WATER CHEMISTRY CHARACTERIZATION AND COMPONENT PERFORMANCE OF A RECIRCULATING AQUACULTURE SYSTEM PRODUCING HYBRID STRIPED BASS

by

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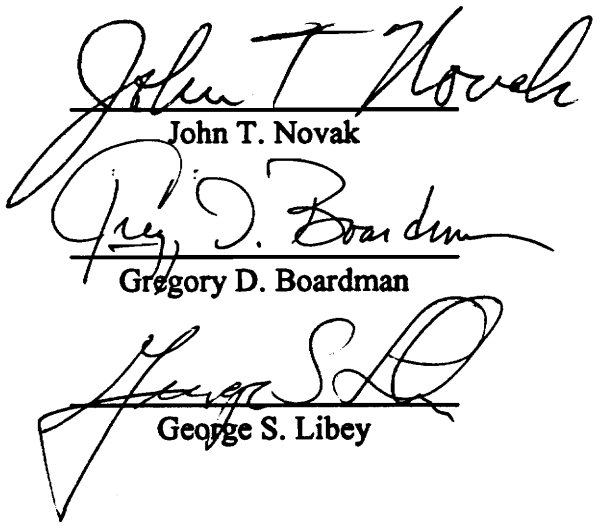

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(ABSTRACT)

Eight identical and independent pilot scale recirculating aquaculture production systems were populated with fingerling hybrid striped bass (*Morone chrysops* female x *Morone saxatilis* male). Three population densities were established with two replicates at 132 fish/m^3 and three replicates each at 66 and 33 fish/m^3. Water chemistry and water quality characteristics were monitored throughout the 228 day growth trial for all eight systems. A system component performance analysis was done for both the multi-tube solids clarifier and rotating biological contactor (RBC).

Water chemistry and water quality analysis included dissolved oxygen (DO), alkalinity, ions, carbonaceous biochemical oxygen demand ($CBOD_5$), chemical oxygen demand (COD), dissolved organic carbon (DOC), total suspended solids (TSS), volatile suspended solids (VSS), total ammonia nitrogen (TAN) , nitrite and nitrate. The major ions present were chloride, nitrate, sulfate, phosphate, sodium, calcium, magnesium and potassium. Trace levels of TAN, nitrite, iron and copper were also observed. Sodium, calcium and chloride levels were controlled based on a preplaned water exchange and chemical management method. TAN, nitrite and nitrate levels increased over time with increasing feed rates but never reached levels toxic to the fish population. $CBOD_5$,COD, DOC, TSS and VSS increased over time increasing as a function of increasing feed rates. No correlation was observed between fish mortality or fish growth rates for the range of organic and solids parameters observed during this study. On average 67% of the TSS present was between 1.5 and 30 microns in size. Diurnal cycles were observed for DO, TAN and alkalinity. The magnitude of these cycles were population dependent.

Multi-tube clarifiers removed an average 56% of all suspended solids in a single pass with 81% removal efficiency for particles above 70 microns in size. Analysis of the system effluent generated by the clarifier indicates a high degree of similarity between the aquaculture effluent and standard municipal waste on a nutrient basis but with much higher levels of nitrogen and phosphate species.

RBC nitrification performance was fitted to an empirical equation. A nearly constant TAN removal rate was observed over the range of mass loading experienced in this growth trial. This implies that within a reasonable range higher flow rates resulting in higher mass loading will yield higher TAN removal rates for a given RBC.

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CHAPTER 1

INTRODUCTION TO THE OVERALL STUDY

Key to understanding the commercial attractiveness and general rising interest in aquaculture is simple supply and demand. Seafood consumption is rising worldwide. This is particularly the case in the United States where a trend toward health-consciousness has blossomed. Concerns about cholesterol levels and risk of heart disease drove the per capita consumption of seafood up to a record of 15.9 pounds in 1990, up nearly 5 percent since 1988. This trend is expected to continue with the National Fisheries Institute setting a goal of 20 pounds per capita by the year 2000 (Egan 1990). While demand is skyrocketing the available natural catch is diminishing because of aggressive harvesting practices such as ocean drift-netting, nicknamed "strip mining of the seas."

Aquaculture systems such as the catfish ponds in the southern United States and shrimp culture systems in South America have helped fill the void providing a dependable high quality supply of seafood. While these aquaculture systems have proven themselves economically, they require a large amount of high quality water which is finite in supply. Clean water supply whether from surface or ground water sources has become a critical issue.

It is clear that intensification of aquaculture systems will continue in an attempt to get the largest harvest possible from a dwindling supply of natural resources, particularly clean water. The situation is further exacerbated by increasing restrictions on waste waters produced by such systems because of concerns about contaminating existing water supplies. The next logical step for intensification of fish culture is the Recirculating Aquaculture System (RAS). These can generally be defined as an assemblage of parts used for the husbandry of aquatic organisms in which water is continuously cleaned and

recycled. The main advantage of these systems is their ability to minimize water use. They can also solve many of the seasonal and site limiting problems of pond systems by being placed inside buildings where the entire fish culture environment can be controlled and managed. Enhanced environmental control can result in increasing growth on a year round production schedule avoiding the seasonal limitations suffered in outdoor systems. These advantages have drawn recent large scale investment from companies such as Seachick, Con-Agra, British petroleum, Chequita Brands and many others.

Along with the benefits of RAS's come new problems. Many of these problems have yet to be well defined or understood. The high degree of intensification coupled with water reuse leads to system contamination from the metabolic byproducts of the fish culture and the biological water treatment systems. Historically, ammonia and nitrite-nitrogen along with dissolved organics and suspended solids have been identified as constituents of concern. Mechanical, chemical and biological wastewater treatment technologies have been applied in recirculating aquaculture systems to recondition the water and attempt to mitigate these problems.

