    |
| 2.07                        | 9             | 7.26±0.05        | 4.48±0.06 | 0.00                            | 57.3±0.5                        | 112±3      | 121±2    |
| 3.45                        | 15            | 7.18±0.02        | 5.00±0.01 | 0.00                            | 58.5±0.3                        | 108±3      | 117±3    |
| 4.60                        | 20            | 7.13±0.02        | 5.27±0.02 | 0.00                            | 59.2±0.6                        | 102±2      | 113±1    |
| 6.90                        | 30            | 7.10±0.03        | 5.80±0.03 | 0.00                            | 60.3±1.0                        | 95±1       | 108±2    |
| Total % rmvd. (-)/prod. (+) |               | -4.05            | 83.54     | -100.00                         | 11.25                           | -19.49     | -22.86   |
| Trial 2, Test 2             |               |                  |           |                                 |                                 |            |          |
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.40±0.04        | 3.16±0.09 | 0.90±0.02                       | 54.2±1.1                        | 118±3      | 140±3    |
| 0.48                        | 3             | 7.37±0.02        | 3.42±0.07 | 0.31±0.03                       | 55.0±0.6                        | 118±1      | 135±3    |
| 0.96                        | 6             | 7.32±0.05        | 3.65±0.02 | 0.00                            | 55.8±0.5                        | 115±3      | 130±3    |
| 1.44                        | 9             | 7.27±0.04        | 3.80±0.06 | 0.00                            | 56.7±0.8                        | 111±2      | 126±2    |
| 2.40                        | 15            | 7.23±0.03        | 4.26±0.06 | 0.00                            | 57.5±0.8                        | 105±2      | 121±2    |
| 3.20                        | 20            | 7.19±0.03        | 4.58±0.04 | 0.00                            | 58.2±1.2                        | 100±3      | 118±1    |
| 4.80                        | 30            | 7.17±0.04        | 5.02±0.05 | 0.00                            | 59.0±0.5                        | 94±2       | 115±2    |
| Total % rmvd. (-)/prod. (+) |               | -3.11            | 58.86     | -100.00                         | 8.86                            | -20.34     | -17.86   |
| Trial 2, Test 3             |               |                  |           |                                 |                                 |            |          |
| Dosage<br>(g/Σ time)        | Time<br>(min) | Parameter (mg/L) |           |                                 |                                 |            |          |
|                             |               | pH               | TAN       | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N | Alkalinity | Hardness |
| 0.00                        | 0             | 7.40±0.04        | 3.16±0.09 | 0.90±0.02                       | 54.2±1.1                        | 118±3      | 140±3    |
| 0.24                        | 3             | 7.38±0.03        | 3.40±0.05 | 0.28±0.04                       | 54.8±0.5                        | 117±1      | 137±4    |
| 0.48                        | 6             | 7.35±0.05        | 3.68±0.04 | 0.05±0.02                       | 55.5±0.4                        | 115±2      | 133±2    |
| 0.72                        | 9             | 7.32±0.01        | 3.92±0.06 | 0.00                            | 56.3±0.5                        | 114±2      | 130±2    |
| 1.20                        | 15            | 7.30±0.01        | 4.23±0.03 | 0.00                            | 56.9±0.8                        | 110±1      | 128±3    |
| 1.60                        | 20            | 7.28±0.02        | 4.50±0.03 | 0.00                            | 57.4±0.2                        | 106±2      | 125±3    |
| 2.40                        | 30            | 7.27±0.04        | 4.79±0.04 | 0.00                            | 58.1±0.9                        | 100±3      | 122±3    |
| Total % rmvd. (-)/prod. (+) |               | -1.76            | 51.58     | -100.00                         | 7.20                            | -15.25     | -12.86   |

Table A 30 continued.

| Trial 1, Test 1      |               |                  |        |         |        |        |        |         |        |         |
|----------------------|---------------|------------------|--------|---------|--------|--------|--------|---------|--------|---------|
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter (mg/L) |        |         |        |        |        |         |        |         |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS     |
| 0.00                 | 0             | 1441±75          | 375±20 | 1066±54 | 229±14 | 181±11 | 48±4   | 1212±44 | 194±12 | 1018±38 |
| 0.69                 | 3             | 1376±55          | 334±15 | 1042±32 | 192±8  | 145±7  | 47±2   | 1184±25 | 189±4  | 995±21  |
| 1.38                 | 6             | 1285±84          | 280±11 | 1005±12 | 160±10 | 114±8  | 46±2   | 1125±21 | 166±3  | 959±30  |
| 2.07                 | 9             | 1244±34          | 249±5  | 995±22  | 127±6  | 85±6   | 42±3   | 1117±22 | 164±5  | 953±23  |
| 3.45                 | 15            | 1202±75          | 218±12 | 984±9   | 97±4   | 62±2   | 35±1   | 1105±16 | 156±2  | 949±16  |
| 4.60                 | 20            | 1134±32          | 189±9  | 945±15  | 81±2   | 50±4   | 31±2   | 1053±18 | 139±2  | 914±8   |
| 6.90                 | 30            | 1070±20          | 169±6  | 901±10  | 65±2   | 39±1   | 27±1   | 1005±5  | 130±3  | 874±11  |
| Total % removed      |               | -25.75           | -54.93 | -15.48  | -71.62 | -78.45 | -43.75 | -17.08  | -32.99 | -14.15  |
| Trial 1, Test 2      |               |                  |        |         |        |        |        |         |        |         |
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter (mg/L) |        |         |        |        |        |         |        |         |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS     |
| 0.00                 | 0             | 1398±51          | 388±12 | 1010±36 | 210±6  | 173±9  | 37±1   | 1188±33 | 215±6  | 973±25  |
| 0.48                 | 3             | 1351±32          | 366±15 | 985±30  | 176±8  | 140±10 | 36±3   | 1175±29 | 226±10 | 949±29  |
| 0.96                 | 6             | 1310±41          | 330±9  | 980±28  | 151±5  | 115±6  | 36±2   | 1159±11 | 215±6  | 944±16  |
| 1.44                 | 9             | 1262±20          | 292±10 | 970±31  | 129±5  | 94±7   | 35±2   | 1133±20 | 198±7  | 935±18  |
| 2.40                 | 15            | 1208±22          | 244±5  | 964±20  | 104±4  | 71±5   | 33±3   | 1104±14 | 173±2  | 931±10  |
| 3.20                 | 20            | 1165±16          | 212±6  | 953±16  | 86±3   | 56±3   | 30±2   | 1079±16 | 156±4  | 923±7   |
| 4.80                 | 30            | 1100±10          | 180±2  | 920±14  | 70±1   | 44±2   | 26±1   | 1030±12 | 136±2  | 894±4   |
| Total % removed      |               | -21.32           | -53.61 | -8.91   | -66.67 | -74.57 | -29.73 | -13.30  | -36.74 | -8.12   |
| Trial 1, Test 3      |               |                  |        |         |        |        |        |         |        |         |
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter        |        |         |        |        |        |         |        |         |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS     |
| 0.00                 | 0             | 1212±22          | 295±8  | 917±27  | 142±3  | 112±3  | 30±3   | 1070±24 | 183±9  | 887±19  |
| 0.24                 | 3             | 1166±8           | 275±9  | 891±19  | 120±4  | 96±2   | 24±2   | 1046±12 | 179±7  | 867±20  |
| 0.48                 | 6             | 1133±16          | 251±4  | 882±16  | 105±2  | 78±1   | 27±2   | 1028±19 | 173±6  | 855±14  |
| 0.72                 | 9             | 1100±12          | 225±6  | 875±21  | 88±2   | 63±2   | 25±1   | 1012±16 | 162±6  | 850±15  |
| 1.20                 | 15            | 1062±10          | 196±5  | 866±14  | 73±3   | 38±2   | 35±1   | 989±12  | 158±7  | 831±11  |
| 1.60                 | 20            | 1014±6           | 152±2  | 862±15  | 60±1   | 28±1   | 32±2   | 954±8   | 124±3  | 830±9   |
| 2.40                 | 30            | 965±8            | 112±4  | 853±9   | 43±2   | 25±2   | 18±1   | 922±10  | 87±2   | 835±8   |
| Total % removed      |               | -20.38           | -62.03 | -6.98   | -69.72 | -77.68 | -40.00 | -13.83  | -52.46 | -5.86   |

Table A 30 continued.

| Trial 2, Test 1      |               |                  |        |         |        |        |        |         |        |        |
|----------------------|---------------|------------------|--------|---------|--------|--------|--------|---------|--------|--------|
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter (mg/L) |        |         |        |        |        |         |        |        |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS    |
| 0.00                 | 0             | 1400±42          | 386±13 | 1014±37 | 218±6  | 176±4  | 42±3   | 1182±44 | 210±8  | 972±22 |
| 0.69                 | 3             | 1338±34          | 342±11 | 996±16  | 180±2  | 140±5  | 40±2   | 1158±26 | 202±5  | 956±15 |
| 1.38                 | 6             | 1260±32          | 278±8  | 982±20  | 145±3  | 103±3  | 42±2   | 1115±25 | 175±4  | 940±16 |
| 2.07                 | 9             | 1182±22          | 228±9  | 954±15  | 108±2  | 71±3   | 37±1   | 1074±16 | 157±3  | 917±11 |
| 3.45                 | 15            | 1136±25          | 192±6  | 944±16  | 90±4   | 55±2   | 35±1   | 1046±19 | 137±2  | 909±7  |
| 4.60                 | 20            | 1090±16          | 170±7  | 920±11  | 69±3   | 40±3   | 29±1   | 1021±21 | 130±3  | 891±8  |
| 6.90                 | 30            | 1028±12          | 138±2  | 890±9   | 50±3   | 27±1   | 23±2   | 978±10  | 111±3  | 867±6  |
| Total % removed      |               | -26.57           | -64.25 | -12.23  | -77.06 | -84.66 | -45.24 | -17.26  | -47.14 | -10.80 |
| Trial 2, Test 2      |               |                  |        |         |        |        |        |         |        |        |
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter (mg/L) |        |         |        |        |        |         |        |        |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS    |
| 0.00                 | 0             | 1400±42          | 386±13 | 1014±37 | 218±6  | 176±4  | 42±3   | 1182±44 | 210±8  | 972±22 |
| 0.48                 | 3             | 1350±33          | 338±12 | 1012±22 | 189±4  | 144±4  | 45±7   | 1161±34 | 194±5  | 967±20 |
| 0.96                 | 6             | 1228±31          | 265±8  | 963±25  | 150±3  | 108±3  | 42±3   | 1078±32 | 157±6  | 921±16 |
| 1.44                 | 9             | 1205±26          | 244±11 | 961±14  | 112±3  | 77±4   | 35±2   | 1093±20 | 167±5  | 926±20 |
| 2.40                 | 15            | 1180±30          | 224±7  | 956±17  | 95±2   | 62±2   | 33±3   | 1085±25 | 162±4  | 923±11 |
| 3.20                 | 20            | 1129±20          | 184±4  | 945±10  | 78±3   | 49±2   | 29±2   | 1051±13 | 135±3  | 916±13 |
| 4.80                 | 30            | 1079±18          | 160±4  | 919±12  | 65±2   | 39±2   | 26±3   | 1014±14 | 121±4  | 893±10 |
| Total % removed      |               | -22.93           | -58.55 | -9.37   | -70.18 | -77.84 | -38.10 | -14.21  | -42.38 | -8.13  |
| Trial 2, Test 3      |               |                  |        |         |        |        |        |         |        |        |
| Dosage<br>(g/Σ time) | Time<br>(min) | Parameter (mg/L) |        |         |        |        |        |         |        |        |
|                      |               | TS               | VTS    | FTS     | TSS    | VSS    | FSS    | TDS     | VDS    | FDS    |
| 0.00                 | 0             | 1400±42          | 386±13 | 1014±37 | 218±6  | 176±4  | 42±3   | 1182±44 | 210±8  | 972±22 |
| 0.24                 | 3             | 1369±36          | 358±5  | 1011±30 | 194±4  | 146±2  | 48±4   | 1175±35 | 212±5  | 963±25 |
| 0.48                 | 6             | 1285±26          | 284±12 | 1001±35 | 159±5  | 117±3  | 42±2   | 1126±29 | 167±7  | 959±16 |
| 0.72                 | 9             | 1240±28          | 241±8  | 999±25  | 121±4  | 86±2   | 35±1   | 1119±30 | 155±6  | 964±16 |
| 1.20                 | 15            | 1205±25          | 216±8  | 989±21  | 106±3  | 70±4   | 36±2   | 1099±22 | 146±5  | 953±13 |
| 1.60                 | 20            | 1168±13          | 194±6  | 974±12  | 91±3   | 60±1   | 31±2   | 1077±15 | 134±6  | 943±14 |
| 2.40                 | 30            | 1120±16          | 180±7  | 940±15  | 82±3   | 53±2   | 29±1   | 1038±17 | 127±4  | 911±12 |
| Total % removed      |               | -20.00           | -53.37 | -7.30   | -62.39 | -69.89 | -30.95 | -12.18  | -39.52 | -6.28  |

Table A31. Raw data for pilot station evaluation study.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) |      | Test 1     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 158  | 11.6       | 9.12  | -    | -                               | -                               |
| SB Effluent                              | 102  | 11.8       | 6.82  | 3.20 | 0.90                            | 13.4                            |
| Sludge SB                                | 365  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 105  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 89   | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 260  | 78.5       | 7.68  | 2.56 | 0.75                            | 7.0                             |
| DR Effluent/OR Influent                  | 208  | 51.2       | 7.36  | 1.72 | 0.34                            | 0.0                             |
| OR Effluent                              | 162  | 52.4       | 4.28  | 7.19 | 0.00                            | 1.1                             |
| Foam from OR                             | 955  | 118.0      | 44.80 | -    | -                               | -                               |
| TF Influent                              | 147  | 51.0       | 3.75  | 6.95 | 0.00                            | 1.2                             |
| TF Effluent                              | 97   | 28.2       | 2.40  | 2.24 | 0.62                            | 4.3                             |
| Final Effluent (CF)                      | 54   | 24.5       | 1.00  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 564  | 10.6       | 27.80 | -    | -                               | -                               |
| SB Effluent                              | 265  | 28.1       | 16.92 | 2.33 | 0.45                            | 62.4                            |
| Sludge SB                                | 2324 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 217  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 248  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 339  | 78.2       | 16.40 | 1.70 | 0.31                            | 32.8                            |
| DR Effluent/OR Influent                  | 241  | 23.4       | 16.22 | 0.72 | 0.10                            | 2.0                             |
| OR Effluent                              | 196  | 28.5       | 9.75  | 3.91 | 0.00                            | 2.6                             |
| Foam from OR                             | 635  | 76.8       | 39.80 | -    | -                               | -                               |
| TF Influent                              | 180  | 27.1       | 8.38  | 3.86 | 0.00                            | 2.8                             |
| TF Effluent                              | 133  | 19.9       | 5.46  | 1.48 | 0.64                            | 3.6                             |
| Final Effluent (CF)                      | 73   | 18.4       | 1.56  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 617  | 12.3       | 30.12 | -    | -                               | -                               |
| SB Effluent                              | 256  | 31.4       | 15.80 | 2.06 | 0.76                            | 44.0                            |
| Sludge SB                                | 2130 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 220  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 240  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 347  | 73.7       | 16.42 | 1.58 | 0.48                            | 33.8                            |
| DR Effluent/OR Influent                  | 250  | 27.0       | 15.00 | 0.90 | 0.08                            | 1.0                             |
| OR Effluent                              | 208  | 31.5       | 9.22  | 3.88 | 0.00                            | 6.4                             |
| Foam from OR                             | 716  | 74.5       | 41.40 | -    | -                               | -                               |
| TF Influent                              | 194  | 30.2       | 8.76  | 3.70 | 0.00                            | 1.1                             |
| TF Effluent                              | 141  | 22.0       | 5.90  | 1.64 | 0.52                            | 2.5                             |
| Final Effluent (CF)                      | 79   | 20.8       | 2.40  | -    | -                               | -                               |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 2     |      |       |      |                                 |                                 |
|--|------------|------|-------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 98         | 10.9 | 6.95  | -    | -                               | -                               |
| SB Effluent                              | 81         | 8.8  | 5.11  | 4.11 | 0.98                            | 11.0                            |
| Sludge SB                                | 168        | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 97         | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 112        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 242        | 71.3 | 6.18  | 3.40 | 0.69                            | 6.9                             |
| DR Effluent/OR Influent                  | 186        | 40.5 | 6.40  | 2.38 | 0.27                            | 0.2                             |
| OR Effluent                              | 145        | 41.9 | 4.20  | 5.14 | 0.02                            | 0.6                             |
| Foam from OR                             | 871        | 94.1 | 49.26 | -    | -                               | -                               |
| TF Influent                              | 126        | 40.8 | 4.00  | 4.96 | 0.00                            | 0.4                             |
| TF Effluent                              | 88         | 31.2 | 2.89  | 2.10 | 0.42                            | 3.0                             |
| Final Effluent (CF)                      | 47         | 29.0 | 1.00  | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 590        | 10.1 | 34.15 | -    | -                               | -                               |
| SB Effluent                              | 248        | 25.1 | 16.70 | 2.42 | 0.71                            | 59.5                            |
| Sludge SB                                | 1810       | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 209        | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 260        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 349        | 75.4 | 16.96 | 1.60 | 0.46                            | 32.4                            |
| DR Effluent/OR Influent                  | 221        | 24.2 | 16.10 | 0.80 | 0.08                            | 1.0                             |
| OR Effluent                              | 176        | 29.7 | 11.42 | 3.12 | 0.00                            | 1.3                             |
| Foam from OR                             | 765        | 86.5 | 44.30 | -    | -                               | -                               |
| TF Influent                              | 164        | 29.1 | 10.60 | 3.00 | 0.00                            | 1.2                             |
| TF Effluent                              | 123        | 22.6 | 7.95  | 1.68 | 0.41                            | 2.7                             |
| Final Effluent (CF)                      | 68         | 20.4 | 2.16  | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 485        | 10.1 | 33.20 | -    | -                               | -                               |
| SB Effluent                              | 212        | 21.4 | 18.15 | 2.10 | 0.90                            | 44.6                            |
| Sludge SB                                | 1638       | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 180        | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 221        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 316        | 72.2 | 16.23 | 1.58 | 0.58                            | 28.0                            |
| DR Effluent/OR Influent                  | 215        | 27.4 | 13.95 | 0.89 | 0.15                            | 0.1                             |
| OR Effluent                              | 169        | 33.7 | 8.90  | 3.68 | 0.00                            | 1.2                             |
| Foam from OR                             | 612        | 76.4 | 37.50 | -    | -                               | -                               |
| TF Influent                              | 161        | 32.9 | 7.25  | 3.65 | 0.02                            | 1.2                             |
| TF Effluent                              | 114        | 25.4 | 4.74  | 1.70 | 0.54                            | 1.9                             |
| Final Effluent (CF)                      | 63         | 24.2 | 1.00  | -    | -                               | -                               |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) |      | Test 3     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 90   | 13.0       | 8.90  | -    | -                               | -                               |
| SB Effluent                              | 81   | 10.5       | 7.60  | 3.92 | 0.75                            | 15.5                            |
| Sludge SB                                | 130  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 87   | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 120  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 295  | 72.3       | 12.30 | 3.55 | 0.58                            | 9.8                             |
| DR Effluent/OR Influent                  | 237  | 42.1       | 12.40 | 2.85 | 0.34                            | 0.0                             |
| OR Effluent                              | 183  | 44.3       | 7.65  | 7.15 | 0.04                            | 0.1                             |
| Foam from OR                             | 891  | 116.0      | 43.80 | -    | -                               | -                               |
| TF Influent                              | 169  | 42.1       | 5.90  | 6.94 | 0.05                            | 0.1                             |
| TF Effluent                              | 110  | 23.2       | 4.40  | 2.12 | 0.22                            | 1.1                             |
| Final Effluent (CF)                      | 59   | 21.8       | 1.70  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 680  | 11.3       | 39.50 | -    | -                               | -                               |
| SB Effluent                              | 252  | 28.4       | 15.60 | 2.45 | 0.85                            | 48.5                            |
| Sludge SB                                | 2465 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 208  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 340  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 358  | 72.2       | 18.20 | 1.71 | 0.59                            | 20.4                            |
| DR Effluent/OR Influent                  | 249  | 24.0       | 17.92 | 1.18 | 0.20                            | 0.6                             |
| OR Effluent                              | 202  | 30.2       | 11.18 | 3.65 | 0.00                            | 1.0                             |
| Foam from OR                             | 810  | 99.0       | 42.20 | -    | -                               | -                               |
| TF Influent                              | 181  | 30.0       | 10.20 | 3.59 | 0.05                            | 1.1                             |
| TF Effluent                              | 134  | 24.4       | 6.64  | 1.54 | 0.62                            | 1.8                             |
| Final Effluent (CF)                      | 76   | 23.2       | 3.10  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 540  | 12.4       | 32.22 | -    | -                               | -                               |
| SB Effluent                              | 221  | 27.4       | 15.60 | 2.82 | 1.08                            | 55.0                            |
| Sludge SB                                | 1820 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 192  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 235  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 345  | 74.2       | 16.44 | 1.90 | 0.62                            | 30.4                            |
| DR Effluent/OR Influent                  | 232  | 22.6       | 16.00 | 0.95 | 0.32                            | 0.5                             |
| OR Effluent                              | 190  | 28.4       | 9.76  | 4.14 | 0.02                            | 1.0                             |
| Foam from OR                             | 794  | 90.5       | 39.90 | -    | -                               | -                               |
| TF Influent                              | 180  | 27.7       | 9.10  | 4.02 | 0.06                            | 1.2                             |
| TF Effluent                              | 131  | 19.8       | 6.80  | 1.45 | 0.30                            | 2.6                             |
| Final Effluent (CF)                      | 70   | 18.5       | 2.62  | -    | -                               | -                               |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 1     |       |       |      |                                 |                                 |
|--|------------|-------|-------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 307        | 7.5   | 14.92 | -    | -                               | -                               |
| SB Effluent                              | 158        | 11.8  | 8.35  | 2.64 | 0.86                            | 14.0                            |
| Sludge SB                                | 930        | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 91         | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 171        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 237        | 62.2  | 8.89  | 2.72 | 0.45                            | 6.0                             |
| DR Effluent/OR Influent                  | 183        | 32.6  | 9.44  | 2.70 | 0.02                            | 0.0                             |
| OR Effluent                              | 121        | 39.4  | 6.92  | 9.24 | 0.01                            | 1.0                             |
| Foam from OR                             | 812        | 68.7  | 46.14 | -    | -                               | -                               |
| TF Influent                              | 110        | 34.1  | 5.95  | 7.32 | 0.02                            | 1.2                             |
| TF Effluent                              | 75         | 14.8  | 4.08  | 0.92 | 0.61                            | 0.8                             |
| Final Effluent (CF)                      | 22         | 13.1  | 1.00  | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 443        | 11.1  | 25.25 | -    | -                               | -                               |
| SB Effluent                              | 228        | 25.2  | 15.15 | 1.72 | 1.49                            | 52.4                            |
| Sludge SB                                | 1312       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 203        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 254        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 268        | 67.4  | 15.80 | 1.40 | 0.98                            | 24.8                            |
| DR Effluent/OR Influent                  | 191        | 22.4  | 15.92 | 0.48 | 0.04                            | 0.4                             |
| OR Effluent                              | 136        | 35.1  | 7.14  | 2.68 | 0.05                            | 0.8                             |
| Foam from OR                             | 884        | 127.5 | 52.89 | -    | -                               | -                               |
| TF Influent                              | 124        | 31.6  | 5.91  | 2.04 | 0.03                            | 2.0                             |
| TF Effluent                              | 87         | 24.4  | 3.85  | 1.24 | 1.66                            | 3.0                             |
| Final Effluent (CF)                      | 45         | 28.0  | 1.14  | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 1102       | 8.5   | 47.36 | -    | -                               | -                               |
| SB Effluent                              | 331        | 28.3  | 17.45 | 1.76 | 1.53                            | 51.6                            |
| Sludge SB                                | 4340       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 264        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 353        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 391        | 70.7  | 18.20 | 1.16 | 0.77                            | 22.0                            |
| DR Effluent/OR Influent                  | 289        | 25.8  | 18.65 | 0.56 | 0.02                            | 0.0                             |
| OR Effluent                              | 221        | 38.4  | 10.14 | 2.56 | 0.02                            | 2.0                             |
| Foam from OR                             | 1035       | 119.5 | 55.17 | -    | -                               | -                               |
| TF Influent                              | 216        | 39.1  | 8.28  | 2.16 | 0.02                            | 1.2                             |
| TF Effluent                              | 152        | 28.6  | 7.94  | 1.52 | 1.68                            | 6.0                             |
| Final Effluent (CF)                      | 78         | 27.7  | 3.11  | -    | -                               | -                               |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) |      | Test 2     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 223  | 10.6       | 12.04 | -    | -                               | -                               |
| SB Effluent                              | 100  | 10.6       | 7.96  | 3.68 | 0.50                            | 6.8                             |
| Sludge SB                                | 722  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 78   | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 139  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 194  | 73.4       | 8.18  | 3.96 | 0.19                            | 3.0                             |
| DR Effluent/OR Influent                  | 202  | 52.8       | 8.96  | 4.12 | 0.02                            | 0.0                             |
| OR Effluent                              | 144  | 46.5       | 5.60  | 9.10 | 0.01                            | 1.6                             |
| Foam from OR                             | 1158 | 116.7      | 77.84 | -    | -                               | -                               |
| TF Influent                              | 124  | 42.8       | 5.10  | 9.10 | 0.03                            | 0.4                             |
| TF Effluent                              | 62   | 20.6       | 4.20  | 2.24 | 0.59                            | 1.6                             |
| Final Effluent (CF)                      | 32   | 19.4       | 1.78  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 700  | 9.1        | 37.10 | -    | -                               | -                               |
| SB Effluent                              | 273  | 24.8       | 15.72 | 1.96 | 0.81                            | 53.6                            |
| Sludge SB                                | 2493 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 192  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 353  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 258  | 60.8       | 13.32 | 1.48 | 0.48                            | 26.8                            |
| DR Effluent/OR Influent                  | 207  | 23.9       | 11.08 | 0.60 | 0.15                            | 0.4                             |
| OR Effluent                              | 144  | 33.7       | 7.60  | 2.35 | 0.02                            | 1.2                             |
| Foam from OR                             | 993  | 112.3      | 53.76 | -    | -                               | -                               |
| TF Influent                              | 134  | 31.9       | 7.52  | 2.15 | 0.03                            | 1.6                             |
| TF Effluent                              | 106  | 26.1       | 5.80  | 1.00 | 0.74                            | 1.6                             |
| Final Effluent (CF)                      | 59   | 25.2       | 2.86  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 785  | 9.7        | 41.60 | -    | -                               | -                               |
| SB Effluent                              | 264  | 25.7       | 15.08 | 2.12 | 0.76                            | 40.0                            |
| Sludge SB                                | 2973 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 225  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 437  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 335  | 68.2       | 15.44 | 1.76 | 0.44                            | 16.2                            |
| DR Effluent/OR Influent                  | 238  | 28.9       | 14.88 | 1.48 | 0.12                            | 0.1                             |
| OR Effluent                              | 180  | 41.8       | 9.12  | 3.56 | 0.00                            | 0.6                             |
| Foam from OR                             | 731  | 78.6       | 39.86 | -    | -                               | -                               |
| TF Influent                              | 146  | 38.6       | 8.54  | 3.10 | 0.04                            | 0.1                             |
| TF Effluent                              | 121  | 24.4       | 6.16  | 1.30 | 0.55                            | 1.0                             |
| Final Effluent (CF)                      | 66   | 22.3       | 2.16  | -    | -                               | -                               |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 3     |       |        |      |                                 |                                 |
|--|------------|-------|--------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |       |        |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN    | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 135        | 10.3  | 8.60   | -    | -                               | -                               |
| SB Effluent                              | 89         | 9.2   | 7.18   | 4.02 | 0.82                            | 11.4                            |
| Sludge SB                                | 298        | -     | -      | -    | -                               | -                               |
| MSF Effluent                             | 99         | -     | -      | -    | -                               | -                               |
| Sludge MSF                               | 102        | -     | -      | -    | -                               | -                               |
| DR. Influent/MB                          | 272        | 59.8  | 9.12   | 3.15 | 0.49                            | 7.0                             |
| DR Effluent/OR Influent                  | 210        | 38.4  | 9.35   | 2.20 | 0.16                            | 0.0                             |
| OR Effluent                              | 146        | 41.9  | 6.85   | 6.32 | 0.00                            | 0.1                             |
| Foam from OR                             | 975        | 108.0 | 101.40 | -    | -                               | -                               |
| TF Influent                              | 110        | 40.2  | 6.15   | 6.25 | 0.04                            | 0.2                             |
| TF Effluent                              | 58         | 18.4  | 4.81   | 1.85 | 0.42                            | 3.6                             |
| Final Effluent (CF)                      | 29         | 16.2  | 1.00   | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |       |        |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN    | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 775        | 9.4   | 38.28  | -    | -                               | -                               |
| SB Effluent                              | 271        | 25.6  | 12.92  | 2.92 | 1.32                            | 42.4                            |
| Sludge SB                                | 2918       | -     | -      | -    | -                               | -                               |
| MSF Effluent                             | 200        | -     | -      | -    | -                               | -                               |
| Sludge MSF                               | 222        | -     | -      | -    | -                               | -                               |
| DR. Influent/MB                          | 310        | 61.7  | 12.20  | 2.04 | 0.97                            | 16.0                            |
| DR Effluent/OR Influent                  | 210        | 29.0  | 12.64  | 1.20 | 0.32                            | 0.8                             |
| OR Effluent                              | 151        | 39.8  | 7.74   | 2.64 | 0.00                            | 1.5                             |
| Foam from OR                             | 1030       | 115.9 | 54.50  | -    | -                               | -                               |
| TF Influent                              | 139        | 32.0  | 7.30   | 2.76 | 0.00                            | 1.4                             |
| TF Effluent                              | 112        | 28.4  | 6.14   | 1.92 | 0.38                            | 2.8                             |
| Final Effluent (CF)                      | 65         | 26.7  | 1.48   | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |       |        |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN    | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 530        | 10.3  | 31.44  | -    | -                               | -                               |
| SB Effluent                              | 240        | 24.8  | 11.00  | 3.60 | 0.64                            | 48.7                            |
| Sludge SB                                | 1822       | -     | -      | -    | -                               | -                               |
| MSF Effluent                             | 203        | -     | -      | -    | -                               | -                               |
| Sludge MSF                               | 256        | -     | -      | -    | -                               | -                               |
| DR. Influent/MB                          | 340        | 64.9  | 11.45  | 2.89 | 0.42                            | 29.0                            |
| DR Effluent/OR Influent                  | 223        | 28.2  | 11.63  | 1.65 | 0.16                            | 1.1                             |
| OR Effluent                              | 157        | 36.1  | 6.92   | 3.48 | 0.00                            | 1.3                             |
| Foam from OR                             | 820        | 97.0  | 45.72  | -    | -                               | -                               |
| TF Influent                              | 144        | 35.1  | 6.34   | 3.36 | 0.04                            | 1.3                             |
| TF Effluent                              | 95         | 26.4  | 6.90   | 1.86 | 0.56                            | 2.8                             |
| Final Effluent (CF)                      | 51         | 24.9  | 1.82   | -    | -                               | -                               |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) |      | Test 1     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 95   | 11.0       | 6.11  | -    | -                               | -                               |
| SB Effluent                              | 84   | 9.2        | 4.96  | 3.40 | 1.15                            | 12.6                            |
| Sludge SB                                | 145  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 97   | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 112  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 256  | 61.3       | 10.85 | 2.95 | 0.68                            | 4.9                             |
| DR Effluent/OR Influent                  | 193  | 37.8       | 11.02 | 2.62 | 0.45                            | 0.0                             |
| OR Effluent                              | 94   | 42.6       | 6.14  | 6.68 | 0.05                            | 0.5                             |
| Foam from OR                             | 1075 | 93.4       | 49.50 | -    | -                               | -                               |
| TF Influent                              | 68   | 41.2       | 5.70  | 6.49 | 0.08                            | 0.7                             |
| TF Effluent                              | 55   | 22.9       | 4.85  | 2.14 | 0.51                            | 1.4                             |
| Final Effluent (CF)                      | 27   | 18.2       | 1.12  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 705  | 10.5       | 33.84 | -    | -                               | -                               |
| SB Effluent                              | 279  | 29.3       | 17.36 | 3.00 | 0.94                            | 40.4                            |
| Sludge SB                                | 2394 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 198  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 333  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 268  | 61.0       | 14.28 | 2.00 | 0.37                            | 14.0                            |
| DR Effluent/OR Influent                  | 220  | 28.9       | 13.16 | 1.04 | 0.01                            | 0.0                             |
| OR Effluent                              | 135  | 36.1       | 8.96  | 2.92 | 0.01                            | 0.1                             |
| Foam from OR                             | 990  | 105.5      | 70.56 | -    | -                               | -                               |
| TF Influent                              | 123  | 34.0       | 8.12  | 3.00 | 0.02                            | 0.1                             |
| TF Effluent                              | 87   | 26.9       | 6.12  | 1.75 | 0.38                            | 2.8                             |
| Final Effluent (CF)                      | 49   | 23.8       | 2.61  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 870  | 9.8        | 36.85 | -    | -                               | -                               |
| SB Effluent                              | 277  | 27.2       | 15.08 | 1.80 | 0.87                            | 37.6                            |
| Sludge SB                                | 3245 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 243  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 260  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 295  | 68.2       | 17.25 | 1.12 | 0.71                            | 19.2                            |
| DR Effluent/OR Influent                  | 224  | 32.4       | 17.92 | 0.60 | 0.56                            | 0.8                             |
| OR Effluent                              | 123  | 39.0       | 14.37 | 2.56 | 0.02                            | 1.2                             |
| Foam from OR                             | 1107 | 121.2      | 76.82 | -    | -                               | -                               |
| TF Influent                              | 112  | 35.5       | 12.72 | 2.60 | 0.02                            | 1.0                             |
| TF Effluent                              | 82   | 26.4       | 12.88 | 1.52 | 0.58                            | 1.4                             |
| Final Effluent (CF)                      | 43   | 25.1       | 2.90  | -    | -                               | -                               |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) | Test 2     |       |       |      |                                 |                                 |
|--|------------|-------|-------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 182        | 10.4  | 14.25 | -    | -                               | -                               |