Many warm water species of fish and crustaceans appear to be potentially promising choices for recirculating systems. Market demand and culture species adaptability to recirculating systems will certainly be among the prime criteria for species selection. The hybrid striped bass has been identified in Virginia as a highly marketable choice for culture in recirculating systems. The hybrid is a cross between the striped bass (*Morone saxatilis*) and the white bass (*Morone chrysops*). This cross provides a degree of hybrid vigor yielding an animal that is more tolerant to environmental challenges with very good growth characteristics.

This thesis is one of two cooperative research efforts conducted simultaneously. The companion thesis (Nunley 1992) concentrated on the production side of the

recirculating system performance along with a basic water chemistry analysis during two growth trials completed over a two year period. This report provides a more in depth investigation of water quality issues and basic performance analysis of the water treatment devices being applied to hybrid striped bass culture during the second of these growth trials. It should be emphasized here that complete understanding of our overall research project can only be gained by studying both reports.

It is also noteworthy that good fish growth rates and low mortality levels were observed during this study. Overall fish survivability averaged greater than 95% in all population levels studied. It was concluded (Nunley 1992) that hybrid striped bass could be successfully cultured at the relatively high densities maintained during this study. The water quality and system component performance analysis that follows characterizes what was considered a successful attempt at culturing hybrid striped bass in a recriculating aquaculture system.

THESIS FORMAT AND RESEARCH OBJECTIVES

Improving the understanding of this relatively new fish culture system and it's water treatment components was the general purpose of this research. Much of this study establishes the baseline characteristics for these systems in hope that the insights gained will lead to improved system designs and management techniques. The report that follows will be broken into separate chapters addressing three related but separate research objectives. These objectives are:

1. To define the basic water chemistry and water quality characteristics of a high density Recirculating Aquaculture System producing hybrid striped bass.
2. To characterize the performance of the multi-tube clarifiers and to establish the characteristics of the system effluent produced by the clarifiers..
3. To characterize the nitrification performance of the Rotating Biological Contactors (RBC's).

Each of the following chapters will contain a separate literature review, materials and methods, results, discussion and conclusions section pertinent to the topics addressed in that chapter.

OVERVIEW OF SYSTEM HARDWARE

Eight identical and independent recirculating aquaculture production systems were used in this study. The systems were located at the Virginia Polytechnic Institute and State University Aquaculture Research Facility in Blacksburg Virginia.

Each system (Figure 1) had a rectangular fiberglass fish culture tank (6.10m long x 1.52m wide x 1.22m tall), a multi-tube solids clarifier or sump (1.52m long x 1.52m wide x 1.22m tall) .and a half cylinder shaped biofilter tank (2.13m dia. x 1.52m long) housing a three stage Rotating Biological Contactor. Water depth in the culture tank and clarifier was maintained near 1 meter resulting in culture tank and clarifier tank volumes of approximately 9270 and 2310 liters respectively. The RBC water level was maintained at or near an emergency overflow by means of a valve in the RBC effluent line providing a volume of 1930 liters. Water flow within each system was maintained by two submerged pumps supported on the multi-tube clarifier media in the top of the sump. Approximately 285 liters per minute of clarified water was pumped through 5.1 cm poly vinyl chloride (PVC) pipe into the elevated RBC tanks for nitrification treatment. The elevation energy provided in the RBC tank allowed gravity flow down a u-tube aerator. Water flowed down the 12.2 meter deep u-tube well inside a central 7.6 cm diameter PVC pipe and back up outside of the 7.6 cm pipe but inside a 15.2 cm PVC well casing. Pure gaseous oxygen was metered into the u-tube inlet water flow and carried down the u-tube well providing for system oxygenation. The reoxygenated water was carried from the u-tube by 5.1 cm diameter PVC pipe and emptied into the culture tank through five inlet ports providing even distribution of the reconditioned water into the culture tank. Water within the culture tank became contaminated with fish waste and depleted of oxygen by the fish culture. This water exited the culture tank through two