| SB Effluent                              | 100        | 10.4  | 9.14  | 3.08 | 0.95                            | 9.8                             |
| Sludge SB                                | 488        | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 96         | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 114        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 241        | 59.8  | 9.92  | 2.80 | 0.48                            | 2.8                             |
| DR Effluent/OR Influent                  | 200        | 32.7  | 10.54 | 1.66 | 0.22                            | 0.0                             |
| OR Effluent                              | 119        | 36.2  | 7.11  | 3.92 | 0.00                            | 0.4                             |
| Foam from OR                             | 705        | 80.5  | 54.85 | -    | -                               | -                               |
| TF Influent                              | 114        | 34.1  | 6.41  | 3.85 | 0.04                            | 0.5                             |
| TF Effluent                              | 60         | 21.4  | 5.88  | 1.65 | 0.62                            | 2.5                             |
| Final Effluent (CF)                      | 31         | 18.9  | 1.82  | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 874        | 10.6  | 43.70 | -    | -                               | -                               |
| SB Effluent                              | 266        | 28.3  | 16.24 | 1.84 | 1.44                            | 68.8                            |
| Sludge SB                                | 3310       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 218        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 248        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 343        | 62.4  | 17.36 | 0.91 | 0.89                            | 28.2                            |
| DR Effluent/OR Influent                  | 252        | 22.3  | 17.92 | 0.45 | 0.68                            | 1.6                             |
| OR Effluent                              | 160        | 30.4  | 10.28 | 2.48 | 0.00                            | 2.2                             |
| Foam from OR                             | 1054       | 109.5 | 73.36 | -    | -                               | -                               |
| TF Influent                              | 136        | 28.8  | 9.50  | 2.52 | 0.01                            | 1.8                             |
| TF Effluent                              | 94         | 22.1  | 7.40  | 0.88 | 0.50                            | 2.1                             |
| Final Effluent (CF)                      | 51         | 21.1  | 2.60  | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 492        | 9.8   | 36.71 | -    | -                               | -                               |
| SB Effluent                              | 239        | 23.4  | 16.41 | 2.12 | 0.90                            | 52.0                            |
| Sludge SB                                | 1514       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 210        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 271        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 314        | 58.8  | 16.75 | 1.65 | 0.79                            | 29.2                            |
| DR Effluent/OR Influent                  | 228        | 22.1  | 17.14 | 1.34 | 0.40                            | 1.5                             |
| OR Effluent                              | 140        | 31.1  | 8.23  | 2.88 | 0.16                            | 1.8                             |
| Foam from OR                             | 628        | 71.3  | 33.18 | -    | -                               | -                               |
| TF Influent                              | 118        | 30.4  | 7.22  | 2.85 | 0.12                            | 1.7                             |
| TF Effluent                              | 82         | 21.5  | 6.85  | 1.22 | 0.61                            | 3.1                             |
| Final Effluent (CF)                      | 43         | 20.1  | 1.40  | -    | -                               | -                               |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) | Test 3     |       |       |      |                                 |                                 |
|--|------------|-------|-------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 104        | 10.5  | 8.75  | -    | -                               | -                               |
| SB Effluent                              | 77         | 8.1   | 8.24  | 4.11 | 1.20                            | 6.8                             |
| Sludge SB                                | 214        | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 92         | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 119        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 198        | 53.4  | 8.75  | 2.82 | 0.65                            | 2.3                             |
| DR Effluent/OR Influent                  | 179        | 25.6  | 8.80  | 1.70 | 0.20                            | 0.0                             |
| OR Effluent                              | 96         | 36.2  | 5.12  | 5.25 | 0.00                            | 0.4                             |
| Foam from OR                             | 924        | 103.5 | 72.35 | -    | -                               | -                               |
| TF Influent                              | 87         | 34.5  | 4.80  | 5.10 | 0.00                            | 0.4                             |
| TF Effluent                              | 53         | 19.6  | 4.45  | 1.86 | 0.72                            | 1.9                             |
| Final Effluent (CF)                      | 25         | 16.8  | 1.00  | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 856        | 12.1  | 39.32 | -    | -                               | -                               |
| SB Effluent                              | 285        | 34.5  | 17.42 | 2.60 | 1.14                            | 58.4                            |
| Sludge SB                                | 3170       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 242        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 316        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 309        | 38.7  | 13.45 | 1.96 | 0.52                            | 24.0                            |
| DR Effluent/OR Influent                  | 236        | 24.2  | 12.68 | 1.00 | 0.05                            | 1.2                             |
| OR Effluent                              | 141        | 37.0  | 9.50  | 3.28 | 0.00                            | 1.8                             |
| Foam from OR                             | 789        | 94.8  | 66.72 | -    | -                               | -                               |
| TF Influent                              | 120        | 33.6  | 6.46  | 2.92 | 0.03                            | 2.8                             |
| TF Effluent                              | 94         | 21.3  | 6.50  | 1.35 | 0.39                            | 1.2                             |
| Final Effluent (CF)                      | 49         | 20.2  | 2.04  | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |       |       |      |                                 |                                 |
| Parameter                                | COD        | DOC   | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 604        | 11.1  | 30.50 | -    | -                               | -                               |
| SB Effluent                              | 224        | 24.8  | 12.39 | 2.25 | 0.88                            | 46.5                            |
| Sludge SB                                | 2212       | -     | -     | -    | -                               | -                               |
| MSF Effluent                             | 189        | -     | -     | -    | -                               | -                               |
| Sludge MSF                               | 197        | -     | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 316        | 63.6  | 10.40 | 1.55 | 0.48                            | 20.0                            |
| DR Effluent/OR Influent                  | 229        | 31.3  | 9.24  | 1.10 | 0.16                            | 0.8                             |
| OR Effluent                              | 138        | 36.5  | 7.17  | 2.84 | 0.00                            | 1.0                             |
| Foam from OR                             | 822        | 81.4  | 68.50 | -    | -                               | -                               |
| TF Influent                              | 129        | 34.2  | 7.00  | 2.80 | 0.04                            | 0.8                             |
| TF Effluent                              | 98         | 22.8  | 6.12  | 1.60 | 0.60                            | 1.3                             |
| Final Effluent (CF)                      | 54         | 21.7  | 1.45  | -    | -                               | -                               |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) |      | Test 1     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 126  | 10.6       | 8.15  | -    | -                               | -                               |
| SB Effluent                              | 93   | 9.9        | 6.95  | 3.00 | 0.95                            | 12.8                            |
| Sludge SB                                | 263  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 104  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 115  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 241  | 53.4       | 8.48  | 2.13 | 0.46                            | 4.0                             |
| DR Effluent/OR Influent                  | 208  | 31.8       | 8.78  | 1.46 | 0.16                            | 0.2                             |
| OR Effluent                              | 140  | 38.1       | 6.12  | 3.90 | 0.00                            | 0.3                             |
| Foam from OR                             | 750  | 88.2       | 46.52 | -    | -                               | -                               |
| TF Influent                              | 118  | 36.9       | 5.70  | 3.87 | 0.02                            | 0.3                             |
| TF Effluent                              | 63   | 21.0       | 3.85  | 1.73 | 0.55                            | 1.8                             |
| Final Effluent (CF)                      | 33   | 18.2       | 1.00  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 670  | 8.4        | 36.91 | -    | -                               | -                               |
| SB Effluent                              | 260  | 21.8       | 14.45 | 2.72 | 1.01                            | 33.6                            |
| Sludge SB                                | 2420 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 190  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 142  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 276  | 55.2       | 14.42 | 1.80 | 0.51                            | 12.8                            |
| DR Effluent/OR Influent                  | 210  | 25.4       | 13.50 | 1.20 | 0.32                            | 1.0                             |
| OR Effluent                              | 142  | 41.5       | 8.87  | 2.36 | 0.01                            | 2.1                             |
| Foam from OR                             | 817  | 108.0      | 56.00 | -    | -                               | -                               |
| TF Influent                              | 130  | 40.2       | 8.12  | 2.32 | 0.02                            | 1.9                             |
| TF Effluent                              | 106  | 28.4       | 5.30  | 1.24 | 0.19                            | 1.2                             |
| Final Effluent (CF)                      | 59   | 24.8       | 1.65  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 592  | 11.0       | 40.85 | -    | -                               | -                               |
| SB Effluent                              | 216  | 23.8       | 14.80 | 3.14 | 0.77                            | 56.0                            |
| Sludge SB                                | 2242 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 194  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 238  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 269  | 57.4       | 15.80 | 2.35 | 0.35                            | 20.4                            |
| DR Effluent/OR Influent                  | 220  | 20.9       | 16.22 | 1.85 | 0.11                            | 2.1                             |
| OR Effluent                              | 161  | 37.0       | 9.36  | 3.40 | 0.00                            | 2.2                             |
| Foam from OR                             | 810  | 94.2       | 51.40 | -    | -                               | -                               |
| TF Influent                              | 147  | 35.1       | 7.90  | 3.21 | 0.00                            | 2.0                             |
| TF Effluent                              | 105  | 24.5       | 6.92  | 1.71 | 0.40                            | 3.2                             |
| Final Effluent (CF)                      | 57   | 22.9       | 2.80  | -    | -                               | -                               |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) |      | Test 2     |       |      |                                 |                                 |
|--|------|------------|-------|------|---------------------------------|---------------------------------|
| Time                                     |      | 2:00 p.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 88   | 11.4       | 5.11  | -    | -                               | -                               |
| SB Effluent                              | 74   | 8.4        | 4.92  | 3.40 | 0.92                            | 8.5                             |
| Sludge SB                                | 146  | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 85   | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 77   | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 195  | 55.7       | 7.15  | 2.14 | 0.35                            | 3.2                             |
| DR Effluent/OR Influent                  | 170  | 34.5       | 7.44  | 1.30 | 0.08                            | 0.0                             |
| OR Effluent                              | 99   | 39.9       | 5.06  | 3.82 | 0.00                            | 0.2                             |
| Foam from OR                             | 690  | 83.4       | 60.18 | -    | -                               | -                               |
| TF Influent                              | 90   | 38.2       | 4.28  | 3.76 | 0.00                            | 0.3                             |
| TF Effluent                              | 50   | 18.0       | 3.15  | 1.80 | 0.46                            | 2.2                             |
| Final Effluent (CF)                      | 25   | 15.7       | 1.00  | -    | -                               | -                               |
| Time                                     |      | 10:00 p.m. |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 911  | 9.7        | 41.42 | -    | -                               | -                               |
| SB Effluent                              | 261  | 25.4       | 15.80 | 2.06 | 0.48                            | 53.0                            |
| Sludge SB                                | 3420 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 214  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 270  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 334  | 68.1       | 16.75 | 1.48 | 0.23                            | 20.9                            |
| DR Effluent/OR Influent                  | 258  | 28.2       | 16.00 | 1.10 | 0.08                            | 1.2                             |
| OR Effluent                              | 192  | 37.0       | 9.02  | 3.20 | 0.00                            | 1.6                             |
| Foam from OR                             | 790  | 82.4       | 45.12 | -    | -                               | -                               |
| TF Influent                              | 176  | 35.2       | 8.17  | 3.14 | 0.03                            | 1.5                             |
| TF Effluent                              | 131  | 25.6       | 6.80  | 1.62 | 0.26                            | 2.8                             |
| Final Effluent (CF)                      | 69   | 23.3       | 2.08  | -    | -                               | -                               |
| Time                                     |      | 6:00 a.m.  |       |      |                                 |                                 |
| Parameter                                | COD  | DOC        | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 525  | 10.7       | 37.65 | -    | -                               | -                               |
| SB Effluent                              | 240  | 25.6       | 15.20 | 1.97 | 0.64                            | 46.2                            |
| Sludge SB                                | 1690 | -          | -     | -    | -                               | -                               |
| MSF Effluent                             | 209  | -          | -     | -    | -                               | -                               |
| Sludge MSF                               | 251  | -          | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 298  | 65.8       | 16.10 | 1.54 | 0.31                            | 16.4                            |
| DR Effluent/OR Influent                  | 220  | 24.1       | 16.42 | 1.18 | 0.10                            | 0.2                             |
| OR Effluent                              | 151  | 39.3       | 8.50  | 3.62 | 0.00                            | 1.6                             |
| Foam from OR                             | 703  | 74.5       | 35.90 | -    | -                               | -                               |
| TF Influent                              | 130  | 36.9       | 8.00  | 3.51 | 0.00                            | 1.3                             |
| TF Effluent                              | 89   | 20.8       | 7.14  | 1.55 | 0.42                            | 3.1                             |
| Final Effluent (CF)                      | 48   | 19.2       | 1.79  | -    | -                               | -                               |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) | Test 3     |      |       |      |                                 |                                 |
|--|------------|------|-------|------|---------------------------------|---------------------------------|
| Time                                     | 2:00 p.m.  |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 114        | 11.3 | 7.12  | -    | -                               | -                               |
| SB Effluent                              | 96         | 10.8 | 6.46  | 3.44 | 1.22                            | 17.4                            |
| Sludge SB                                | 175        | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 126        | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 140        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 236        | 60.4 | 7.80  | 2.43 | 0.59                            | 6.3                             |
| DR Effluent/OR Influent                  | 190        | 29.7 | 7.86  | 1.88 | 0.19                            | 0.1                             |
| OR Effluent                              | 122        | 35.8 | 4.38  | 4.16 | 0.00                            | 0.4                             |
| Foam from OR                             | 835        | 97.2 | 52.66 | -    | -                               | -                               |
| TF Influent                              | 112        | 34.1 | 4.06  | 4.07 | 0.04                            | 0.4                             |
| TF Effluent                              | 70         | 19.5 | 2.90  | 2.14 | 0.93                            | 3.1                             |
| Final Effluent (CF)                      | 36         | 17.8 | 1.00  | -    | -                               | -                               |
| Time                                     | 10:00 p.m. |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 665        | 10.1 | 44.38 | -    | -                               | -                               |
| SB Effluent                              | 248        | 25.0 | 16.40 | 2.00 | 0.80                            | 55.0                            |
| Sludge SB                                | 2380       | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 218        | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 260        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 322        | 64.2 | 16.15 | 1.28 | 0.41                            | 21.8                            |
| DR Effluent/OR Influent                  | 237        | 26.3 | 16.80 | 0.75 | 0.12                            | 1.0                             |
| OR Effluent                              | 169        | 34.8 | 7.90  | 3.36 | 0.00                            | 1.4                             |
| Foam from OR                             | 910        | 95.9 | 43.25 | -    | -                               | -                               |
| TF Influent                              | 156        | 31.9 | 7.00  | 3.20 | 0.02                            | 1.4                             |
| TF Effluent                              | 113        | 19.8 | 5.84  | 1.86 | 0.40                            | 0.6                             |
| Final Effluent (CF)                      | 70         | 19.0 | 2.23  | -    | -                               | -                               |
| Time                                     | 6:00 a.m.  |      |       |      |                                 |                                 |
| Parameter                                | COD        | DOC  | TKN   | TAN  | NO <sub>2</sub> <sup>-</sup> -N | NO <sub>3</sub> <sup>-</sup> -N |
| Raw Water                                | 512        | 11.3 | 35.82 | -    | -                               | -                               |
| SB Effluent                              | 208        | 23.6 | 13.47 | 2.42 | 0.52                            | 50.8                            |
| Sludge SB                                | 1788       | -    | -     | -    | -                               | -                               |
| MSF Effluent                             | 187        | -    | -     | -    | -                               | -                               |
| Sludge MSF                               | 236        | -    | -     | -    | -                               | -                               |
| DR. Influent/MB                          | 300        | 58.9 | 14.40 | 1.70 | 0.19                            | 18.8                            |
| DR Effluent/OR Influent                  | 225        | 23.8 | 14.32 | 1.18 | 0.08                            | 0.6                             |
| OR Effluent                              | 161        | 37.4 | 6.48  | 4.00 | 0.02                            | 0.9                             |
| Foam from OR                             | 765        | 84.4 | 39.00 | -    | -                               | -                               |
| TF Influent                              | 147        | 33.4 | 6.10  | 3.84 | 0.05                            | 1.1                             |
| TF Effluent                              | 100        | 21.9 | 5.22  | 1.46 | 0.60                            | 3.6                             |
| Final Effluent (CF)                      | 58         | 20.4 | 1.86  | -    | -                               | -                               |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 1     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 30.5       | -    | -     | 2.88 | 21.0  |
| SB Effluent                              | 30.5       | 76   | 148   | 1.56 | 17.7  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.9       | -    | -     | 0.60 | 18.4  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 30.4       | 92   | 146   | 0.54 | 18.3  |
| DR Effluent/OR Influent                  | 30.5       | 102  | 145   | 0.00 | 18.4  |
| OR Effluent                              | 30.5       | 93   | 141   | -    | 16.6  |
| Foam from OR                             | -          | -    | -     | -    | 140.6 |
| TF Influent                              | 30.5       | 93   | 140   | 5.58 | 16.6  |
| TF Effluent                              | 31.4       | 68   | 140   | 5.06 | 16.1  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.6   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 27.8       | -    | -     | 0.12 | 118.8 |
| SB Effluent                              | 28         | 108  | 154   | 0.08 | 82.4  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.9       | -    | -     | 0.32 | 61.5  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.3       | 145  | 150   | 0.10 | 64.3  |
| DR Effluent/OR Influent                  | 28.4       | 214  | 148   | 0.03 | 65.2  |
| OR Effluent                              | 28.6       | 193  | 142   | -    | 25.0  |
| Foam from OR                             | -          | -    | -     | -    | 153.3 |
| TF Influent                              | 28.8       | 191  | 142   | 5.78 | 17.3  |
| TF Effluent                              | 28         | 174  | 141   | 5.14 | 18.8  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 5.5   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.2       | -    | -     | 0.10 | 151.5 |
| SB Effluent                              | 28         | 115  | 148   | 0.07 | 75.2  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.7       | -    | -     | 0.20 | 58.8  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.8       | 144  | 144   | 0.08 | 62.4  |
| DR Effluent/OR Influent                  | 28.9       | 189  | 142   | 0.00 | 63.3  |
| OR Effluent                              | 29.4       | 173  | 138   | -    | 24.6  |
| Foam from OR                             | -          | -    | -     | -    | 175.5 |
| TF Influent                              | 29.4       | 172  | 138   | 5.66 | 20.6  |
| TF Effluent                              | 28.8       | 160  | 137   | 5.10 | 18.8  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 5.2   |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 2     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 30         | -    | -     | 3.20 | 24.3  |
| SB Effluent                              | 29.8       | 85   | 146   | 1.32 | 37.0  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.7       | -    | -     | 1.08 | 30.6  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 30.1       | 95   | 143   | 0.60 | 26.1  |
| DR Effluent/OR Influent                  | 30.2       | 113  | 143   | 0.00 | 27.0  |
| OR Effluent                              | 30.4       | 100  | 140   | -    | 14.8  |
| Foam from OR                             | -          | -    | -     | -    | 138.4 |
| TF Influent                              | 30.4       | 100  | 140   | 5.44 | 12.4  |
| TF Effluent                              | 29.8       | 88   | 138   | 5.12 | 13.3  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.8   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.2       | -    | -     | 0.22 | 137.0 |
| SB Effluent                              | 28         | 107  | 146   | 0.08 | 76.1  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.9       | -    | -     | 0.60 | 56.1  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.4       | 148  | 143   | 0.25 | 58.8  |
| DR Effluent/OR Influent                  | 28.5       | 212  | 142   | 0.04 | 60.6  |
| OR Effluent                              | 28.9       | 194  | 139   | -    | 21.5  |
| Foam from OR                             | -          | -    | -     | -    | 171.5 |
| TF Influent                              | 28.8       | 192  | 139   | 6.02 | 22.4  |
| TF Effluent                              | 28.1       | 183  | 138   | 5.47 | 20.6  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 7.2   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.9       | -    | -     | 0.10 | 157.0 |
| SB Effluent                              | 29         | 115  | 150   | 0.07 | 73.3  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28.6       | -    | -     | 0.82 | 46.1  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.1       | 147  | 144   | 0.16 | 50.6  |
| DR Effluent/OR Influent                  | 29.1       | 197  | 144   | 0.00 | 51.5  |
| OR Effluent                              | 29.3       | 183  | 140   | -    | 26.6  |
| Foam from OR                             | -          | -    | -     | -    | 164.3 |
| TF Influent                              | 29.4       | 182  | 138   | 5.44 | 16.6  |
| TF Effluent                              | 29         | 175  | 138   | 4.87 | 15.2  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 6.1   |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 3     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29.9       | -    | -     | 1.20 | 23.3  |
| SB Effluent                              | 30         | 100  | 152   | 0.86 | 22.4  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.8       | -    | -     | 0.12 | 25.2  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 30         | 116  | 148   | 0.60 | 19.9  |
| DR Effluent/OR Influent                  | 30.2       | 132  | 149   | 0.06 | 18.8  |
| OR Effluent                              | 30.2       | 125  | 143   | -    | 14.3  |
| Foam from OR                             | -          | -    | -     | -    | 187.2 |
| TF Influent                              | 30.6       | 126  | 142   | 8.45 | 21.3  |
| TF Effluent                              | 30.9       | 116  | 139   | 6.40 | 18.8  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 1.8   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.6       | -    | -     | 0.18 | 139.7 |
| SB Effluent                              | 29.3       | 136  | 160   | 0.15 | 84.6  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.9       | -    | -     | 0.12 | 61.7  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.7       | 180  | 151   | 0.52 | 67.0  |
| DR Effluent/OR Influent                  | 30.1       | 216  | 150   | 0.06 | 65.3  |
| OR Effluent                              | 30.2       | 210  | 140   | -    | 34.1  |
| Foam from OR                             | -          | -    | -     | -    | 166.4 |
| TF Influent                              | 30.3       | 212  | 141   | 7.66 | 23.9  |
| TF Effluent                              | 29.8       | 204  | 138   | 6.10 | 22.8  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 7.4   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.4       | -    | -     | 0.26 | 129.7 |
| SB Effluent                              | 28.5       | 120  | 152   | 0.08 | 75.2  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.5       | -    | -     | 0.46 | 56.3  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.7       | 148  | 148   | 0.36 | 61.0  |
| DR Effluent/OR Influent                  | 28.9       | 200  | 148   | 0.04 | 61.7  |
| OR Effluent                              | 29         | 194  | 137   | -    | 23.2  |
| Foam from OR                             | -          | -    | -     | -    | 172.3 |
| TF Influent                              | 29.2       | 192  | 137   | 6.10 | 23.3  |
| TF Effluent                              | 29         | 184  | 135   | 5.58 | 22.1  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 7.3   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 1     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.7       | -    | -     | 0.64 | 57.9  |
| SB Effluent                              | 28.6       | 111  | 144   | 0.08 | 43.3  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28.3       | -    | -     | 0.64 | 34.3  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.5       | 107  | 140   | 0.32 | 36.1  |
| DR Effluent/OR Influent                  | 30         | 125  | 139   | 0.06 | 36.3  |
| OR Effluent                              | 30.2       | 118  | 133   | -    | 16.3  |
| Foam from OR                             | -          | -    | -     | -    | 189.7 |