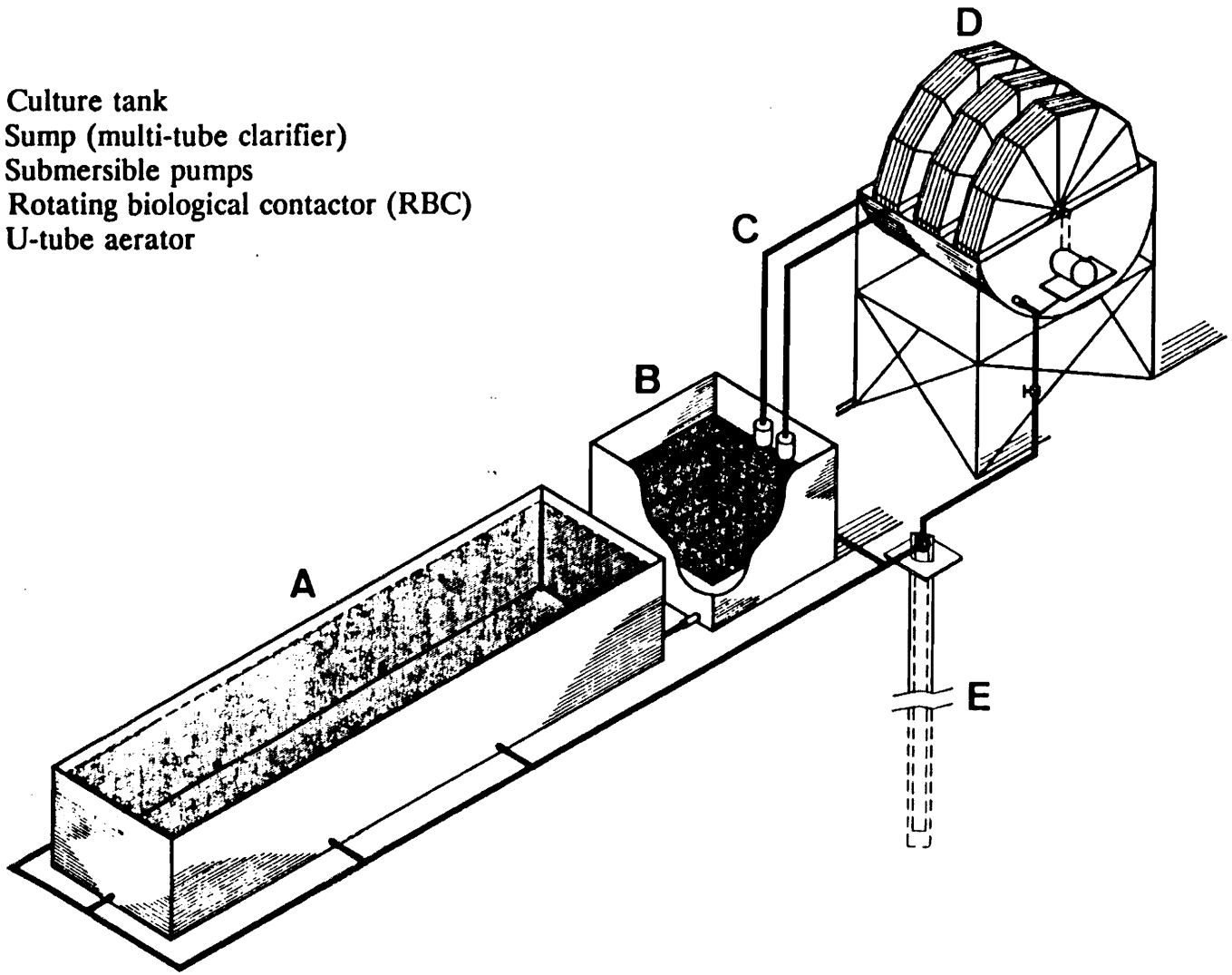


Figure 1. Schematic diagram of the recirculating aquaculture system used in this study.

screened 7.6 cm outlet ports into the bottom of the clarifier and up through the mutli-tube clarifier media to once again be recycled and reconditioned. More details of the multi-tube clarifier and the RBC system will be provided in the chapters addressing these systems. Specifics regarding the u-tube aerator system were not addressed in this study. The u-tube aerator was evaluated as a part of a thesis by Wood (1992).

The RAS,s were housed in a well insulated post-frame building (35m x15.2m x4.8m). The building was covered inside and out with aluminum siding. RAS water temperature was maintained near 24 ^{o}C by controlling the building temperature with four propane heaters suspended from the ceiling in the corners of the building. The RBC's provided an excellent heat exchanger allowing the system temperatures to adjust rapidly to changes in the room temperature.

The research facility lighting was kept at a minimum to reduce fish stress and RAS algal growth, using six 150 watt incandescent light fixtures. Lighting intensity was gradually adjusted in the morning and evening by a manually operated rheostat avoiding abrupt changes which upset the fish population. Diurnal lighting was maintained at an approximate 14 hour light :10 hour dark cycle.

FISH STOCKING

Based on the results of Nunley's (1992) first growth trial it was decided that appropriate stocking densities and the related effects of density on fish production and RAS water quality needed to be investigated. Three stocking densities were used in this study. Tanks were populated with 450 (RAS 4,8 and 9), 900 (RAS 3,6 and 7) and 1800 (RAS 2 and 5) fish per system resulting in densities of 33 fish/m^3, 66 fish/m^3 and 132 fish/m^3 respectively. The two lower densities had three replicate tanks each with the highest densities (repeats of Nunley's first growth trial) only having two replicate tanks. Tanks were populated with 7 to 12 cm fingerling reciprocal cross hybrid striped bass

(female white bass x male striped bass) purchased from KEO FISH FARMS, (Keo AR.). Stocking took place during late December 1990 and early January 1991, allowing an acclimation period of at least two weeks before beginning the study.

FEEDING

The fish were fed a 44% crude protein, floating pellet formulated for hybrid bass and manufactured by BIOSPONGE AQUACULTURE PRODUCTS, (Sheridan Wyoming). Feed was offered by hand twice daily around 0830 every morning and again in the mid-afternoon. Random samples of 5.5% of each systems fish population were weighed every 28 days during the growth trial to continuously update estimates of total biomass and to obtain growth rate data. Feeding rates were calculated based on fish biomass estimates from these samplings. Tanks were fed on a percentile basis starting with 3% of the total biomass weight and adjusted weekly based on an assumed feed conversion ratio of 1.5:1. Feeding levels were dropped gradually to a low of 1% as the fish grew in size and percentile feeding rates decreased. The goal was to keep the feeding rates between tanks of different populations similar on a mass of feed to mass of fish basis. Although a target level of feeding (based on percent biomass) was desired, feed was offered to a given tank only as the fish would accept the feed. This was possible because the pelleted feed floated, which allowed a visual determination of feed consumption.

WATER EXCHANGE

All eight systems were originally filled with well water before being stocked with fish. After this initial filling, all RAS replacement water was taken from the municipal water supply system because the well water system could not support the daily water requirements of the entire research facility. Fresh municipal water was added to each system to make up for the minor evaporative losses and to flush out and refill the multi-tube solids clarifiers. Clarifiers were periodically isolated from the overall system and then sprayed clean and refilled whenever three or more kilograms of feed had been accepted by an individual RAS. However, clarifiers were not cleaned and refilled more often than once each day no matter what level of feed had been accepted during that day. Each exchange of a clarifier replaced 15% of a total system volume. This resulted in no more than one complete water volume exchange per week for any given system.

The well water was characteristically high in alkalinity while the city water was a relatively low 70 mg/l as $CaCO_3$ (Table 1). In response to alkalinity destruction by the RBC nitrification process, sodium bicarbonate ($NaHCO_3$) was added to the municipal water to maintain a minimal alkalinity and stabilize the pH.

Table 1. Characterization of well and municipal water supplies.

Parameter	Well Water	Municipal Water
Alkalinity as $CaCO_3$ mg/L	270	70
Hardness as $CaCO_3$ mg/L	370	120
Ammonia-nitrogen	nd*	nd*
Nitrite-nitrogen	nd*	nd*
Nitrate-nitrogen	nd*	nd*
pH	7.9	8.4

nd = not detected

The amount of sodium bicarbonate added to each refilled clarifier was based on the assumption that one kilogram of 44 % protein feed produces approximately 30 to 40 grams of total ammonia-nitrogen (TAN) and the fact that oxidation of 1 gram of TAN to NO_3^- destroys approximately 7.14 grams of alkalinity.

Based on this, the alkalinity present in the municipal water and the feed based wastage rate it was estimated that 1 kg of $NaHCO_3$ should be added each time a clarifier was cleaned and refilled in order to maintain alkalinity. In an effort to maintain an approximate 1:1 ratio of Na^+ and Cl^- ions and to maintain hardness levels with calcium addition, 660 grams of $CaCl_2$ was added along with the sodium bicarbonate. Any time a system alkalinity was observed below 100mg/l as $CaCO_3$ both sodium bicarbonate and calcium chloride inputs were doubled for that day.

CHAPTER II

CHARACTERIZATION OF WATER CHEMISTRY AND WATER QUALITY PARAMETERS

INTRODUCTION

The advantages and flexibility gained in intensive recirculating systems are purchased at the cost of increasing management complexity and system sensitivity. The fish population impacts water quality by producing metabolic waste such as solid feces and excreted ammonia. Concurrently, a complex suspended and attached microbial population is consuming and releasing compounds into the aquaculture system. Water chemistry and water quality can be expected to change as more and more metabolic activity occurs.

It has been shown that the accumulation of contaminants can lead to fish toxicity. By establishing three widely varied population levels it was hoped that the effects of these buildups could be characterized. In fact, based on a preliminary growth trial study, the highest population level was expected to cause problems. It was therefore expected that these various population densities would allow an evaluation of water quality characteristic of safe and unsafe conditions. This part of the evaluation included the implementation of a bioluminescent bacterial toxicity bioassay (Microtox).

How the water chemistry and water qualities change from the stocking of fingerlings to the harvest of the mature fish crop is an important part of the basis for management decisions. Understanding these changes can be a starting point for sound decisions and help in the task of minimizing environmental stress to and maximizing the growth of the fish culture .

OBJECTIVE

The purpose of this chapter was to quantify the basic water chemistry and water quality parameters of a high density recirculating aquaculture system producing hybrid striped bass. In order to accomplish this, the overall study program was divided into the following specific objectives:

1. To identify and monitor over time the major anions and cations present in solution.
2. To monitor transitional organic water quality parameters and investigate their effects if any on fish growth and mortality.
3. To monitor the build up of suspended solids and to characterize the particle size distribution of those solids.
4. To monitor for toxic events using the bioluminescent bacterial bioassay "Microtox" and attempt to correlate the bioassays response to any negative responses by the fish population.
5. To identify and describe the major diurnal cycles being established within the RAS.

LITERATURE REVIEW

Optimum growth conditions must be maintained for the hybrid striped bass for the RAS's to be successful. These culture requirements include a wide variety of physical and chemical parameters. More critical parameters are summarized in the following pages.

Woiwode and Adelman (1984) found that maximum growth of hybrid striped bass fingerlings occurred at 31°C. This is in agreement with the suggestion by Parker (1989) to raise water temperature to 30°C or higher once larvae are 10 days old or older. However, best feed conversion occurs at 19 to 23°C (Woiwode and Adelman 1984). Kerby *et al.* (1983) reported best growth of pond raised hybrids when temperatures were between 23 and 29°C. Temperature will also become an issue when discussing the nitrification system.

Carbon dioxide and pH are two related chemical parameters of concern. CO_2 respired into the water will produce carbonic acid and might alter the pH unless it is removed. Continuous stripping of carbon dioxide will largely alleviate this problem. This stripping is accomplished in our case by the RBC system (Wood 1992). Parker (1989) reported on studies by Bonn *et al.* (1976) where slightly basic pH (7.3) and well buffered water proved to be most productive in striped bass hatcheries.

The sustained oxygen needs of hybrid striped bass is reported to be 5 mg/L or higher (Lewis 1981).

Hybrids are an anadromous fish and therefore euryhaline. They can tolerate a wide range of salinity and have been observed to rapidly adjust from freshwater conditions to as high as 3.6 % salinity (sea water) (Bayless 1968).

TOXICITY OF NITROGENOUS COMPOUNDS

In recirculating aquaculture systems ammonia-nitrogen has historically been identified as the principal excretory product of the fish population effecting water quality. It has been well established that the unionized form of aqueous ammonia is the most toxic with temperature and pH controlling the proportion of the total ammonia-nitrogen that will be present in the unionized form (Smart 1978). The unionized ammonia levels can be calculated using the following equation derived from basic chemical equilibrium expressions.

$$\text{Unionized ammonia-nitrogen} = \text{TAN}/(1+10^{(pKa-pH)})$$

where: TAN = Total ammonia-nitrogen (mg/L)

pKa= equilibrium constant

pH = measured pH of the solution

The equilibrium constant is a function of temperature and can be determined using the following empirically fitted equation proposed by Emerson *et al.* (1975).

$$pKa = 0.09018 + 2729.92/T$$

where: T = solution temperature in ^{o}K.