| TF Influent                              | 30.6       | 107  | 133   | 8.26 | 13.9  |
| TF Effluent                              | 30.3       | 81   | 132   | 6.26 | 14.3  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 1.8   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29.3       | -    | -     | 0.44 | 142.4 |
| SB Effluent                              | 29.3       | 116  | 152   | 0.09 | 66.1  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.3       | -    | -     | 0.52 | 47.9  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 30.8       | 160  | 148   | 0.40 | 48.8  |
| DR Effluent/OR Influent                  | 31         | 214  | 148   | 0.03 | 51.7  |
| OR Effluent                              | 31.1       | 195  | 138   | -    | 18.6  |
| Foam from OR                             | -          | -    | -     | -    | 163.3 |
| TF Influent                              | 31.8       | 194  | 138   | 5.60 | 16.6  |
| TF Effluent                              | 31.4       | 190  | 138   | 6.06 | 14.8  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 3.8   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.9       | -    | -     | 0.08 | 199.7 |
| SB Effluent                              | 29.2       | 126  | 153   | 0.06 | 101.3 |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.1       | -    | -     | 0.25 | 72.4  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 30.5       | 165  | 147   | 0.18 | 74.8  |
| DR Effluent/OR Influent                  | 30.6       | 225  | 144   | 0.03 | 78.8  |
| OR Effluent                              | 30.7       | 204  | 135   | -    | 25.7  |
| Foam from OR                             | -          | -    | -     | -    | 185.2 |
| TF Influent                              | 31.4       | 204  | 134   | 7.65 | 20.6  |
| TF Effluent                              | 30.4       | 196  | 132   | 5.75 | 19.7  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 7.4   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 2     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29.2       | -    | -     | 2.05 | 55.2  |
| SB Effluent                              | 29.8       | 108  | 150   | 1.35 | 30.6  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.9       | -    | -     | 1.18 | 22.4  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.8       | 123  | 146   | 0.61 | 24.3  |
| DR Effluent/OR Influent                  | 30         | 128  | 144   | 0.03 | 27.9  |
| OR Effluent                              | 30         | 120  | 137   | -    | 11.7  |
| Foam from OR                             | -          | -    | -     | -    | 227.9 |
| TF Influent                              | 30.4       | 122  | 135   | 6.34 | 10.1  |
| TF Effluent                              | 30.2       | 102  | 134   | 5.81 | 11.0  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.0   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.7       | -    | -     | 0.13 | 173.3 |
| SB Effluent                              | 29.4       | 126  | 170   | 0.15 | 79.7  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.7       | -    | -     | 0.13 | 62.4  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.5       | 168  | 164   | 0.55 | 63.9  |
| DR Effluent/OR Influent                  | 30.3       | 225  | 162   | 0.05 | 63.9  |
| OR Effluent                              | 30.3       | 218  | 150   | -    | 21.7  |
| Foam from OR                             | -          | -    | -     | -    | 164.1 |
| TF Influent                              | 30.3       | 222  | 148   | 7.66 | 20.3  |
| TF Effluent                              | 29.6       | 216  | 146   | 6.10 | 17.7  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.7   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.5       | -    | -     | 0.10 | 181.5 |
| SB Effluent                              | 28.2       | 109  | 144   | 0.12 | 84.3  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.5       | -    | -     | 1.00 | 66.1  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.8       | 138  | 140   | 0.57 | 68.8  |
| DR Effluent/OR Influent                  | 29.7       | 184  | 140   | 0.06 | 69.7  |
| OR Effluent                              | 29.7       | 162  | 132   | -    | 22.4  |
| Foam from OR                             | -          | -    | -     | -    | 174.8 |
| TF Influent                              | 29.7       | 162  | 134   | 8.26 | 17.9  |
| TF Effluent                              | 29.8       | 152  | 134   | 6.26 | 17.3  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.5   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 3     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28         | -    | -     | 1.35 | 44.3  |
| SB Effluent                              | 28.1       | 108  | 152   | 1.18 | 27.0  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.8       | -    | -     | 1.25 | 22.8  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.5       | 124  | 146   | 0.55 | 25.3  |
| DR Effluent/OR Influent                  | 28.8       | 140  | 144   | 0.03 | 27.2  |
| OR Effluent                              | 30         | 130  | 138   | -    | 15.0  |
| Foam from OR                             | -          | -    | -     | -    | 171.7 |
| TF Influent                              | 30.1       | 129  | 138   | 7.25 | 9.9   |
| TF Effluent                              | 29         | 114  | 136   | 6.18 | 9.8   |
| Final Effluent (CF)                      | -          | -    | -     | -    | 1.6   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 27.8       | -    | -     | 0.26 | 165.2 |
| SB Effluent                              | 27.7       | 114  | 152   | 0.06 | 77.0  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.6       | -    | -     | 0.28 | 61.5  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.7       | 142  | 148   | 0.12 | 63.5  |
| DR Effluent/OR Influent                  | 28.9       | 198  | 148   | 0.00 | 63.2  |
| OR Effluent                              | 28.2       | 186  | 140   | -    | 27.2  |
| Foam from OR                             | -          | -    | -     | -    | 149.0 |
| TF Influent                              | 28.9       | 184  | 142   | 7.44 | 19.0  |
| TF Effluent                              | 28         | 180  | 140   | 6.33 | 19.9  |
| Final Effl.                              | -          | -    | -     | -    | 7.1   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29.1       | -    | -     | 0.10 | 142.1 |
| SB Effluent                              | 29         | 126  | 144   | 0.08 | 67.0  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28.8       | -    | -     | 0.40 | 51.5  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.6       | 165  | 137   | 0.15 | 53.3  |
| DR Effluent/OR Influent                  | 29.7       | 217  | 137   | 0.03 | 53.3  |
| OR Effluent                              | 30.3       | 192  | 131   | -    | 19.7  |
| Foam from OR                             | -          | -    | -     | -    | 177.0 |
| TF Influent                              | 30.3       | 185  | 130   | 7.38 | 17.0  |
| TF Effluent                              | 29.5       | 177  | 128   | 6.51 | 16.1  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.0   |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) | Test 1     |      |       |       |       |
|--|------------|------|-------|-------|-------|
| Time                                     | 2:00 p.m.  |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 28.2       | -    | -     | 2.20  | 38.8  |
| SB Effluent                              | 28.2       | 112  | 145   | 1.42  | 28.8  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 28         | -    | -     | 1.00  | 31.9  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 28.6       | 120  | 142   | 0.38  | 33.3  |
| DR Effluent/OR Influent                  | 29         | 138  | 140   | 0.00  | 33.3  |
| OR Effluent                              | 29.3       | 131  | 129   | -     | 11.8  |
| Foam from OR                             | -          | -    | -     | -     | 160.6 |
| TF Influent                              | 29.5       | 137  | 130   | 10.42 | 11.2  |
| TF Effluent                              | 29.1       | 122  | 128   | 6.31  | 10.6  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 1.8   |
| Time                                     | 10:00 p.m. |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 29.1       | -    | -     | 0.10  | 147.9 |
| SB Effluent                              | 29         | 136  | 165   | 0.08  | 80.6  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 28.9       | -    | -     | 0.54  | 64.3  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 30.4       | 170  | 154   | 0.37  | 69.7  |
| DR Effluent/OR Influent                  | 30.3       | 210  | 152   | 0.04  | 74.6  |
| OR Effluent                              | 30.4       | 183  | 140   | -     | 17.5  |
| Foam from OR                             | -          | -    | -     | -     | 163.3 |
| TF Influent                              | 30.3       | 184  | 138   | 10.67 | 16.1  |
| TF Effluent                              | 29.5       | 176  | 136   | 6.39  | 13.3  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 5.1   |
| Time                                     | 6:00 a.m.  |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 28         | -    | -     | 0.22  | 167.2 |
| SB Effluent                              | 28.2       | 123  | 139   | 0.09  | 78.8  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 28.1       | -    | -     | 0.45  | 61.5  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 29.6       | 160  | 136   | 0.32  | 68.8  |
| DR Effluent/OR Influent                  | 29.9       | 196  | 134   | 0.03  | 68.8  |
| OR Effluent                              | 30         | 170  | 125   | -     | 18.3  |
| Foam from OR                             | -          | -    | -     | -     | 190.6 |
| TF Influent                              | 29.7       | 173  | 126   | 10.44 | 16.1  |
| TF Effluent                              | 28         | 168  | 126   | 6.35  | 15.2  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 3.4   |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) |       | Test 2     |       |       |       |  |
|--|-------|------------|-------|-------|-------|--|
| Time                                     |       | 2:00 p.m.  |       |       |       |  |
| Parameter                                | Temp. | Alk.       | Hard. | D.O.  | NTU   |  |
| Raw Water                                | 28.8  | -          | -     | 1.25  | 47.5  |  |
| SB Effluent                              | 28.2  | 126        | 140   | 0.90  | 31.5  |  |
| Sludge SB                                | -     | -          | -     | -     | 4.3   |  |
| MSF Effluent                             | 29.9  | -          | -     | 0.82  | 33.3  |  |
| Sludge MSF                               | -     | -          | -     | -     | 4.3   |  |
| DR. Influent/MB                          | 29.3  | 132        | 139   | 0.32  | 39.9  |  |
| DR Effluent/OR Influent                  | 29.2  | 148        | 138   | 0.00  | 39.7  |  |
| OR Effluent                              | 29.6  | 130        | 126   | -     | 12.1  |  |
| Foam from OR                             | -     | -          | -     | -     | 179.7 |  |
| TF Influent                              | 29.9  | 130        | 128   | 10.26 | 11.3  |  |
| TF Effluent                              | 29.6  | 120        | 124   | 6.20  | 11.5  |  |
| Final Effluent (CF)                      | -     | -          | -     | -     | 1.8   |  |
| Time                                     |       | 10:00 p.m. |       |       |       |  |
| Parameter                                | Temp. | Alk.       | Hard. | D.O.  | NTU   |  |
| Raw Water                                | 28.6  | -          | -     | 0.18  | 160.6 |  |
| SB Effluent                              | 29.2  | 128        | 156   | 0.12  | 97.0  |  |
| Sludge SB                                | -     | -          | -     | -     | 4.3   |  |
| MSF Effluent                             | 29.1  | -          | -     | 0.58  | 74.3  |  |
| Sludge MSF                               | -     | -          | -     | -     | 4.3   |  |
| DR. Influent/MB                          | 29.9  | 192        | 150   | 0.15  | 85.2  |  |
| DR Effluent/OR Influent                  | 31    | 245        | 148   | 0.03  | 83.3  |  |
| OR Effluent                              | 29.9  | 222        | 137   | -     | 21.9  |  |
| Foam from OR                             | -     | -          | -     | -     | 188.3 |  |
| TF Influent                              | 30.8  | 225        | 136   | 9.83  | 23.7  |  |
| TF Effluent                              | 30    | 220        | 135   | 6.28  | 22.1  |  |
| Final Effluent (CF)                      | -     | -          | -     | -     | 4.3   |  |
| Time                                     |       | 6:00 a.m.  |       |       |       |  |
| Parameter                                | Temp. | Alk.       | Hard. | D.O.  | NTU   |  |
| Raw Water                                | 29.1  | -          | -     | 0.15  | 135.2 |  |
| SB Effluent                              | 28.9  | 141        | 145   | 0.09  | 59.7  |  |
| Sludge SB                                | -     | -          | -     | -     | 4.3   |  |
| MSF Effluent                             | 28.7  | -          | -     | 0.48  | 47.0  |  |
| Sludge MSF                               | -     | -          | -     | -     | 49.7  |  |
| DR. Influent/MB                          | 29.5  | 202        | 140   | 0.22  | 52.4  |  |
| DR Effluent/OR Influent                  | 29.7  | 247        | 137   | 0.00  | 15.5  |  |
| OR Effluent                              | 29.7  | 214        | 128   | -     | 18.4  |  |
| Foam from OR                             | -     | -          | -     | -     | 144.1 |  |
| TF Influent                              | 29.9  | 214        | 128   | 10.78 | 14.3  |  |
| TF Effluent                              | 29.2  | 200        | 128   | 6.51  | 14.6  |  |
| Final Effluent (CF)                      | -     | -          | -     | -     | 3.7   |  |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) | Test 3     |      |       |       |       |
|--|------------|------|-------|-------|-------|
| Time                                     | 2:00 p.m.  |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 28.5       | -    | -     | 1.80  | 41.3  |
| SB Effluent                              | 28.6       | 125  | 146   | 1.15  | 27.2  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 28.3       | -    | -     | 0.80  | 28.6  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 28.9       | 130  | 140   | 0.37  | 31.5  |
| DR Effluent/OR Influent                  | 30.2       | 138  | 140   | 0.00  | 32.6  |
| OR Effluent                              | 30.3       | 128  | 127   | -     | 13.4  |
| Foam from OR                             | -          | -    | -     | -     | 185.0 |
| TF Influent                              | 30.2       | 130  | 128   | 10.15 | 10.4  |
| TF Effluent                              | 29.4       | 113  | 126   | 6.80  | 10.6  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 1.7   |
| Time                                     | 10:00 p.m. |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 28.3       | -    | -     | 0.09  | 182.4 |
| SB Effluent                              | 28.2       | 135  | 148   | 0.08  | 94.1  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 27.8       | -    | -     | 0.59  | 76.6  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 28.4       | 170  | 144   | 0.28  | 78.3  |
| DR Effluent/OR Influent                  | 28.2       | 243  | 146   | 0.03  | 79.2  |
| OR Effluent                              | 28         | 218  | 136   | -     | 21.5  |
| Foam from OR                             | -          | -    | -     | -     | 166.1 |
| TF Influent                              | 28.2       | 218  | 136   | 10.46 | 21.2  |
| TF Effluent                              | 28         | 212  | 137   | 6.18  | 20.6  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 6.4   |
| Time                                     | 6:00 a.m.  |      |       |       |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O.  | NTU   |
| Raw Water                                | 28.8       | -    | -     | 0.06  | 153.3 |
| SB Effluent                              | 28.6       | 140  | 142   | 0.07  | 80.6  |
| Sludge SB                                | -          | -    | -     | -     | 4.3   |
| MSF Effluent                             | 28.5       | -    | -     | 0.78  | 65.7  |
| Sludge MSF                               | -          | -    | -     | -     | 4.3   |
| DR. Influent/MB                          | 29.2       | 188  | 135   | 0.44  | 71.9  |
| DR Effluent/OR Influent                  | 29.6       | 220  | 134   | 0.03  | 74.3  |
| OR Effluent                              | 29.8       | 196  | 122   | -     | 20.3  |
| Foam from OR                             | -          | -    | -     | -     | 180.6 |
| TF Influent                              | 30.1       | 196  | 122   | 10.59 | 22.4  |
| TF Effluent                              | 29.6       | 184  | 120   | 6.65  | 21.9  |
| Final Effluent (CF)                      | -          | -    | -     | -     | 5.6   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) | Test 1     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.2       | -    | -     | 1.60 | 42.4  |
| SB Effluent                              | 28         | 117  | 148   | 1.28 | 27.2  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 27.9       | -    | -     | 1.16 | 22.4  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.4       | 130  | 142   | 0.38 | 24.3  |
| DR Effluent/OR Influent                  | 28.6       | 139  | 140   | 0.03 | 25.0  |
| OR Effluent                              | 28.9       | 126  | 132   | -    | 14.4  |
| Foam from OR                             | -          | -    | -     | -    | 155.2 |
| TF Influent                              | 30         | 126  | 132   | 8.90 | 9.9   |
| TF Effluent                              | 29.5       | 115  | 130   | 6.05 | 9.5   |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.5   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29         | -    | -     | 0.14 | 168.1 |
| SB Effluent                              | 29.4       | 114  | 150   | 0.06 | 81.5  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.4       | -    | -     | 0.32 | 67.9  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 31         | 148  | 145   | 0.08 | 74.3  |
| DR Effluent/OR Influent                  | 31         | 175  | 144   | 0.03 | 73.7  |
| OR Effluent                              | 31.1       | 167  | 135   | -    | 23.9  |
| Foam from OR                             | -          | -    | -     | -    | 177.9 |
| TF Influent                              | 31.2       | 164  | 134   | 8.48 | 18.4  |
| TF Effluent                              | 30.1       | 155  | 134   | 5.60 | 18.1  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 5.1   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.6       | -    | -     | 0.10 | 148.8 |
| SB Effluent                              | 28.9       | 122  | 156   | 0.08 | 75.2  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29.4       | -    | -     | 0.26 | 53.3  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.5       | 161  | 152   | 0.10 | 54.1  |
| DR Effluent/OR Influent                  | 29.7       | 228  | 156   | 0.00 | 52.1  |
| OR Effluent                              | 29.8       | 209  | 148   | -    | 21.3  |
| Foam from OR                             | -          | -    | -     | -    | 174.3 |
| TF Influent                              | 29.8       | 207  | 150   | 8.56 | 18.1  |
| TF Effluent                              | 29.1       | 192  | 147   | 5.89 | 17.0  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.6   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) | Test 2     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.6       | -    | -     | 1.82 | 35.2  |
| SB Effluent                              | 28.7       | 110  | 156   | 1.50 | 22.8  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28.3       | -    | -     | 1.28 | 25.2  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 29.4       | 123  | 148   | 0.46 | 25.7  |
| DR Effluent/OR Influent                  | 29.6       | 132  | 148   | 0.03 | 26.1  |
| OR Effluent                              | 29.7       | 120  | 141   | -    | 12.1  |
| Foam from OR                             | -          | -    | -     | -    | 150.8 |
| TF Influent                              | 29.9       | 120  | 140   | 8.88 | 7.5   |
| TF Effluent                              | 29.2       | 110  | 142   | 6.45 | 7.7   |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.4   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 29         | -    | -     | 0.10 | 177.0 |
| SB Effluent                              | 29.1       | 108  | 140   | 0.08 | 81.5  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 29         | -    | -     | -    | 71.2  |
| Sludge MSF                               | -          | -    | -     | 0.44 | 4.3   |
| DR. Influent/MB                          | 29.4       | 146  | 132   | 0.15 | 76.3  |
| DR Effluent/OR Influent                  | 29.7       | 200  | 132   | -    | 76.6  |
| OR Effluent                              | 29.8       | 185  | 130   | -    | 25.9  |
| Foam from OR                             | -          | -    | -     | -    | 176.1 |
| TF Influent                              | 30         | 185  | 130   | 8.60 | 17.0  |
| TF Effluent                              | 29.1       | 173  | 126   | 5.75 | 16.1  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.5   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28         | -    | -     | 0.18 | 147.9 |
| SB Effluent                              | 27.8       | 117  | 148   | 0.08 | 55.5  |
| Sludge SB                                | -          | -    | -     | -    | 66.1  |
| MSF Effluent                             | 27.7       | -    | -     | 0.39 | 46.1  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.2       | 155  | 142   | 0.12 | 49.7  |
| DR Effluent/OR Influent                  | 28.5       | 210  | 140   | 0.03 | 52.4  |
| OR Effluent                              | 29         | 195  | 130   | -    | 20.4  |
| Foam from OR                             | -          | -    | -     | -    | 149.0 |
| TF Influent                              | 29.4       | 192  | 128   | 8.68 | 16.1  |
| TF Effluent                              | 29         | 181  | 130   | 6.10 | 13.3  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 3.8   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) | Test 3     |      |       |      |       |
|--|------------|------|-------|------|-------|
| Time                                     | 2:00 p.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.3       | -    | -     | 1.92 | 42.4  |
| SB Effluent                              | 28.2       | 79   | 156   | 1.70 | 24.3  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28         | -    | -     | 1.00 | 26.8  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | -          | 106  | 150   | 0.56 | 31.2  |
| DR Effluent/OR Influent                  | 28.8       | 117  | 150   | 0.21 | 31.5  |
| OR Effluent                              | 28.9       | 100  | 141   | -    | 13.2  |
| Foam from OR                             | -          | -    | -     | -    | 151.9 |
| TF Influent                              | 29.7       | 98   | 140   | 8.94 | 7.9   |
| TF Effluent                              | 29.4       | 86   | 136   | 6.40 | 8.1   |
| Final Effluent (CF)                      | -          | -    | -     | -    | 2.8   |
| Time                                     | 10:00 p.m. |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 27.9       | -    | -     | 0.15 | 157.0 |
| SB Effluent                              | 28         | 114  | 146   | 0.08 | 77.0  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28         | -    | -     | 0.46 | 63.3  |
| Sludge MSF                               | -          | -    | -     | -    | 67.9  |
| DR. Influent/MB                          | 28.6       | 174  | 137   | 0.16 | 68.8  |
| DR Effluent/OR Influent                  | 28.9       | 212  | 137   | 0.00 | 19.7  |
| OR Effluent                              | 29.1       | 197  | 130   | -    | 21.7  |
| Foam from OR                             | -          | -    | -     | -    | 175.2 |
| TF Influent                              | 29.1       | 195  | 128   | 8.14 | 15.2  |
| TF Effluent                              | 28.5       | 186  | 126   | 5.60 | 15.5  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 5.3   |
| Time                                     | 6:00 a.m.  |      |       |      |       |
| Parameter                                | Temp.      | Alk. | Hard. | D.O. | NTU   |
| Raw Water                                | 28.8       | -    | -     | 0.10 | 135.2 |
| SB Effluent                              | 29         | 110  | 145   | 0.10 | 66.1  |
| Sludge SB                                | -          | -    | -     | -    | 4.3   |
| MSF Effluent                             | 28.4       | -    | -     | 0.35 | 45.2  |
| Sludge MSF                               | -          | -    | -     | -    | 4.3   |
| DR. Influent/MB                          | 28.9       | 168  | 138   | 0.10 | 58.8  |
| DR Effluent/OR Influent                  | 29.4       | 215  | 138   | 0.03 | 60.6  |
| OR Effluent                              | 29.5       | 198  | 130   | -    | 21.5  |
| Foam from OR                             | -          | -    | -     | -    | 163.3 |
| TF Influent                              | 29.7       | 198  | 130   | 8.50 | 16.1  |
| TF Effluent                              | 29.2       | 181  | 128   | 6.00 | 15.5  |
| Final Effluent (CF)                      | -          | -    | -     | -    | 4.7   |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 1     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1080       | 179  | 901  | 66   | 54   | 12  |
| SB Effluent                              | 1021       | 136  | 885  | 40   | 34   | 6   |
| Sludge SB                                | 1280       | 360  | 920  | 168  | 130  | 38  |
| MSF Effluent                             | 998        | 126  | 872  | 34   | 29   | 5   |
| Sludge MSF                               | 1005       | 116  | 889  | 52   | 44   | 8   |
| DR. Influent/MB                          | 1062       | 190  | 872  | 42   | 35   | 7   |
| DR Effluent/OR Influent                  | 1116       | 215  | 901  | 54   | 45   | 9   |
| OR Effluent                              | 978        | 130  | 848  | 26   | 21   | 5   |
| Foam from OR                             | 2088       | 1140 | 948  | 612  | 520  | 92  |
| TF Influent                              | 960        | 124  | 836  | 22   | 18   | 4   |
| TF Effluent                              | 932        | 95   | 837  | 25   | 20   | 5   |
| Final Effluent (CF)                      | 835        | 74   | 761  | 5    | 4    | 1   |
| Time                                     | 10.00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1660       | 608  | 1052 | 505  | 412  | 93  |
| SB Effluent                              | 1362       | 348  | 1014 | 143  | 126  | 17  |
| Sludge SB                                | 2850       | 1680 | 1170 | 2024 | 1592 | 432 |
| MSF Effluent                             | 1314       | 306  | 1008 | 130  | 111  | 19  |
| Sludge MSF                               | 1365       | 320  | 1045 | 160  | 127  | 33  |
| DR. Influent/MB                          | 1363       | 342  | 1021 | 158  | 131  | 27  |
| DR Effluent/OR Influent                  | 1412       | 350  | 1062 | 180  | 159  | 21  |
| OR Effluent                              | 1240       | 248  | 992  | 45   | 39   | 6   |
| Foam from OR                             | 1695       | 682  | 1013 | 668  | 570  | 98  |
| TF Influent                              | 1218       | 216  | 1002 | 42   | 38   | 4   |
| TF Effluent                              | 1210       | 212  | 998  | 46   | 41   | 5   |
| Final Effluent (CF)                      | 1092       | 182  | 910  | 10   | 8    | 2   |
| Time                                     | 6.00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1796       | 710  | 1086 | 535  | 437  | 98  |
| SB Effluent                              | 1385       | 355  | 1030 | 138  | 116  | 22  |
| Sludge SB                                | 3385       | 2115 | 1270 | 2190 | 1790 | 400 |
| MSF Effluent                             | 1310       | 298  | 1012 | 108  | 94   | 14  |
| Sludge MSF                               | 1414       | 375  | 1039 | 158  | 133  | 25  |
| DR. Influent/MB                          | 1430       | 368  | 1062 | 137  | 122  | 15  |
| DR Effluent/OR Influent                  | 1486       | 392  | 1094 | 179  | 158  | 21  |
| OR Effluent                              | 1286       | 265  | 1021 | 42   | 37   | 5   |
| Foam from OR                             | 1916       | 814  | 1102 | 518  | 385  | 133 |
| TF Influent                              | 1262       | 250  | 1012 | 40   | 34   | 6   |
| TF Effluent                              | 1240       | 236  | 1004 | 45   | 37   | 8   |
| Final Effluent (CF)                      | 1118       | 196  | 922  | 10   | 9    | 1   |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 2     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1224       | 118  | 1106 | 55   | 45   | 10  |
| SB Effluent                              | 1114       | 94   | 1020 | 36   | 30   | 6   |
| Sludge SB                                | 1586       | 208  | 1378 | 146  | 121  | 25  |
| MSF Effluent                             | 1119       | 130  | 989  | 31   | 26   | 5   |
| Sludge MSF                               | 1152       | 148  | 1004 | 68   | 58   | 10  |
| DR. Influent/MB                          | 1171       | 153  | 1018 | 45   | 38   | 7   |
| DR Effluent/OR Influent                  | 1229       | 186  | 1043 | 53   | 46   | 7   |
| OR Effluent                              | 1095       | 108  | 987  | 22   | 19   | 3   |
| Foam from OR                             | 2240       | 1060 | 1180 | 202  | 116  | 86  |
| TF Influent                              | 1079       | 102  | 977  | 22   | 18   | 4   |
| TF Effluent                              | 1042       | 72   | 970  | 27   | 22   | 5   |
| Final Effluent (CF)                      | 996        | 59   | 937  | 5    | 4    | 1   |
| Time                                     | 10:00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1678       | 675  | 1003 | 554  | 464  | 90  |
| SB Effluent                              | 1280       | 350  | 930  | 171  | 142  | 29  |
| Sludge SB                                | 3264       | 2011 | 1253 | 2080 | 1761 | 319 |
| MSF Effluent                             | 1252       | 312  | 940  | 113  | 96   | 17  |
| Sludge MSF                               | 1334       | 372  | 962  | 180  | 152  | 28  |
| DR. Influent/MB                          | 1304       | 351  | 953  | 162  | 135  | 27  |
| DR Effluent/OR Influent                  | 1364       | 418  | 946  | 184  | 163  | 21  |
| OR Effluent                              | 1169       | 260  | 909  | 39   | 31   | 8   |
| Foam from OR                             | 1895       | 814  | 1081 | 674  | 525  | 149 |
| TF Influent                              | 1137       | 255  | 882  | 34   | 28   | 6   |
| TF Effluent                              | 1142       | 242  | 900  | 39   | 32   | 7   |
| Final Effluent (CF)                      | 1023       | 168  | 855  | 8    | 6    | 2   |
| Time                                     | 6:00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1880       | 720  | 1160 | 592  | 493  | 99  |
| SB Effluent                              | 1405       | 371  | 1034 | 206  | 172  | 34  |
| Sludge SB                                | 3742       | 2185 | 1557 | 2088 | 1779 | 309 |
| MSF Effluent                             | 1373       | 328  | 1045 | 154  | 129  | 25  |
| Sludge MSF                               | 1456       | 360  | 1096 | 230  | 189  | 41  |
| DR. Influent/MB                          | 1408       | 349  | 1059 | 164  | 141  | 23  |
| DR Effluent/OR Influent                  | 1468       | 411  | 1057 | 198  | 169  | 29  |
| OR Effluent                              | 1204       | 220  | 984  | 44   | 37   | 7   |
| Foam from OR                             | 2095       | 915  | 1180 | 642  | 289  | 353 |
| TF Influent                              | 1238       | 214  | 1024 | 38   | 34   | 4   |
| TF Effluent                              | 1230       | 216  | 1014 | 44   | 39   | 5   |
| Final Effluent (CF)                      | 1114       | 151  | 963  | 7    | 5    | 2   |

Table A31 continued.

| Stage 1 (22 mg O <sub>3</sub> /L; 6 Lpm) | Test 3     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1156       | 145  | 1011 | 69   | 58   | 11  |
| SB Effluent                              | 1015       | 120  | 895  | 32   | 27   | 5   |
| Sludge SB                                | 1660       | 246  | 1414 | 225  | 190  | 35  |
| MSF Effluent                             | 1005       | 103  | 902  | 35   | 29   | 6   |
| Sludge MSF                               | 1082       | 160  | 922  | 55   | 42   | 13  |
| DR. Influent/MB                          | 1112       | 164  | 948  | 41   | 35   | 6   |
| DR Effluent/OR Influent                  | 1120       | 170  | 950  | 50   | 43   | 7   |
| OR Effluent                              | 980        | 95   | 885  | 18   | 16   | 2   |
| Foam from OR                             | 1962       | 982  | 980  | 421  | 341  | 80  |
| TF Influent                              | 964        | 85   | 879  | 21   | 18   | 3   |
| TF Effluent                              | 981        | 90   | 891  | 25   | 20   | 5   |
| Final Effluent (CF)                      | 912        | 68   | 844  | 3    | 2    | 1   |
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2260       | 895  | 1365 | 650  | 548  | 102 |
| SB Effluent                              | 1657       | 532  | 1125 | 208  | 175  | 33  |
| Sludge SB                                | 4692       | 2374 | 2318 | 2418 | 2040 | 378 |
| MSF Effluent                             | 1522       | 498  | 1024 | 170  | 151  | 19  |
| Sludge MSF                               | 1639       | 570  | 1069 | 250  | 218  | 32  |
| DR. Influent/MB                          | 1596       | 545  | 1051 | 168  | 150  | 18  |
| DR Effluent/OR Influent                  | 1620       | 558  | 1062 | 187  | 164  | 23  |
| OR Effluent                              | 1382       | 392  | 990  | 54   | 46   | 8   |
| Foam from OR                             | 2026       | 928  | 1098 | 344  | 265  | 79  |
| TF Influent                              | 1330       | 341  | 989  | 47   | 42   | 5   |
| TF Effluent                              | 1327       | 314  | 1013 | 51   | 45   | 6   |
| Final Effluent (CF)                      | 1187       | 235  | 952  | 12   | 10   | 2   |
| Time                                     | 10:00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1634       | 712  | 922  | 523  | 452  | 71  |
| SB Effluent                              | 1248       | 390  | 858  | 144  | 125  | 19  |
| Sludge SB                                | 3265       | 2112 | 1153 | 2110 | 1818 | 292 |
| MSF Effluent                             | 1148       | 356  | 792  | 112  | 92   | 20  |
| Sludge MSF                               | 1156       | 395  | 761  | 165  | 139  | 26  |
| DR. Influent/MB                          | 1267       | 422  | 845  | 129  | 104  | 25  |
| DR Effluent/OR Influent                  | 1285       | 422  | 863  | 159  | 127  | 32  |
| OR Effluent                              | 1088       | 295  | 793  | 35   | 28   | 7   |
| Foam from OR                             | 1816       | 831  | 985  | 360  | 286  | 74  |
| TF Influent                              | 1098       | 291  | 807  | 29   | 22   | 7   |
| TF Effluent                              | 1065       | 255  | 810  | 35   | 27   | 8   |
| Final Effluent (CF)                      | 962        | 194  | 768  | 8    | 6    | 2   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 1     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1380       | 405  | 975  | 264  | 226  | 38  |
| SB Effluent                              | 1120       | 216  | 904  | 88   | 72   | 16  |
| Sludge SB                                | 2308       | 1156 | 1152 | 980  | 858  | 122 |
| MSF Effluent                             | 1026       | 192  | 834  | 55   | 47   | 8   |
| Sludge MSF                               | 1082       | 244  | 838  | 129  | 104  | 25  |
| DR. Influent/MB                          | 1068       | 208  | 860  | 65   | 56   | 9   |
| DR Effluent/OR Influent                  | 1106       | 252  | 854  | 60   | 50   | 10  |
| OR Effluent                              | 997        | 180  | 817  | 24   | 20   | 4   |
| Foam from OR                             | 1968       | 872  | 1096 | 487  | 390  | 97  |
| TF Influent                              | 960        | 168  | 792  | 26   | 21   | 5   |
| TF Effluent                              | 958        | 160  | 798  | 29   | 22   | 7   |
| Final Effluent (CF)                      | 816        | 55   | 761  | 5    | 4    | 1   |
| Time                                     | 10.00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1920       | 621  | 1299 | 660  | 541  | 119 |
| SB Effluent                              | 1348       | 360  | 988  | 140  | 122  | 18  |
| Sludge SB                                | 4280       | 1710 | 2570 | 2665 | 2166 | 499 |
| MSF Effluent                             | 1272       | 340  | 932  | 83   | 69   | 14  |
| Sludge MSF                               | 1360       | 400  | 960  | 180  | 140  | 40  |
| DR. Influent/MB                          | 1274       | 344  | 930  | 114  | 96   | 18  |
| DR Effluent/OR Influent                  | 1352       | 368  | 984  | 118  | 97   | 21  |
| OR Effluent                              | 1186       | 268  | 918  | 30   | 27   | 3   |
| Foam from OR                             | 2075       | 960  | 1115 | 382  | 276  | 106 |
| TF Influent                              | 1156       | 256  | 900  | 28   | 26   | 2   |
| TF Effluent                              | 1102       | 216  | 886  | 30   | 27   | 3   |
| Final Effluent (CF)                      | 939        | 118  | 821  | 6    | 5    | 1   |
| Time                                     | 6.00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2426       | 1362 | 1064 | 993  | 824  | 169 |
| SB Effluent                              | 1456       | 500  | 956  | 214  | 188  | 26  |
| Sludge SB                                | 6329       | 4845 | 1484 | 4265 | 3493 | 772 |
| MSF Effluent                             | 1312       | 384  | 928  | 128  | 108  | 20  |
| Sludge MSF                               | 1472       | 577  | 895  | 232  | 200  | 32  |
| DR. Influent/MB                          | 1384       | 440  | 944  | 159  | 126  | 33  |
| DR Effluent/OR Influent                  | 1416       | 476  | 940  | 164  | 128  | 36  |
| OR Effluent                              | 1212       | 344  | 868  | 45   | 37   | 8   |
| Foam from OR                             | 2246       | 1122 | 1124 | 634  | 496  | 138 |
| TF Influent                              | 1170       | 318  | 852  | 40   | 34   | 6   |
| TF Effluent                              | 1185       | 326  | 859  | 43   | 35   | 8   |
| Final Effluent (CF)                      | 1034       | 217  | 817  | 11   | 9    | 2   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) |            | Test 2 |      |      |      |     |
|--|------------|--------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1205       | 248    | 957  | 198  | 170  | 28  |
| SB Effluent                              | 1056       | 116    | 940  | 59   | 52   | 7   |
| Sludge SB                                | 1790       | 802    | 988  | 765  | 645  | 120 |
| MSF Effluent                             | 964        | 100    | 864  | 35   | 30   | 5   |
| Sludge MSF                               | 1064       | 180    | 884  | 95   | 80   | 15  |
| DR. Influent/MB                          | 1011       | 148    | 863  | 43   | 38   | 5   |
| DR Effluent/OR Influent                  | 1088       | 242    | 846  | 49   | 40   | 9   |
| OR Effluent                              | 932        | 128    | 804  | 19   | 17   | 2   |
| Foam from OR                             | 2992       | 1984   | 1008 | 547  | 433  | 114 |
| TF Influent                              | 925        | 120    | 805  | 18   | 16   | 2   |
| TF Effluent                              | 912        | 98     | 814  | 25   | 21   | 4   |
| Final Effluent (CF)                      | 832        | 68     | 764  | 5    | 4    | 1   |
| Time                                     | 10.00 p.m. |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1978       | 982    | 996  | 716  | 602  | 114 |
| SB Effluent                              | 1445       | 512    | 933  | 265  | 228  | 37  |
| Sludge SB                                | 4135       | 2892   | 1243 | 2582 | 2148 | 434 |
| MSF Effluent                             | 1320       | 442    | 878  | 125  | 105  | 20  |
| Sludge MSF                               | 1424       | 556    | 868  | 272  | 220  | 52  |
| DR. Influent/MB                          | 1415       | 490    | 925  | 138  | 115  | 23  |
| DR Effluent/OR Influent                  | 1409       | 502    | 907  | 142  | 116  | 26  |
| OR Effluent                              | 1158       | 324    | 834  | 40   | 33   | 7   |
| Foam from OR                             | 2076       | 1100   | 976  | 340  | 284  | 56  |
| TF Influent                              | 1120       | 296    | 824  | 35   | 29   | 6   |
| TF Effluent                              | 1085       | 256    | 829  | 39   | 32   | 7   |
| Final Effluent (CF)                      | 962        | 182    | 780  | 9    | 7    | 2   |
| Time                                     | 6.00 a.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1924       | 992    | 932  | 848  | 710  | 138 |
| SB Effluent                              | 1368       | 440    | 928  | 241  | 202  | 39  |
| Sludge SB                                | 4192       | 3268   | 924  | 3350 | 2811 | 539 |
| MSF Effluent                             | 1316       | 433    | 883  | 142  | 121  | 21  |
| Sludge MSF                               | 1388       | 518    | 870  | 364  | 251  | 113 |
| DR. Influent/MB                          | 1375       | 476    | 899  | 163  | 126  | 37  |
| DR Effluent/OR Influent                  | 1374       | 490    | 884  | 170  | 128  | 42  |
| OR Effluent                              | 1138       | 324    | 814  | 44   | 38   | 6   |
| Foam from OR                             | 1891       | 896    | 995  | 382  | 314  | 68  |
| TF Influent                              | 1136       | 318    | 818  | 41   | 34   | 7   |
| TF Effluent                              | 1106       | 295    | 811  | 43   | 35   | 8   |
| Final Effluent (CF)                      | 1008       | 204    | 804  | 10   | 8    | 2   |