Colt and Tchobanoglous (1976) reported a 96-hour LC50 for channel catfish exposure to unionized-ammonia ranging from 2.4 mg/L at 22 ^{o}C to 3.8 mg/L at 30 ^{o}C. They also report a 96-hour LC50 for rainbow trout as low as 0.4 mg/L indicating the relative sensitivity of different species. Sub lethal levels of ammonia-nitrogen might also impact fish production facilities by reducing growth, causing damage to gills and other organs, and predisposing fish to disease (Colt and Armstrong 1981, Luchetti and Gray 1988). Meade (1985) reported a wide variety of symptoms indicative of ammonia toxicity ranging from gill damage to damaged kidneys and possible neurological

dysfunction. Manci and Quigley (1981) observed gill damage in yellow perch in 120 day bioassays with total ammonia-nitrogen ranging from 0.5 to 2.0 mg/L. No specific literature recommendation was identified for hybrid striped bass. However, Colt and Orwicz (1991) set a conservative allowable design criteria for flow through systems at 0.0125 mg/L unionized ammonia-nitrogen.

Toxicity due to nitrite-nitrogen is also of concern in aquaculture systems. Nitrite is known to cause conversion of hemoglobin to methhemoglobin which is incapable of binding and transporting oxygen. It is however unclear based on literature whether nitrite toxicity in fish is caused by methhemoglobinemia effects or by a toxic reaction to the NO_2^- itself. Like ammonia-nitrogen, the degree of sensitivity exhibited by a given culture species varies widely. Mazik *et al.* (1991) reported a 96-hour LC50 for rainbow trout at 22 mg/l but 453 mg/L for largemouth bass. Mazik also shows that chloride ions will reduce nitrite toxicity effects. This concept is in agreement with findings by Russo and Thurston (1977) studying rainbow trout and Palachek and Tomasso (1984) studying channel catfish. Mazik found striped bass to be highly tolerant of environmental nitrite. Nitrite is transported via gill membranes into freshwater fish by the same mechanism used to transport chloride. It is suggested that striped bass are among a list of fish species capable of selectively passing chloride vice nitrite across the gill membranes. This ion selectivity provides a protection against high blood nitrite levels. Mazik *et al.* (1991) list a 24-hour LC50 of 163 mg/L for striped bass in the presence of relatively low chloride levels (38 mg/L) with markedly higher tolerances for higher levels of chloride.

Nitrate is the end product of biological nitrification. This metabolic product will build over time in recirculating systems as more and more feed is introduced. Fish are generally very tolerant of nitrate-nitrogen. Colt and Tchobanoglous (1976) report a 96-hour LC50 for channel catfish at 6,200 mg/L. Bohl (1977) reported on work by Braun

(1972) which demonstrated a "tolerance limit" to nitrate of 800 mg/L by rainbow trout. Nitrate levels of 90 to 200 mg/L had no reported effect on channel catfish during a study by Knepp and Arkin (1972) as reported by Colt and Armstrong (1981). Generally it appears that nitrate toxicity to aquatic species is not a serious problem.

EFFECTS OF ORGANICS

It has long been recognized that intensification of aquaculture systems results in a buildup of dissolved organic compounds. These organics are excreted by the fish or leached into the water directly from uneaten feed. Murphy and Lipper (1970) demonstrated that channel catfish produced more Biochemical Oxygen Demand (BOD) on a per pound live weight basis than chickens, swine or beef cattle. Chieng *et al.* (1989) studied the oxidation requirements of a high protein micro pulverized feed and reported an ultimate BOD of 660 mg O_2 per gram of feed. Dry trout feed BOD_5 ranged from 500 to 800 mg O_2 per gram of feed. Hirayama *et al.* (1988) observed the build up of dissolved organic substances (as DOC) in recirculating culture systems. Using ultra filtration techniques they observed that more than half of the total organic carbon existing had a molecular weight on the order of 10^4.

The concern with organics is similar to the more well established toxicity problems observed with ammonia-nitrogen, although the effects are perhaps less straight forward. Many fish species exhibit acute and chronic sensitivity to a wide range of organic compounds. Yu and Perlmutter (1970) even report on a growth inhibiting factor being released by zebra fish and gourami's living in crowded recirculating conditions. Perlmutter *et al.* (1973) continued this study investigating crowding effects on the immune response of blue gourami when challenged by infectious pancreatic necrosis virus. They suggests that an observed reduced immune response resulted only from crowding. However, systems with water cleaned of organics before recirculation showed

less suppression of the immune response. Hirayama and his colleges suggest from their study that build ups of organic substances might lead to the suppression of growth of fish cultured in recirculating systems.

MICROTOX

Surrogate organisms have become a well established tool used to study toxicity to the general aquatic population. Rainbow trout, fathead minnows, daphnia and an array of other organisms have long been used in bioassay studies attempting to determine toxicity effects of individual and complex solutions of toxicants. Microtox is a bioluminescent bacterial bioassay produced by Beckman Instruments Inc., (Carlsbad CA 92008). The Microtox toxicity test measures the reduction of light emitted by a bioluminescent bacterium (*Photobacterium phosphoreum)* when it is exposed to a toxic environment. The test is reproducible because of well established standardized procedures, hardware and reagents as well as fast (minutes vice hours or days) relative to the longer bioassays needed for other surrogates (Blum and Speece 1990). Blum and Speece are among many researchers who report good correlation between the Microtox assay and bioassays using fish species such as fathead minnows, rainbow trout and blue gill (Munkittrick *et al.* 1991, Ribo and Kaiser 1983.) In fact, Microtox is reported to be a more sensitive bioassay when exposed to complex solutions of multiple toxins, especially organic complexes such as those found in waste effluents(Munkittrick *et al.* 1991). For all of these reasons Microtox was chosen for use in this study to investigate potential toxic situations caused by contamination build ups in the aquaculture water over time.

METHODS AND MATERIALS

EXPERIMENTAL DESIGN

A primary goal of this study was to characterize water chemistry and water quality changes with time as feeding rates were increased. This was accomplished by establishing the three fish population levels of 33 fish/m^3, 66 fish/m^3 and 132 fish/m^3. These population densities were chosen based on an earlier growth trial (Nunley 1992) where all tanks were populated at 132 fish/m^3. During this earlier trial, three episodes of presumed toxicity occurred, resulting in sudden high mortalities. By running duplicate systems at this same intensity, it was expected that toxicity would occur. By operating the other six systems at lower populations it was anticipated that a non-toxic level would also be observed. Observing a toxic and non toxic situation would allow characterization of a full range of water qualities defining safe and unsafe conditions.

ANION ANALYSIS

Anions were evaluated using a DIONEX 2010i Ion Chromatograph (DIONEX INC. Sunnyvale California) with an AS4A separation column and conductometric detection. Samples were collected from the center of each culture tank and evaluated that same day. After being diluted with milli-Q water and just prior to injection into the ion chromatograph, samples were filtered through a 1.5 micron glass fiber filter to protect the AS4A separation column. Chromatograph traces were analyzed by comparison to standards prepared for each analysis.

CATION ANALYSIS

Cations were evaluated by atomic absorption using a PERKIN-ELMER 703 Atomic Absorption Spectrophotometer (PERKIN-ELMER CORP. Norwalk, Conn.). The exception to this was lead, which required use of graphite furnace atomic absorption methods because of the extremely low levels being analyzed. For lead a PERKIN-

ELMER 5100PC was used with a ZEEMAN 5100 BACKGROUND CORRECTION. Spectrophotometers were calibrated using commercially available standard solutions purchased from FISHER SCIENTIFIC. Water samples were again collected from near the center of the culture tanks but unlike those samples collected for anion analysis these were fixed by adjusting the sample pH to below 2 with HCl and sealed in filled clean glass containers for analysis at a latter convenient date.

ORGANICS and SUSPENDED SOLIDS ANALYSIS

The organic indicators assayed were five day carbonaceous biochemical oxygen demand ($CBOD_5$), dissolved organic carbon (DOC) and chemical oxygen demand (COD).

$CBOD_5$ was analyzed using unfiltered water samples following Standard Methods procedure 5210 B for the 5-day 20 ^oC BOD test and inhibiting nitrification using 2-chloro-6(trichloro methyl) pyridine (TCMP).

Dissolved organic carbon levels were analyzed using a DOHRMANN MODEL DC 80 Total organic carbon analyzer (DOHRMANN INC., Santa Clara California). DOC analysis required access to an off site analyzer located across campus. Samples ,once collected, were immediately filtered through 0.45um filters into new 5ml scintillation viles, acidified with phosphoric acid to below pH 2 and refrigerated (not frozen). Analysis was completed as soon as possible , usually the same day but always within 24 hours.

Analysis of DOC was carried one step further in attempt to characterize the molecular weight distribution of the soluble organics. SERIES 8200 AMICON Stirred cells (AMICON INC., Beverly MA) were used with AMICON YM1, YM3, YM10 and YM30 ultrafiltration membranes providing molecular weight cut offs of 1,000, 3,000, 10,000 and 30,000 respectively. Water samples had to be prefiltered through 1.5 micron

glass fiber filters before using a 0.45 micron filter to clear the sample of solids. This was necessary to prevent excessive clogging of the 0.45 micron filters while generating enough sample for the ultrafiltration procedure. Ultrafiltration membranes were cleaned of residual organics by purging them with carbon free milli-Q water. Once the membranes were cleaned and the sample water filtered, samples were sequentially forced through smaller and smaller membranes starting with the largest YM30 and ending with the smallest YM1, using nitrogen gas at 40 psi,. By measuring the starting DOC levels and the DOC levels present in the effluent water collected from each membrane the fraction of DOC retained between a given pair of membranes was calculated by differences . This procedure was performed at the end of the growth trial on August 25 for one RAS representing each of the three population levels.

COD analysis was based on the Closed reflux titrimetric method listed in Standard Methods as procedure 5220 C. Culture tank COD samples were filtered through 0.45 micron filters prior to analysis.

Total, fixed and volatile suspended solids were analyzed following the "Total Suspended Solids Dried at 103-105°C" procedure listed as 2540 D in Standard Methods followed by the "Fixed and Volatile Solids Ignited at 550°C" procedure 2540E.

The particle size distribution of the total suspended solids (TSS) was also evaluated by sequentially washing water samples through 105, 70, 30 and 1.5 micron filters. The 105, 70 and 30 micron filters were cut from sheets of SPECTRA mesh woven polymer screens purchased from FISHER SCIENTIFIC. Other than altering the filter ratings, procedure 2540.D from Standard Methods was followed.

MICROTOX ANALYSIS

Microtox evaluations were completed on sight, following instructions provided in a 1982 BECKMAN INSTRUMENTS system operating manual using a BECKMAN MODEL 2055 ANALYZER AND RECORDER. The 2:1 serial dilution was used with unfiltered aquaculture samples being adjusted to the minimum 2% NaCl required by the marine bioluminescent bacteria. The maximum percent aquaculture water tested following the Microtox procedure was 45.0 %. Sample dilution's were analyzed at 20 ^{o}C for 5, 15 and 30 minute runs.

OTHER DAILY ANALYSIS

As a preplanned part of the daily management scheme, water quality parameters were measured in the early morning in order to evaluate a system's daily status and determine if a given RAS was operating properly before it was fed at the preplanned level. These parameters included total ammonia nitrogen, dissolved oxygen, temperature, alkalinity and pH. In addition nitrite, nitrate and hardness values were evaluated at least once a week. Portions of this data taken from Nunley (1992) will be used in this thesis and therefore those methods will be briefly summarized herein.