Table A31 continued.

| Stage 2 (33 mg O <sub>3</sub> /L; 6 Lpm) | Test 3     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1215       | 174  | 1041 | 58   | 48   | 10  |
| SB Effluent                              | 1105       | 120  | 985  | 40   | 33   | 7   |
| Sludge SB                                | 1625       | 385  | 1240 | 120  | 101  | 19  |
| MSF Effluent                             | 1072       | 105  | 967  | 32   | 26   | 6   |
| Sludge MSF                               | 1160       | 148  | 1012 | 67   | 51   | 16  |
| DR. Influent/MB                          | 1212       | 229  | 983  | 48   | 38   | 10  |
| DR Effluent/OR Influent                  | 1220       | 252  | 968  | 54   | 45   | 9   |
| OR Effluent                              | 1095       | 175  | 920  | 23   | 20   | 3   |
| Foam from OR                             | 2086       | 1082 | 1004 | 412  | 326  | 86  |
| TF Influent                              | 1074       | 158  | 916  | 21   | 19   | 2   |
| TF Effluent                              | 1056       | 135  | 921  | 24   | 18   | 6   |
| Final Effluent (CF)                      | 958        | 70   | 888  | 4    | 3    | 1   |
| Time                                     | 10.00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2214       | 958  | 1256 | 680  | 563  | 117 |
| SB Effluent                              | 1614       | 475  | 1139 | 190  | 160  | 30  |
| Sludge SB                                | 4811       | 3070 | 1741 | 2675 | 2215 | 460 |
| MSF Effluent                             | 1522       | 512  | 1010 | 106  | 92   | 14  |
| Sludge MSF                               | 1690       | 640  | 1050 | 170  | 139  | 31  |
| DR. Influent/MB                          | 1628       | 563  | 1065 | 112  | 94   | 18  |
| DR Effluent/OR Influent                  | 1642       | 580  | 1062 | 119  | 98   | 21  |
| OR Effluent                              | 1354       | 364  | 990  | 50   | 44   | 6   |
| Foam from OR                             | 2288       | 1110 | 1178 | 326  | 234  | 92  |
| TF Influent                              | 1305       | 332  | 973  | 43   | 38   | 5   |
| TF Effluent                              | 1254       | 284  | 970  | 48   | 41   | 7   |
| Final Effluent (CF)                      | 1096       | 194  | 902  | 6    | 5    | 1   |
| Time                                     | 6.00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1935       | 722  | 1213 | 553  | 455  | 98  |
| SB Effluent                              | 1492       | 391  | 1101 | 158  | 130  | 28  |
| Sludge SB                                | 3710       | 2185 | 1525 | 2118 | 1744 | 374 |
| MSF Effluent                             | 1363       | 325  | 1038 | 122  | 105  | 17  |
| Sludge MSF                               | 1470       | 410  | 1060 | 187  | 135  | 52  |
| DR. Influent/MB                          | 1526       | 432  | 1094 | 139  | 126  | 13  |
| DR Effluent/OR Influent                  | 1529       | 429  | 1100 | 160  | 150  | 10  |
| OR Effluent                              | 1256       | 243  | 1013 | 38   | 31   | 7   |
| Foam from OR                             | 2204       | 912  | 1292 | 435  | 381  | 54  |
| TF Influent                              | 1214       | 218  | 996  | 24   | 21   | 3   |
| TF Effluent                              | 1154       | 166  | 988  | 29   | 25   | 4   |
| Final Effluent (CF)                      | 1033       | 114  | 919  | 5    | 4    | 1   |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) | Test 1     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1148       | 139  | 1009 | 60   | 51   | 9   |
| SB Effluent                              | 1118       | 120  | 998  | 41   | 35   | 6   |
| Sludge SB                                | 1269       | 220  | 1049 | 121  | 105  | 16  |
| MSF Effluent                             | 1099       | 132  | 967  | 39   | 33   | 6   |
| Sludge MSF                               | 1148       | 153  | 995  | 88   | 59   | 29  |
| DR. Influent/MB                          | 1154       | 173  | 981  | 45   | 35   | 10  |
| DR Effluent/OR Influent                  | 1196       | 210  | 986  | 44   | 35   | 9   |
| OR Effluent                              | 1079       | 128  | 951  | 21   | 17   | 4   |
| Foam from OR                             | 2389       | 1264 | 1125 | 368  | 262  | 106 |
| TF Influent                              | 1068       | 122  | 946  | 18   | 16   | 2   |
| TF Effluent                              | 1032       | 94   | 938  | 21   | 15   | 6   |
| Final Effluent (CF)                      | 912        | 35   | 877  | 3    | 2    | 1   |
| Time                                     | 10.00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1684       | 840  | 844  | 664  | 554  | 110 |
| SB Effluent                              | 1220       | 416  | 804  | 166  | 139  | 27  |
| Sludge SB                                | 3595       | 2610 | 985  | 2555 | 2126 | 429 |
| MSF Effluent                             | 1154       | 360  | 794  | 115  | 100  | 15  |
| Sludge MSF                               | 1266       | 468  | 798  | 270  | 222  | 48  |
| DR. Influent/MB                          | 1168       | 354  | 814  | 124  | 108  | 16  |
| DR Effluent/OR Influent                  | 1236       | 424  | 812  | 130  | 110  | 20  |
| OR Effluent                              | 1042       | 288  | 754  | 39   | 31   | 8   |
| Foam from OR                             | 1940       | 1085 | 855  | 378  | 321  | 57  |
| TF Influent                              | 1028       | 280  | 748  | 34   | 28   | 6   |
| TF Effluent                              | 1019       | 272  | 747  | 36   | 29   | 7   |
| Final Effluent (CF)                      | 842        | 128  | 714  | 6    | 5    | 1   |
| Time                                     | 6.00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2154       | 1016 | 1138 | 718  | 585  | 133 |
| SB Effluent                              | 1564       | 510  | 1054 | 192  | 162  | 30  |
| Sludge SB                                | 4640       | 3226 | 1414 | 2796 | 2280 | 516 |
| MSF Effluent                             | 1322       | 376  | 946  | 103  | 93   | 10  |
| Sludge MSF                               | 1416       | 472  | 944  | 180  | 156  | 24  |
| DR. Influent/MB                          | 1328       | 364  | 964  | 131  | 116  | 15  |
| DR Effluent/OR Influent                  | 1380       | 416  | 964  | 131  | 119  | 12  |
| OR Effluent                              | 1159       | 267  | 892  | 37   | 31   | 6   |
| Foam from OR                             | 2166       | 1196 | 970  | 436  | 372  | 64  |
| TF Influent                              | 1148       | 262  | 886  | 30   | 26   | 4   |
| TF Effluent                              | 1129       | 248  | 881  | 33   | 26   | 7   |
| Final Effluent (CF)                      | 918        | 114  | 804  | 5    | 4    | 1   |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) |            | Test 2 |      |      |      |     |
|--|------------|--------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1405       | 241    | 1164 | 111  | 92   | 19  |
| SB Effluent                              | 1197       | 133    | 1064 | 64   | 54   | 10  |
| Sludge SB                                | 2115       | 660    | 1455 | 280  | 226  | 54  |
| MSF Effluent                             | 1180       | 132    | 1048 | 39   | 31   | 8   |
| Sludge MSF                               | 1218       | 146    | 1072 | 92   | 63   | 29  |
| DR. Influent/MB                          | 1345       | 250    | 1095 | 67   | 52   | 15  |
| DR Effluent/OR Influent                  | 1340       | 255    | 1085 | 67   | 53   | 14  |
| OR Effluent                              | 1206       | 165    | 1041 | 20   | 17   | 3   |
| Foam from OR                             | 2021       | 860    | 1161 | 385  | 316  | 69  |
| TF Influent                              | 1195       | 157    | 1038 | 17   | 15   | 2   |
| TF Effluent                              | 1131       | 85     | 1046 | 19   | 16   | 3   |
| Final Effluent (CF)                      | 1020       | 43     | 977  | 3    | 2    | 1   |
| Time                                     | 10.00 p.m. |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2120       | 995    | 1125 | 839  | 676  | 163 |
| SB Effluent                              | 1432       | 492    | 940  | 194  | 163  | 31  |
| Sludge SB                                | 4896       | 3212   | 1684 | 3490 | 2806 | 684 |
| MSF Effluent                             | 1360       | 436    | 924  | 152  | 129  | 23  |
| Sludge MSF                               | 1398       | 464    | 934  | 462  | 370  | 92  |
| DR. Influent/MB                          | 1402       | 456    | 946  | 174  | 148  | 26  |
| DR Effluent/OR Influent                  | 1446       | 494    | 952  | 178  | 154  | 24  |
| OR Effluent                              | 1187       | 302    | 885  | 43   | 35   | 8   |
| Foam from OR                             | 2096       | 1094   | 1002 | 476  | 380  | 96  |
| TF Influent                              | 1160       | 296    | 864  | 38   | 30   | 8   |
| TF Effluent                              | 1091       | 232    | 859  | 41   | 34   | 7   |
| Final Effluent (CF)                      | 944        | 156    | 788  | 6    | 5    | 1   |
| Time                                     | 6.00 a.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1744       | 712    | 1032 | 518  | 419  | 99  |
| SB Effluent                              | 1336       | 420    | 916  | 165  | 140  | 25  |
| Sludge SB                                | 3298       | 1842   | 1456 | 1796 | 1410 | 386 |
| MSF Effluent                             | 1248       | 355    | 893  | 114  | 82   | 32  |
| Sludge MSF                               | 1324       | 418    | 906  | 388  | 339  | 49  |
| DR. Influent/MB                          | 1288       | 375    | 913  | 135  | 108  | 27  |
| DR Effluent/OR Influent                  | 1306       | 368    | 938  | 140  | 112  | 28  |
| OR Effluent                              | 1084       | 217    | 867  | 32   | 28   | 4   |
| Foam from OR                             | 1634       | 652    | 982  | 296  | 219  | 77  |
| TF Influent                              | 1170       | 194    | 976  | 27   | 23   | 4   |
| TF Effluent                              | 1081       | 116    | 965  | 29   | 24   | 5   |
| Final Effluent (CF)                      | 981        | 87     | 894  | 4    | 3    | 1   |

Table A31 continued.

| Stage 3 (33 mg O <sub>3</sub> /L; 4 Lpm) |            | Test 3 |      |      |      |     |
|--|------------|--------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1226       | 145    | 1081 | 53   | 44   | 9   |
| SB Effluent                              | 1168       | 112    | 1056 | 40   | 34   | 6   |
| Sludge SB                                | 1425       | 280    | 1145 | 102  | 85   | 17  |
| MSF Effluent                             | 1188       | 127    | 1061 | 36   | 29   | 7   |
| Sludge MSF                               | 1209       | 151    | 1058 | 93   | 61   | 32  |
| DR. Influent/MB                          | 1306       | 230    | 1076 | 64   | 52   | 12  |
| DR Effluent/OR Influent                  | 1295       | 223    | 1072 | 60   | 49   | 11  |
| OR Effluent                              | 1159       | 138    | 1021 | 24   | 19   | 5   |
| Foam from OR                             | 2320       | 1188   | 1132 | 460  | 402  | 58  |
| TF Influent                              | 1145       | 120    | 1025 | 20   | 16   | 4   |
| TF Effluent                              | 1090       | 82     | 1008 | 21   | 17   | 4   |
| Final Effluent (CF)                      | 986        | 34     | 952  | 3    | 2    | 1   |
| Time                                     | 10.00 p.m. |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2132       | 1061   | 1071 | 766  | 641  | 125 |
| SB Effluent                              | 1545       | 513    | 1032 | 202  | 170  | 32  |
| Sludge SB                                | 4650       | 3382   | 1268 | 3072 | 2567 | 505 |
| MSF Effluent                             | 1433       | 421    | 1012 | 151  | 125  | 26  |
| Sludge MSF                               | 1559       | 520    | 1039 | 286  | 239  | 47  |
| DR. Influent/MB                          | 1612       | 584    | 1028 | 162  | 138  | 24  |
| DR Effluent/OR Influent                  | 1628       | 687    | 941  | 155  | 135  | 20  |
| OR Effluent                              | 1342       | 475    | 867  | 37   | 32   | 5   |
| Foam from OR                             | 1935       | 832    | 1103 | 412  | 339  | 73  |
| TF Influent                              | 1270       | 308    | 962  | 34   | 29   | 5   |
| TF Effluent                              | 1233       | 278    | 955  | 37   | 31   | 6   |
| Final Effluent (CF)                      | 1020       | 129    | 891  | 5    | 4    | 1   |
| Time                                     | 6.00 a.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1948       | 789    | 1159 | 584  | 481  | 103 |
| SB Effluent                              | 1384       | 388    | 996  | 160  | 135  | 25  |
| Sludge SB                                | 4352       | 2560   | 1792 | 2307 | 1876 | 431 |
| MSF Effluent                             | 1298       | 342    | 956  | 108  | 87   | 21  |
| Sludge MSF                               | 1361       | 396    | 965  | 252  | 210  | 42  |
| DR. Influent/MB                          | 1342       | 371    | 971  | 130  | 109  | 21  |
| DR Effluent/OR Influent                  | 1386       | 408    | 978  | 141  | 121  | 20  |
| OR Effluent                              | 1150       | 234    | 916  | 33   | 28   | 5   |
| Foam from OR                             | 1823       | 790    | 1033 | 432  | 332  | 100 |
| TF Influent                              | 1116       | 208    | 908  | 30   | 26   | 4   |
| TF Effluent                              | 1056       | 144    | 912  | 32   | 27   | 5   |
| Final Effluent (CF)                      | 961        | 109    | 852  | 5    | 4    | 1   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) |            | Test 1 |      |      |      |     |
|--|------------|--------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1148       | 165    | 983  | 64   | 53   | 11  |
| SB Effluent                              | 1097       | 136    | 961  | 42   | 36   | 6   |
| Sludge SB                                | 1315       | 272    | 1043 | 145  | 119  | 26  |
| MSF Effluent                             | 1071       | 137    | 934  | 31   | 26   | 5   |
| Sludge MSF                               | 1135       | 158    | 977  | 75   | 64   | 11  |
| DR. Influent/MB                          | 1128       | 175    | 953  | 50   | 43   | 7   |
| DR Effluent/OR Influent                  | 1178       | 196    | 982  | 55   | 48   | 7   |
| OR Effluent                              | 1044       | 136    | 908  | 24   | 19   | 5   |
| Foam from OR                             | 1890       | 886    | 1004 | 302  | 259  | 43  |
| TF Influent                              | 1021       | 125    | 896  | 22   | 18   | 4   |
| TF Effluent                              | 985        | 97     | 888  | 24   | 18   | 6   |
| Final Effluent (CF)                      | 872        | 53     | 819  | 4    | 3    | 1   |
| Time                                     | 10.00 p.m. |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1952       | 868    | 1084 | 724  | 592  | 132 |
| SB Effluent                              | 1376       | 431    | 945  | 167  | 142  | 25  |
| Sludge SB                                | 4358       | 2681   | 1677 | 2940 | 2381 | 559 |
| MSF Effluent                             | 1340       | 386    | 954  | 126  | 107  | 19  |
| Sludge MSF                               | 1288       | 312    | 976  | 180  | 139  | 41  |
| DR. Influent/MB                          | 1426       | 444    | 982  | 144  | 125  | 19  |
| DR Effluent/OR Influent                  | 1466       | 479    | 987  | 152  | 130  | 22  |
| OR Effluent                              | 1229       | 308    | 921  | 42   | 36   | 6   |
| Foam from OR                             | 1916       | 848    | 1068 | 392  | 316  | 76  |
| TF Influent                              | 1191       | 279    | 912  | 36   | 31   | 5   |
| TF Effluent                              | 1136       | 225    | 911  | 39   | 33   | 6   |
| Final Effluent (CF)                      | 1016       | 170    | 846  | 6    | 5    | 1   |
| Time                                     | 6.00 a.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1790       | 820    | 970  | 705  | 580  | 125 |
| SB Effluent                              | 1258       | 411    | 847  | 145  | 122  | 23  |
| Sludge SB                                | 3866       | 2430   | 1436 | 3062 | 2515 | 547 |
| MSF Effluent                             | 1205       | 383    | 822  | 109  | 93   | 16  |
| Sludge MSF                               | 1255       | 430    | 825  | 164  | 135  | 29  |
| DR. Influent/MB                          | 1271       | 421    | 850  | 131  | 112  | 19  |
| DR Effluent/OR Influent                  | 1328       | 453    | 875  | 144  | 118  | 26  |
| OR Effluent                              | 1086       | 286    | 800  | 38   | 32   | 6   |
| Foam from OR                             | 1958       | 862    | 1096 | 371  | 309  | 62  |
| TF Influent                              | 1056       | 259    | 797  | 32   | 26   | 6   |
| TF Effluent                              | 1003       | 211    | 792  | 36   | 27   | 9   |
| Final Effluent (CF)                      | 902        | 165    | 737  | 6    | 5    | 1   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) |            | Test 2 |      |      |      |     |
|--|------------|--------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1196       | 132    | 1064 | 54   | 46   | 8   |
| SB Effluent                              | 1138       | 105    | 1033 | 41   | 36   | 5   |
| Sludge SB                                | 1396       | 235    | 1161 | 103  | 88   | 15  |
| MSF Effluent                             | 1162       | 125    | 1037 | 28   | 23   | 5   |
| Sludge MSF                               | 1190       | 112    | 1078 | 60   | 50   | 10  |
| DR. Influent/MB                          | 1205       | 158    | 1047 | 42   | 36   | 6   |
| DR Effluent/OR Influent                  | 1232       | 189    | 1043 | 44   | 37   | 7   |
| OR Effluent                              | 1122       | 124    | 998  | 14   | 11   | 3   |
| Foam from OR                             | 1845       | 720    | 1125 | 285  | 250  | 35  |
| TF Influent                              | 1097       | 112    | 985  | 12   | 10   | 2   |
| TF Effluent                              | 1066       | 78     | 988  | 15   | 12   | 3   |
| Final Effluent (CF)                      | 974        | 36     | 938  | 3    | 2    | 1   |
| Time                                     | 10.00 p.m. |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2334       | 1136   | 1198 | 832  | 675  | 157 |
| SB Effluent                              | 1490       | 494    | 996  | 226  | 190  | 36  |
| Sludge SB                                | 5795       | 3805   | 1990 | 3366 | 2730 | 636 |
| MSF Effluent                             | 1402       | 428    | 974  | 131  | 113  | 18  |
| Sludge MSF                               | 1418       | 440    | 978  | 269  | 220  | 49  |
| DR. Influent/MB                          | 1453       | 456    | 997  | 165  | 140  | 25  |
| DR Effluent/OR Influent                  | 1532       | 510    | 1022 | 174  | 146  | 28  |
| OR Effluent                              | 1281       | 328    | 953  | 46   | 39   | 7   |
| Foam from OR                             | 1943       | 876    | 1067 | 416  | 353  | 63  |
| TF Influent                              | 1263       | 316    | 947  | 39   | 35   | 4   |
| TF Effluent                              | 1242       | 288    | 954  | 44   | 38   | 6   |
| Final Effluent (CF)                      | 1076       | 181    | 895  | 9    | 7    | 2   |
| Time                                     | 6.00 a.m.  |        |      |      |      |     |
| Parameter                                | TS         | VTS    | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1730       | 689    | 1041 | 510  | 411  | 99  |
| SB Effluent                              | 1336       | 408    | 928  | 180  | 149  | 31  |
| Sludge SB                                | 3411       | 1905   | 1506 | 1860 | 1522 | 338 |
| MSF Effluent                             | 1274       | 362    | 912  | 115  | 97   | 18  |
| Sludge MSF                               | 1372       | 435    | 937  | 390  | 330  | 60  |
| DR. Influent/MB                          | 1316       | 370    | 946  | 139  | 117  | 22  |
| DR Effluent/OR Influent                  | 1413       | 445    | 968  | 146  | 123  | 23  |
| OR Effluent                              | 1224       | 326    | 898  | 31   | 27   | 4   |
| Foam from OR                             | 1762       | 742    | 1020 | 348  | 295  | 53  |
| TF Influent                              | 1189       | 299    | 890  | 27   | 23   | 4   |
| TF Effluent                              | 1186       | 294    | 892  | 30   | 24   | 6   |
| Final Effluent (CF)                      | 975        | 130    | 845  | 5    | 4    | 1   |