Total ammonia-nitrogen (TAN) was analyzed on a HACH DR2000 spectrophotometer (HACH Co., Loveland CO) using Nessler method # 380. Nitrite-nitrogen was analyzed on the same spectrophotometer using the diazotization method # 371. TAN and nitrite-nitrogen samples were analyzed on site immediately after sampling. Ammonia-nitrogen standards purchased from HACH were used to periodically check the procedure accuracy. Sodium nitrite was used to mix the unstable standard used to ensure accuracy in the nitrite analysis.

Dissolved Oxygen and temperature were evaluated using a YSI MODEL 58 dissolved oxygen meter (YSI Co.,Yellow Springs, OH.). A HACH pH pen was used for

pH measurements. Hardness and alkalinity were measured as $CaCO_3$ following HACH digital titration procedures. Hardness was evaluated using HACH ManVer 2 powder pillows and titrated with the HACH digital titrator using an ethylenediamineetraacectic acid (EDTA) titration cartridge. Alkalinity was evaluated using HACH bromcresol green methyl red powder pillows and titrated with a sulfuric acid HACH titration cartridge to the pink color change corresponding to a pH of 4.5.

DIURNAL CYCLE ANALYSIS

On August 22 1991 three RAS were chosen for a 24 hour analysis, intended to characterize diurnal behavior. One system was selected at each population level. Starting at 9AM on the morning of August 22 water quality parameters were monitored around the clock until 9AM August 23. This analysis included bihourly evaluations of culture tank temperature, pH, TAN, nitrite-nitrogen, dissolved oxygen and alkalinity.

RESULTS

IONS

The major anions observed were chloride, nitrate, sulfate, phosphate and bicarbonate. Observed concentration levels are summarized in Appendix A. Bicarbonate levels are available through Nunley (1992) measured as alkalinity at a known pH. Figure 2 shows the variation in chloride over time. All eight systems started at different levels of chloride but were nearly equal by day 75 into the growth trial. Generally, systems were higher in chloride at the beginning of the growth trial because of therapeutic doses of NaCl (solar rock salt) added when fingerlings were initially stocked into the systems. This is particularly true of RAS 2 which was slower to acclimate than the other seven systems and more heavily salted. System nitrate levels presented in Figure 3 rose steadily from very low levels early in the growth trial to as high as 170 mg/L near the end.

Sulfate levels began near 30 to 35 mg/L and slowly rose over time to 40 to 50 mg/L. Initial sulfate levels are attributed to sulfate present in the well water used to fill the systems at the beginning of the growth trial.

Phosphate levels rose slowly from near zero to a range of 4 to 8.5 mg/L as phosphorus by the end of the trial depending on the particular RAS being analyzed.

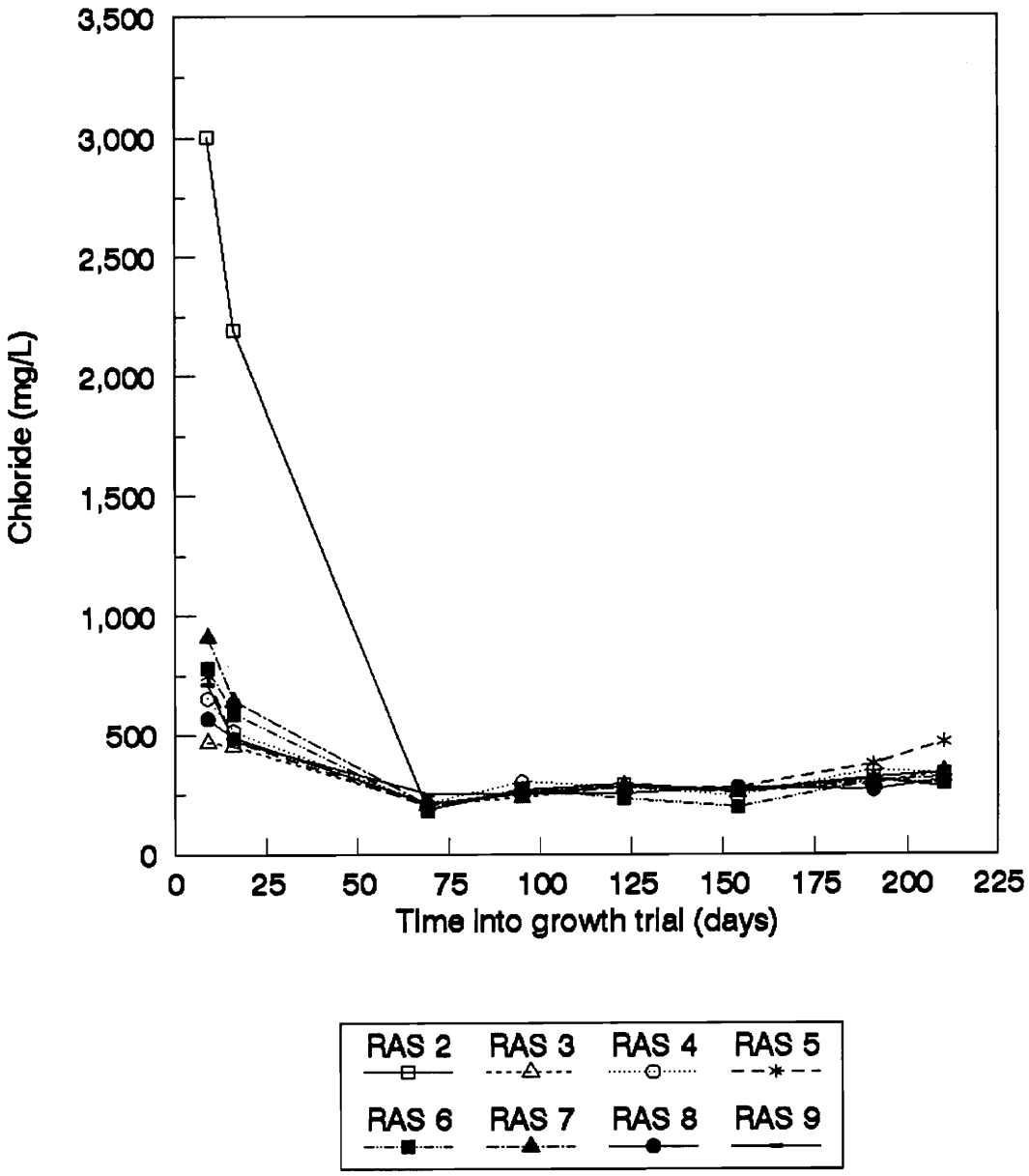


Figure 2. System chloride levels as a function of days into the growth trial.