Table A31 continued.

| Stage 4 (22 mg O <sub>3</sub> /L; 4 Lpm) | Test 3     |      |      |      |      |     |
|--|------------|------|------|------|------|-----|
| Time                                     | 2:00 p.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1275       | 165  | 1110 | 55   | 46   | 9   |
| SB Effluent                              | 1221       | 134  | 1087 | 41   | 35   | 6   |
| Sludge SB                                | 1480       | 285  | 1195 | 117  | 96   | 21  |
| MSF Effluent                             | 1230       | 169  | 1061 | 36   | 30   | 6   |
| Sludge MSF                               | 1258       | 174  | 1084 | 80   | 68   | 12  |
| DR. Influent/MB                          | 1262       | 180  | 1082 | 46   | 39   | 7   |
| DR Effluent/OR Influent                  | 1276       | 189  | 1087 | 51   | 43   | 8   |
| OR Effluent                              | 1161       | 130  | 1031 | 20   | 17   | 3   |
| Foam from OR                             | 1984       | 864  | 1120 | 280  | 231  | 49  |
| TF Influent                              | 1145       | 120  | 1025 | 17   | 15   | 2   |
| TF Effluent                              | 1130       | 102  | 1028 | 19   | 16   | 3   |
| Final Effluent (CF)                      | 1036       | 58   | 978  | 5    | 4    | 1   |
| Time                                     | 10.00 p.m. |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 2015       | 875  | 1140 | 729  | 594  | 135 |
| SB Effluent                              | 1440       | 435  | 1005 | 196  | 166  | 30  |
| Sludge SB                                | 4396       | 2715 | 1681 | 2755 | 2218 | 537 |
| MSF Effluent                             | 1392       | 380  | 1012 | 122  | 104  | 18  |
| Sludge MSF                               | 1362       | 365  | 997  | 210  | 174  | 36  |
| DR. Influent/MB                          | 1448       | 418  | 1030 | 132  | 111  | 21  |
| DR Effluent/OR Influent                  | 1524       | 462  | 1062 | 140  | 115  | 25  |
| OR Effluent                              | 1311       | 326  | 985  | 39   | 33   | 6   |
| Foam from OR                             | 1962       | 880  | 1082 | 378  | 321  | 57  |
| TF Influent                              | 1297       | 319  | 978  | 34   | 29   | 5   |
| TF Effluent                              | 1260       | 270  | 990  | 36   | 30   | 6   |
| Final Effluent (CF)                      | 1071       | 141  | 930  | 8    | 6    | 2   |
| Time                                     | 6.00 a.m.  |      |      |      |      |     |
| Parameter                                | TS         | VTS  | FTS  | TSS  | VSS  | FSS |
| Raw Water                                | 1790       | 714  | 1076 | 516  | 426  | 90  |
| SB Effluent                              | 1315       | 349  | 966  | 141  | 119  | 22  |
| Sludge SB                                | 3840       | 2310 | 1530 | 2091 | 1712 | 379 |
| MSF Effluent                             | 1288       | 349  | 939  | 96   | 82   | 14  |
| Sludge MSF                               | 1345       | 392  | 953  | 189  | 163  | 26  |
| DR. Influent/MB                          | 1360       | 388  | 972  | 121  | 102  | 19  |
| DR Effluent/OR Influent                  | 1450       | 445  | 1005 | 130  | 110  | 20  |
| OR Effluent                              | 1255       | 320  | 935  | 37   | 30   | 7   |
| Foam from OR                             | 1832       | 728  | 1104 | 328  | 280  | 48  |
| TF Influent                              | 1229       | 298  | 931  | 30   | 25   | 5   |
| TF Effluent                              | 1214       | 281  | 933  | 34   | 29   | 5   |
| Final Effluent (CF)                      | 1025       | 142  | 883  | 5    | 4    | 1   |

Table A32. Raw data for tests including cBOD<sub>5</sub>.

Stage 1 (22 mg O<sub>3</sub>/L; 6 Lpm)

| Test #                  | 1                 |           |      | 2                 |          |       |
|-------------------------|-------------------|-----------|------|-------------------|----------|-------|
|                         | cBOD <sub>5</sub> | COD       | DOC  | cBOD <sub>5</sub> | COD      | DOC   |
| SB Effluent             | 71±5.2            | 248± 11.2 | 27.9 | 78±2.6            | 266±5.2  | 32.4  |
| MSF Effluent            | 60±3.4            | 199±5.2   | 23.2 | 63±0.8            | 215±7.0  | 27.9  |
| DR. Influent/MB         | 143±8.0           | 315±7.0   | 58.3 | 155±6.0           | 302±3.0  | 59.5  |
| DR Effluent/OR Influent | 61±1.6            | 197±2.5   | 26.7 | 71±3.3            | 220±5.2  | 31.1  |
| OR Effluent             | 49±2.5            | 154±2.3   | 34.9 | 58±1.5            | 172±1.3  | 36.8  |
| Foam from OR            | 392±14.6          | 823±21.6  | 90.0 | 388±26.6          | 875±12.3 | 114.0 |
| TF Influent             | 43±2.2            | 146±3.5   | 34.1 | 53±5.3            | 160±2.3  | 36.0  |
| TF Effluent             | 24±1.0            | 110±5.3   | 25.2 | 28±1.6            | 122±4.5  | 23.8  |
| Final Effluent (CF)     | 13±0.5            | 66±1.0    | 21.1 | 17±0.5            | 73±3.0   | 22.4  |

Stage 2 (33 mg O<sub>3</sub>/L; 6 Lpm)

| Test #                  | 1                 |          |      | 2                 |           |       |
|-------------------------|-------------------|----------|------|-------------------|-----------|-------|
|                         | cBOD <sub>5</sub> | COD      | DOC  | cBOD <sub>5</sub> | COD       | DOC   |
| SB Effluent             | 78±4.5            | 284±9.0  | 29.2 | 82±3.8            | 297±11.2  | 28.8  |
| MSF Effluent            | 54±2.3            | 212±3.3  | 26.5 | 58±3.0            | 207±2.3   | 27.2  |
| DR. Influent/MB         | 153±3.3           | 344±6.4  | 58.2 | 169±5.5           | 338±5.0   | 67.9  |
| DR Effluent/OR Influent | 61±1.0            | 225±4.0  | 20.7 | 64±4.3            | 233±4.6   | 33.3  |
| OR Effluent             | 46±0.5            | 162±1.3  | 37.7 | 48±2.6            | 168±4.5   | 39.8  |
| Foam from OR            | 528±30.6          | 951±17.5 | 98.3 | 638±42.5          | 1069±27.0 | 107.4 |
| TF Influent             | 43±1.5            | 150±8.6  | 36.5 | 47±3.3            | 159±7.2   | 39.2  |
| TF Effluent             | 25±1.3            | 112±4.2  | 30.5 | 28±2.0            | 118±5.3   | 30.7  |
| Final Effluent (CF)     | 15±0.0            | 68±1.3   | 26.5 | 16±0.5            | 74±2.0    | 28.0  |

Stage 3 (33 mg O<sub>3</sub>/L; 4 Lpm)

| Test #                  | 1                 |          |      | 2                 |          |      |
|-------------------------|-------------------|----------|------|-------------------|----------|------|
|                         | cBOD <sub>5</sub> | COD      | DOC  | cBOD <sub>5</sub> | COD      | DOC  |
| SB Effluent             | 78±2.0            | 276±6.3  | 28.4 | 73±5.3            | 255±7.5  | 28.4 |
| MSF Effluent            | 63±2.3            | 218±4.0  | 26.5 | 55±2.2            | 198±4.0  | 23.2 |
| DR. Influent/MB         | 117±4.0           | 323±7.2  | 51.1 | 136±4.0           | 282±4.6  | 46.5 |
| DR Effluent/OR Influent | 64±1.5            | 253±11.3 | 20.1 | 53±4.3            | 208±5.3  | 19.2 |
| OR Effluent             | 48±0.5            | 158±5.0  | 37.9 | 37±1.6            | 112±4.0  | 34.8 |
| Foam from OR            | 503±22.5          | 1054±6.2 | 95.5 | 414±20.0          | 885±24.0 | 86.6 |
| TF Influent             | 48±3.3            | 151±5.3  | 37.1 | 36±1.0            | 106±6.0  | 34.4 |
| TF Effluent             | 24±1.6            | 102±4.0  | 28.8 | 19±0              | 74±2.3   | 24.5 |
| Final Effluent (CF)     | 10±0.3            | 46±2.5   | 25.6 | 8±0.3             | 39±1.5   | 22.7 |

Stage 4 (22 mg O<sub>3</sub>/L; 4 Lpm)

| Test #                  | 1                 |          |       | 2                 |          |      |
|-------------------------|-------------------|----------|-------|-------------------|----------|------|
|                         | cBOD <sub>5</sub> | COD      | DOC   | cBOD <sub>5</sub> | COD      | DOC  |
| SB Effluent             | 89±2.0            | 310±9.6  | 34.1  | 58±1.5            | 237±7.4  | 26.1 |
| MSF Effluent            | 66±2.3            | 244±2.4  | 32.4  | 42±0.5            | 179±3.0  | 22.8 |
| DR. Influent/MB         | 179±3.8           | 345±2.6  | 64.5  | 141±2.0           | 260±4.2  | 58.6 |
| DR Effluent/OR Influent | 69±1.8            | 265±3.4  | 37.3  | 44±1.1            | 185±3.0  | 23.6 |
| OR Effluent             | 55±0.7            | 198±2.0  | 48.8  | 35±0.6            | 122±2.5  | 29.4 |
| Foam from OR            | 546±24.0          | 880±31.2 | 111.3 | 429±28.0          | 794±15.0 | 92.2 |
| TF Influent             | 49±2.4            | 177±4.6  | 47.7  | 29±0.5            | 114±2.2  | 28.1 |
| TF Effluent             | 27±1.1            | 114±4.0  | 38.9  | 20±0.5            | 81±1.4   | 18.5 |
| Final Effluent (CF)     | 15±0.5            | 67±2.5   | 27.2  | 10±0.5            | 52±1.0   | 16.5 |

Table A33. Raw data for volumes of foam collected.

| Stage #    | 1           |         | 2           |         | 3           |         | 4           |         |
|------------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|
|            | V (L/6 min) | V (L/L) | V (L/6 min) | V (L/L) | V (L/6 min) | V (L/L) | V (L/6 min) | V (L/L) |
| 2:00 p.m.  | 0.180       | 0.030   | 0.250       | 0.042   | 0.245       | 0.061   | 0.230       | 0.058   |
| 10:00 p.m. | 0.235       | 0.039   | 0.215       | 0.036   | 0.230       | 0.058   | 0.225       | 0.056   |
| 6:00 a.m.  | 0.210       | 0.035   | 0.260       | 0.043   | 0.250       | 0.063   | 0.170       | 0.043   |
| 2:00 p.m.  | 0.150       | 0.025   | 0.170       | 0.028   | 0.285       | 0.071   | 0.275       | 0.069   |
| 10:00 p.m. | 0.220       | 0.037   | 0.240       | 0.040   | 0.245       | 0.061   | 0.205       | 0.051   |
| 6:00 a.m.  | 0.285       | 0.048   | 0.275       | 0.046   | 0.340       | 0.085   | 0.275       | 0.069   |
| 2:00 p.m.  | 0.245       | 0.041   | 0.235       | 0.039   | 0.245       | 0.061   | 0.210       | 0.053   |
| 10:00 p.m. | 0.205       | 0.034   | 0.210       | 0.035   | 0.310       | 0.078   | 0.200       | 0.050   |
| 6:00 a.m.  | 0.195       | 0.033   | 0.285       | 0.048   | 0.300       | 0.075   | 0.195       | 0.049   |

Table A34. Raw data for volumes of foam collected in tests measuring cBOD<sub>5</sub>.

| Stage # | Test | V (L/6 min) | V (L/L) |
|---------|------|-------------|---------|
| 1       | 1    | 0.230       | 0.038   |
|         | 2    | 0.205       | 0.034   |
| 2       | 1    | 0.250       | 0.042   |
|         | 2    | 0.220       | 0.037   |
| 3       | 1    | 0.250       | 0.063   |
|         | 2    | 0.290       | 0.073   |
| 4       | 1    | 0.190       | 0.048   |
|         | 2    | 0.215       | 0.054   |

Table A35. Raw data for nitrogen budget determination (Production Subsystem).

| Time          | Parameter |                                 |                                 |       |      |      |
|---------------|-----------|---------------------------------|---------------------------------|-------|------|------|
|               | TAN       | NO <sub>3</sub> <sup>-</sup> -N | NO <sub>2</sub> <sup>-</sup> -N | Temp. | pH   | Alk. |
| Sept 24. 2003 |           |                                 |                                 |       |      |      |
| 2:00 p.m.     | 3.24      | 11.2                            | 1.12                            | 28.6  | 7.11 | 98   |
| 5:00 p.m.     | 2.71      | 34.5                            | 0.52                            | 28.2  | 7.09 | 125  |
| 8:00 p.m.     | 4.00      | 36.0                            | 1.55                            | 27.9  | 7.16 | 109  |
| 11:00 p.m.    | 1.12      | 80.4                            | 0.92                            | 29.0  | 7.12 | 132  |
| 2:00 a.m.     | 2.48      | 41.8                            | 1.16                            | 28.3  | 7.16 | 122  |
| 5:00 a.m.     | 3.62      | 33.8                            | 0.77                            | 28.5  | 7.22 | 112  |
| 8:00 a.m.     | 2.12      | 55.9                            | 1.05                            | 27.8  | 7.06 | 110  |
| 11:00 a.m.    | 3.38      | 18.0                            | 1.31                            | 29.0  | 7.16 | 104  |
| Sept 30. 2003 |           |                                 |                                 |       |      |      |
| 3:30 p.m.     | 2.61      | 24.9                            | 0.97                            | 29.2  | 7.14 | 102  |
| 6:30 p.m.     | 2.37      | 41.2                            | 0.56                            | 28.4  | 7.08 | 115  |
| 9:30 p.m.     | 4.56      | 58.8                            | 1.56                            | 28.0  | 7.25 | 120  |
| 12:30 a.m.    | 3.45      | 75                              | 0.84                            | 28.9  | 7.18 | 111  |
| 3:30 a.m.     | 2.52      | 66.5                            | 1.11                            | 27.9  | 7.12 | 134  |
| 6:30 a.m.     | 3.21      | 36.4                            | 1.89                            | 28.6  | 7.16 | 126  |
| 9:30 a.m.     | 2.75      | 31.5                            | 1.18                            | 29.6  | 7.08 | 112  |
| 12:30 p.m.    | 3.36      | 14.4                            | 1.66                            | 29.0  | 7.15 | 95   |

Table A36. Raw data for nitrogen budget determination (Broodstock Subsystem).

| Day         |  | Aug. 5 | Aug. 12 | Aug. 19 |
|-------------|--|--------|---------|---------|
| 8:00 a.m.   | TAN (mg/L)                             | 0.75   | 0.82    | 0.8     |
| 12:00 p.m.  |  | 0.47   | 0.42    | 0.4     |
| Total rmvd. |  | 0.28   | 0.4     | 0.4     |
| Day         |  | Aug. 5 | Aug. 9  | Aug. 16 |
| 8:00 a.m.   | NO <sub>2</sub> <sup>-</sup> -N (mg/L) | 0.03   | 0.02    | 0.03    |
| 12:00 p.m.  |  | 0.02   | 0.01    | 0.01    |
| Total rmvd. |  | 0.01   | 0.01    | 0.02    |
| Day         |  | Aug. 5 | Aug. 9  | Aug. 16 |
| 8:00 a.m.   | NO <sub>3</sub> <sup>-</sup> -N (mg/L) | 0.8    | 0.6     | 0.7     |
| 12:00 p.m.  |  | 0.7    | 0.3     | 0.3     |
| Total rmvd. |  | 0.1    | 0.3     | 0.4     |
| Day         |  | Aug. 5 | Aug. 9  | Aug. 16 |
| 8:00 a.m.   | TKN (mg/L)                             | 13.22  | 10.56   | 11.86   |
| 12:00 p.m.  |  | 5.22   | 3.64    | 3.88    |
| Total rmvd. |  | 8      | 6.92    | 7.98    |
| Day         |  | Aug. 5 | Aug. 9  | Aug. 16 |
| 8:00 a.m.   | N <sub>TON</sub> (mg/L)                | 12.47  | 9.74    | 11.06   |
| 12:00 p.m.  |  | 4.75   | 3.22    | 3.48    |

|             |      |      |      |
|-------------|------|------|------|
| Total rmvd. | 7.72 | 6.52 | 7.58 |
|-------------|------|------|------|

Table A37. Raw data for nitrogen budget determination (Hatchery Subsystem).

| Day         |  | Jul. 24 | Jul. 28 | Aug. 1  | Aug. 5  | Aug. 9 |
|-------------|--|---------|---------|---------|---------|--------|
| 8:00 a.m.   | TAN (mg/L)                             | 0.36    | 2.75    | 4.56    | 7.88    | 9.86   |
| 12:00 p.m.  |  | 0.12    | 0.95    | 1.86    | 2.18    | 3.24   |
| Total rmvd. |  | 0.24    | 1.8     | 2.7     | 5.7     | 6.62   |
| Day         |  | Jul. 24 | Jul. 28 | Jul. 24 | Jul. 28 | Aug. 1 |
| 8:00 a.m.   | NO <sub>2</sub> <sup>-</sup> -N (mg/L) | 0.15    | 0.48    | 0.68    | 0.87    | 1.12   |
| 12:00 p.m.  |  | 0.1     | 0.12    | 0.15    | 0.18    | 0.22   |
| Total rmvd. |  | 0.05    | 0.36    | 0.53    | 0.69    | 0.9    |
| Day         |  | Jul. 24 | Jul. 28 | Jul. 24 | Jul. 28 | Aug. 1 |
| 8:00 a.m.   | NO <sub>3</sub> <sup>-</sup> -N (mg/L) | 0.8     | 1.8     | 2.4     | 3.5     | 4.1    |
| 12:00 p.m.  |  | 0.2     | 0.3     | 0.4     | 0.4     | 0.6    |
| Total rmvd. |  | 0.6     | 1.5     | 2       | 3.1     | 3.5    |
| Day         |  | Jul. 24 | Jul. 28 | Jul. 24 | Jul. 28 | Aug. 1 |
| 8:00 a.m.   | TKN (mg/L)                             | 2.12    | 5.24    | 9.38    | 15.9    | 19.64  |
| 12:00 p.m.  |  | 1.3     | 1.86    | 2.58    | 3.29    | 4.56   |
| Total rmvd. |  | 0.82    | 3.38    | 6.8     | 12.61   | 15.08  |
| Day         |  | Jul. 24 | Jul. 28 | Jul. 24 | Jul. 28 | Aug. 1 |
| 8:00 a.m.   | N <sub>TON</sub> (mg/L)                | 1.76    | 2.49    | 4.82    | 8.02    | 9.78   |
| 12:00 p.m.  |  | 1.18    | 0.91    | 0.72    | 1.11    | 1.32   |
| Total rmvd. |  | 0.58    | 1.58    | 4.1     | 6.91    | 8.46   |

Table A38. Raw data for nitrogen budget determination (Fingerlings Subsystem 1).

| Day         |  | Aug. 10 | Aug. 21 | Aug. 31 | Sept. 11 | Sept. 22 |
|-------------|--|---------|---------|---------|----------|----------|
| 8:00 a.m.   | TAN (mg/L)                             | 0.82    | 3.52    | 5.65    | 7.54     | 8.52     |
| 12:00 p.m.  |  | 0.16    | 0.94    | 1.64    | 2.33     | 2.62     |
| Total rmvd. |  | 0.66    | 2.58    | 4.01    | 5.21     | 5.9      |
| Day         |  | Aug. 10 | Aug. 21 | Aug. 31 | Sept. 11 | Sept. 22 |
| 8:00 a.m.   | NO <sub>2</sub> <sup>-</sup> -N (mg/L) | 0.1     | 0.38    | 0.77    | 0.94     | 1.16     |
| 12:00 p.m.  |  | 0.07    | 0.16    | 0.38    | 0.51     | 0.6      |
| Total rmvd. |  | 0.03    | 0.22    | 0.39    | 0.43     | 0.56     |
| Day         |  | Aug. 10 | Aug. 21 | Aug. 31 | Sept. 11 | Sept. 22 |
| 8:00 a.m.   | NO <sub>3</sub> <sup>-</sup> -N (mg/L) | 1.6     | 2.9     | 3.68    | 4.85     | 5.92     |
| 12:00 p.m.  |  | 0.4     | 0.18    | 1.84    | 2.12     | 2.56     |
| Total rmvd. |  | 1.2     | 2.72    | 1.84    | 2.73     | 3.36     |
| Day         |  | Aug. 10 | Aug. 21 | Aug. 31 | Sept. 11 | Sept. 22 |
| 8:00 a.m.   | TKN (mg/L)                             | 13.4    | 14.5    | 15.96   | 17.9     | 19.68    |
| 12:00 p.m.  |  | 1.86    | 2.6     | 3.12    | 4.62     | 5.35     |
| Total rmvd. |  | 11.54   | 11.9    | 12.84   | 13.28    | 14.33    |
| Day         |  | Aug. 10 | Aug. 21 | Aug. 31 | Sept. 11 | Sept. 22 |
| 8:00 a.m.   | N <sub>TON</sub> (mg/L)                | 12.58   | 10.98   | 10.31   | 10.36    | 11.16    |

|             |  |       |      |      |      |      |
|-------------|--|-------|------|------|------|------|
| 12:00 p.m.  |  | 1.7   | 1.66 | 1.48 | 2.29 | 2.73 |
| Total rmvd. |  | 10.88 | 9.32 | 8.83 | 8.07 | 8.43 |

Table A39. Raw data for nitrogen budget determination (Fingerlings Subsystem 2).

| Day         |  | Aug. 10 | Aug. 17 | Aug. 24 | Aug. 31 | Sept. 7 |
|-------------|--|---------|---------|---------|---------|---------|
| 8:00 a.m.   | TAN (mg/L)                             | 8.26    | 11.52   | 13.4    | 14.28   | 14.96   |
| 12:00 p.m.  |  | 2.36    | 2.7     | 2.88    | 3.15    | 3.62    |
| Total rmvd. |  | 5.9     | 8.82    | 10.52   | 11.13   | 11.34   |
| Day         |  | Aug. 10 | Aug. 17 | Aug. 24 | Aug. 31 | Sept. 7 |
| 8:00 a.m.   | NO <sub>2</sub> <sup>-</sup> -N (mg/L) | 0.46    | 0.38    | 0.52    | 0.6     | 0.65    |
| 12:00 p.m.  |  | 0.1     | 0.12    | 0.15    | 0.17    | 0.18    |
| Total rmvd. |  | 0.36    | 0.26    | 0.37    | 0.43    | 0.47    |
| Day         |  | Aug. 10 | Aug. 17 | Aug. 24 | Aug. 31 | Sept. 7 |
| 8:00 a.m.   | NO <sub>3</sub> <sup>-</sup> -N (mg/L) | 6.1     | 7.3     | 8.2     | 10.2    | 12      |
| 12:00 p.m.  |  | 2.1     | 2.3     | 2.8     | 3.1     | 3.4     |
| Total rmvd. |  | 4       | 5       | 5.4     | 7.1     | 8.6     |
| Day         |  | Aug. 10 | Aug. 17 | Aug. 24 | Aug. 31 | Sept. 7 |
| 8:00 a.m.   | TKN (mg/L)                             | 18.96   | 22.45   | 26.52   | 29.06   | 31.14   |
| 12:00 p.m.  |  | 3.46    | 3.82    | 4.12    | 4.62    | 5.35    |
| Total rmvd. |  | 15.5    | 18.63   | 22.4    | 24.44   | 25.79   |
| Day         |  | Aug. 10 | Aug. 17 | Aug. 24 | Aug. 31 | Sept. 7 |
| 8:00 a.m.   | N <sub>TON</sub> (mg/L)                | 10.7    | 10.93   | 13.12   | 14.78   | 16.18   |
| 12:00 p.m.  |  | 1.1     | 1.12    | 1.24    | 1.47    | 1.73    |
| Total rmvd. |  | 9.6     | 9.81    | 11.88   | 13.31   | 14.45   |

Table A40. Raw data for maximum carrying capacity modeling (Tank A12).

| Time    | Place   | Parameter |                                 |                                 |      |       |      |      |
|---------|---------|-----------|---------------------------------|---------------------------------|------|-------|------|------|
|         |         | TAN       | NO <sub>3</sub> <sup>-</sup> -N | NO <sub>2</sub> <sup>-</sup> -N | pH   | Temp. | DO   | Alk. |
| 4 a.m.  | Tank    | 1.53      | 49.0                            | 0.170                           | 7.09 | 28.0  | 4.71 | 81   |
|         | RBC in  | 1.55      | 31.0                            | 0.183                           | 7.08 | 28.1  | 4.38 | 78   |
|         | RBC out | 0.61      | 41.4                            | 0.148                           | 7.32 | 28.2  | -    | 81   |
| 8 a.m.  | Tank    | 2.33      | 41.2                            | 0.164                           | 7.02 | 28.3  | 6.67 | 92   |
|         | RBC in  | 2.19      | 37.2                            | 0.170                           | 7.01 | 28.3  | 6.16 | 98   |
|         | RBC out | 1.26      | 45.0                            | 0.114                           | 7.23 | 28.6  | -    | 90   |
| 12 p.m. | Tank    | 1.65      | 46.2                            | 0.188                           | 7.1  | 28.2  | 6.08 | 90   |
|         | RBC in  | 1.53      | 40.8                            | 0.202                           | 7.1  | 28.2  | 5.67 | 95   |
|         | RBC out | 0.78      | 50.6                            | 0.152                           | 7.36 | 28.4  | -    | 92   |
| 4 p.m.  | Tank    | 1.46      | 54.5                            | 0.200                           | 7.12 | 28.4  | 6.33 | 91   |
|         | RBC in  | 1.34      | 65.1                            | 0.284                           | 7.14 | 28.6  | 5.92 | 86   |
|         | RBC out | 0.54      | 44.2                            | 0.146                           | 7.33 | 28.6  | -    | 71   |
| 8 p.m.  | Tank    | 1.74      | 48.2                            | 0.233                           | 7.04 | 28.6  | 6.64 | 71   |
|         | RBC in  | 1.63      | 52.1                            | 0.285                           | 7.02 | 28.8  | 6.24 | 73   |
|         | RBC out | 0.78      | 66.8                            | 0.200                           | 7.26 | 28.9  | -    | 79   |
| 12 a.m. | Tank    | 1.94      | 53.5                            | 0.220                           | 7    | 28.4  | 5.56 | 80   |
|         | RBC in  | 2         | 52.0                            | 0.246                           | 7    | 28.5  | 5.15 | 83   |
|         | RBC out | 0.92      | 55.7                            | 0.178                           | 7.28 | 28.6  | -    | 80   |
| 4 a.m.  | Tank    | 2.06      | 60.2                            | 0.262                           | 6.98 | 28.0  | 6.03 | 92   |
|         | RBC in  | 2.08      | 53.4                            | 0.324                           | 7.02 | 28.1  | 5.68 | 99   |
|         | RBC out | 1.02      | 62.0                            | 0.214                           | 7.31 | 28.3  | -    | 90   |
| 8 a.m.  | Tank    | 1.75      | 50.6                            | 0.236                           | 7.06 | 27.9  | 5.16 | 88   |
|         | RBC in  | 1.78      | 54.2                            | 0.275                           | 7.08 | 28.0  | 4.76 | 94   |
|         | RBC out | 0.88      | 59.0                            | 0.246                           | 7.36 | 28.1  | -    | 90   |
| 12 p.m. | Tank    | 1.80      | 64.0                            | 0.315                           | 7.12 | 27.6  | 5.06 | 91   |
|         | RBC in  | 1.84      | 49.8                            | 0.437                           | 7.11 | 27.7  | 4.65 | 87   |
|         | RBC out | 0.80      | 58.0                            | 0.265                           | 7.40 | 28.0  | -    | 78   |

Table A41. Raw data for maximum carrying capacity modeling (Tank A11).

| Time    | Place   | Parameter |                                 |                                 |      |       |      |      |
|---------|---------|-----------|---------------------------------|---------------------------------|------|-------|------|------|
|         |         | TAN       | NO <sub>3</sub> <sup>-</sup> -N | NO <sub>2</sub> <sup>-</sup> -N | pH   | Temp. | DO   | Alk. |
| 2 a.m.  | Tank    | 2.18      | 37.8                            | 0.152                           | 6.94 | 28.5  | 6.93 | 96   |
|         | RBC in  | 2.08      | 47.4                            | 0.154                           | 6.92 | 28.6  | 6.41 | 105  |
|         | RBC out | 0.85      | 35.1                            | 0.115                           | 7.21 | 28.8  | -    | 86   |
| 6 a.m.  | Tank    | 2.65      | 30.8                            | 0.231                           | 6.96 | 27.7  | 6.05 | 91   |
|         | RBC in  | 2.42      | 48.0                            | 0.252                           | 7.23 | 28.3  | 5.52 | 82   |
|         | RBC out | 0.98      | 36.6                            | 0.175                           | 6.97 | 27.9  | -    | 104  |
| 10 a.m. | Tank    | 2.3       | 44.6                            | 0.244                           | 7    | 27.9  | 5.45 | 95   |
|         | RBC in  | 2.36      | 41.8                            | 0.281                           | 7.02 | 28.0  | 4.96 | 98   |
|         | RBC out | 1.2       | 46.5                            | 0.196                           | 7.35 | 28.2  | -    | 90   |
| 2 p.m.  | Tank    | 2.26      | 48.0                            | 0.260                           | 7.08 | 28.0  | 5.26 | 92   |
|         | RBC in  | 2.3       | 46.2                            | 0.326                           | 7.08 | 28.0  | 4.86 | 94   |
|         | RBC out | 1.1       | 50.2                            | 0.214                           | 7.3  | 28.5  | -    | 93   |
| 4 p.m.  | Tank    | 2.37      | 52.6                            | 0.295                           | 7.03 | 28.5  | 5.85 | 91   |
|         | RBC in  | 2.43      | 51.0                            | 0.378                           | 6.98 | 29.0  | 5.32 | 94   |
|         | RBC out | 0.98      | 55.0                            | 0.204                           | 7.23 | 29.1  | -    | 92   |
| 9 p.m.  | Tank    | 2.25      | 50.8                            | 0.334                           | 6.94 | 27.9  | 3.39 | 81   |
|         | RBC in  | 2.33      | 55.6                            | 0.442                           | 6.94 | 28.3  | 3.4  | 83   |
|         | RBC out | 0.95      | 65.4                            | 0.256                           | 7.19 | 28.7  | -    | 88   |

Table A42. Raw data for maximum carrying capacity modeling (Tank B16).

| Time    | Place   | Parameter |                                 |                                 |      |       |      |      |
|---------|---------|-----------|---------------------------------|---------------------------------|------|-------|------|------|
|         |         | TAN       | NO <sub>3</sub> <sup>-</sup> -N | NO <sub>2</sub> <sup>-</sup> -N | pH   | Temp. | DO   | Alk. |
| 6 p.m.  | Tank    | 1.72      | 44.8                            | 0.148                           | 7.06 | 26.8  | 6.17 | 85   |
|         | RBC in  | 1.82      | 30.0                            | 0.180                           | 7.05 | 27.0  | 5.85 | 89   |
|         | RBC out | 0.82      | 42.0                            | 0.120                           | 7.28 | 27.1  | -    | 83   |
| 10 p.m. | Tank    | 1.94      | 28.2                            | 0.174                           | 7.11 | 27.1  | 7.8  | 85   |
|         | RBC in  | 2         | 26.4                            | 0.220                           | 7.04 | 27.3  | 7.16 | 87   |
|         | RBC out | 0.96      | 29.2                            | 0.140                           | 7.28 | 27.7  | -    | 86   |
| 2 a.m.  | Tank    | 2         | 49.5                            | 0.096                           | 7.10 | 27.5  | 7.05 | 94   |
|         | RBC in  | 2.14      | 27.4                            | 0.118                           | 7.08 | 27.6  | 6.61 | 98   |
|         | RBC out | 1.12      | 44.9                            | 0.075                           | 7.33 | 27.7  | -    | 96   |
| 4 a.m.  | Tank    | 2.02      | 37.1                            | 0.212                           | 6.97 | 26.6  | 6.75 | 103  |
|         | RBC in  | 2.01      | 46.3                            | 0.234                           | 6.98 | 26.7  | 6.18 | 89   |
|         | RBC out | 0.9       | 56.3                            | 0.168                           | 7.27 | 27.0  | -    | 94   |
| 8 a.m.  | Tank    | 1.7       | 47.3                            | 0.190                           | 7.08 | 27.6  | 5.72 | 75   |
|         | RBC in  | 1.74      | 41.6                            | 0.219                           | 7.08 | 27.7  | 5.25 | 83   |
|         | RBC out | 0.84      | 49.5                            | 0.140                           | 7.26 | 27.9  | -    | 71   |
| 12 p.m. | Tank    | 2.06      | 40.1                            | 0.245                           | 7.01 | 28.0  | 5.15 | 90   |
|         | RBC in  | 2.12      | 41.6                            | 0.313                           | 7.03 | 28.1  | 4.79 | 94   |
|         | RBC out | 1.06      | 49.5                            | 0.204                           | 7.29 | 28.3  | -    | 88   |
| 4 p.m.  | Tank    | 2.24      | 52.2                            | 0.238                           | 6.98 | 28.8  | 4.12 | 92   |
|         | RBC in  | 2.21      | 50.0                            | 0.316                           | 6.95 | 28.5  | 3.92 | 96   |
|         | RBC out | 1.07      | 56.2                            | 0.192                           | 7.18 | 28.7  |      | 86   |
| 8 p.m.  | Tank    | 2.24      | 54.0                            | 0.215                           | 6.94 | 28.9  | 5.7  | 101  |
|         | RBC in  | 2.3       | 46.8                            | 0.280                           | 6.94 | 28.9  | 5.28 | 95   |
|         | RBC out | 1.14      | 52.0                            | 0.194                           | 7.16 | 29.1  | -    | 88   |

Table A43. Raw data for maximum carrying capacity modeling (Tank A18).

| Time    | Place   | Parameter |                                 |                                 |      |       |      |      |
|---------|---------|-----------|---------------------------------|---------------------------------|------|-------|------|------|
|         |         | TAN       | NO <sub>3</sub> <sup>-</sup> -N | NO <sub>2</sub> <sup>-</sup> -N | pH   | Temp. | DO   | Alk. |
| 2 a.m.  | Tank    | 1.94      | 33.5                            | 0.254                           | 7.05 | 26.9  | 8.58 | 86   |
|         | RBC in  | 1.99      | 47.8                            | 0.116                           | 7.03 | 26.5  | 8.02 | 88   |
|         | RBC out | 0.87      | 38.8                            | 0.084                           | 7.31 | 26.8  | -    | 87   |
| 6 a.m.  | Tank    | 2.12      | 30.4                            | 0.226                           | 7.05 | 27.3  | 6.75 | 95   |
|         | RBC in  | 2.27      | 42.4                            | 0.238                           | 7.03 | 27.3  | 6.25 | 92   |
|         | RBC out | 0.83      | 37.2                            | 0.166                           | 7.31 | 27.4  | -    | 83   |
| 10 a.m. | Tank    | 1.96      | 41.2                            | 0.215                           | 7    | 27.5  | 5.94 | 92   |
|         | RBC in  | 2.02      | 38.0                            | 0.260                           | 7.01 | 27.6  | 5.56 | 98   |
|         | RBC out | 0.86      | 45.1                            | 0.204                           | 7.26 | 27.8  | -    | 90   |
| 2 p.m.  | Tank    | 2.08      | 47.9                            | 0.230                           | 6.92 | 28.0  | 6.21 | 81   |
|         | RBC in  | 2.02      | 27.0                            | 0.359                           | 6.92 | 28.1  | 5.81 | 89   |
|         | RBC out | 0.82      | 44.7                            | 0.310                           | 7.18 | 28.1  | -    | 80   |
| 6 p.m.  | Tank    | 2.08      | 48.9                            | 0.305                           | 7    | 28.0  | 4.68 | 75   |
|         | RBC in  | 2.08      | 43.5                            | 0.357                           | 6.89 | 28.4  | 4.31 | 78   |
|         | RBC out | 0.92      | 52.3                            | 0.244                           | 7.22 | 28.4  | -    | 73   |
| 8 p.m.  | Tank    | 2.36      | 44.4                            | 0.320                           | 6.92 | 27.9  | 5.54 | 76   |
|         | RBC in  | 2.26      | 42.4                            | 0.419                           | 6.87 | 28.0  | 5.12 | 77   |
|         | RBC out | 1.1       | 60.5                            | 0.249                           | 7.17 | 28.1  | -    | 71   |

## **VITAE**

Simonel Ioan Sandu was born in Havirna, Botosani, Romania, on May 30, 1968. He attended Dunarea de Jos University located in Galati, Romania, where he received his B.S. in Aquaculture and Fishing Engineering in July of 1994. Afterwards, Simonel came to the United States and enrolled in the Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University in the fall of 1999. He graduated in December 2000 with a Master of Science degree. He then began working on his Ph.D. at the same university in the fall of 2001 and on September 13, 2004, he successfully defended his dissertation work and received his Doctorate of Philosophy degree.