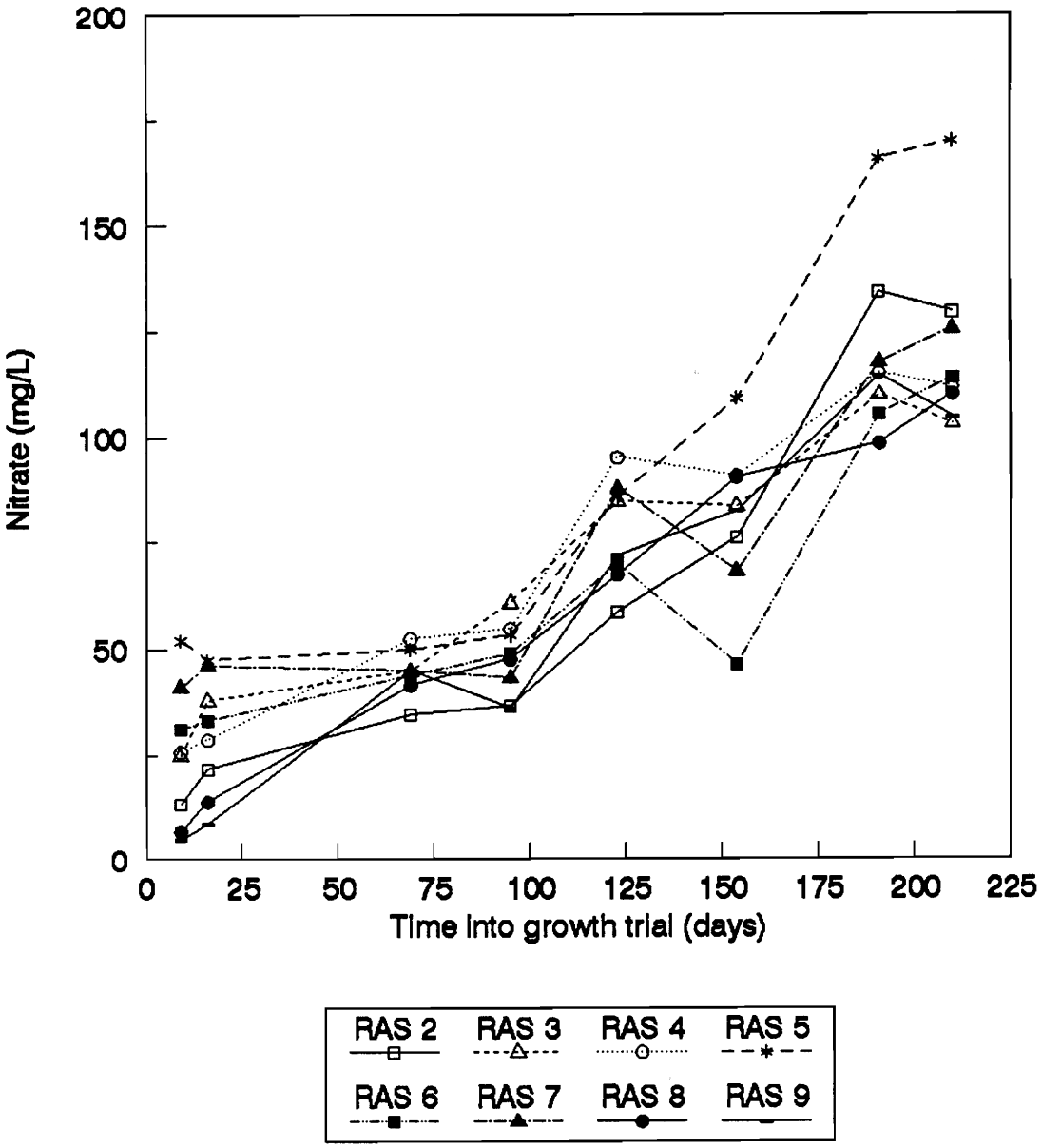


Figure 3. System nitrate levels as a function of days into the growth trial.

The major cations observed were sodium, calcium, magnesium and potassium. Trace levels of copper and iron were also observed. Lead was intermittently detected at 1-2 ppb. Cation data is tabulated in Appendix B.

The effects of the chemical management approach to treating the replacement clarifier water is shown in Figure 4 where, like chloride, the sodium started at different levels dependent on the degree of therapeutic salting and adjusted with time to a level largely dictated by this managed approach. Figure 5 shows a comparison of a representative set of RAS sodium and chloride concentrations observed near the halfway point in the growth trial. The $Na^+$to Cl^- ratios represented in Figure 5 ranged from 0.78 to 1.12, a good approximation of the expected 1:1 ratio. Calcium and Magnesium levels were initially relatively high because of the hard well water used at the beginning of the study. Calcium levels for all RAS's gradually fell until around day 70. After day 70 calcium levels slowly rose in all systems in response to increasing rates of $CaCl_2$ addition matching the increasing $NaHCO_3$ required to maintain alkalinity as increasing feeding rates generated more ammonia-nitrogen.

Potassium rose gradually over time from very low levels around 10 mg/L up to 33 to 90 mg/L depending on the individual system. Figure 6 shows an ion balance for the major contributing anions and cations for a representative system during the middle of the growth trial (RAS 5 on April 19 1991).

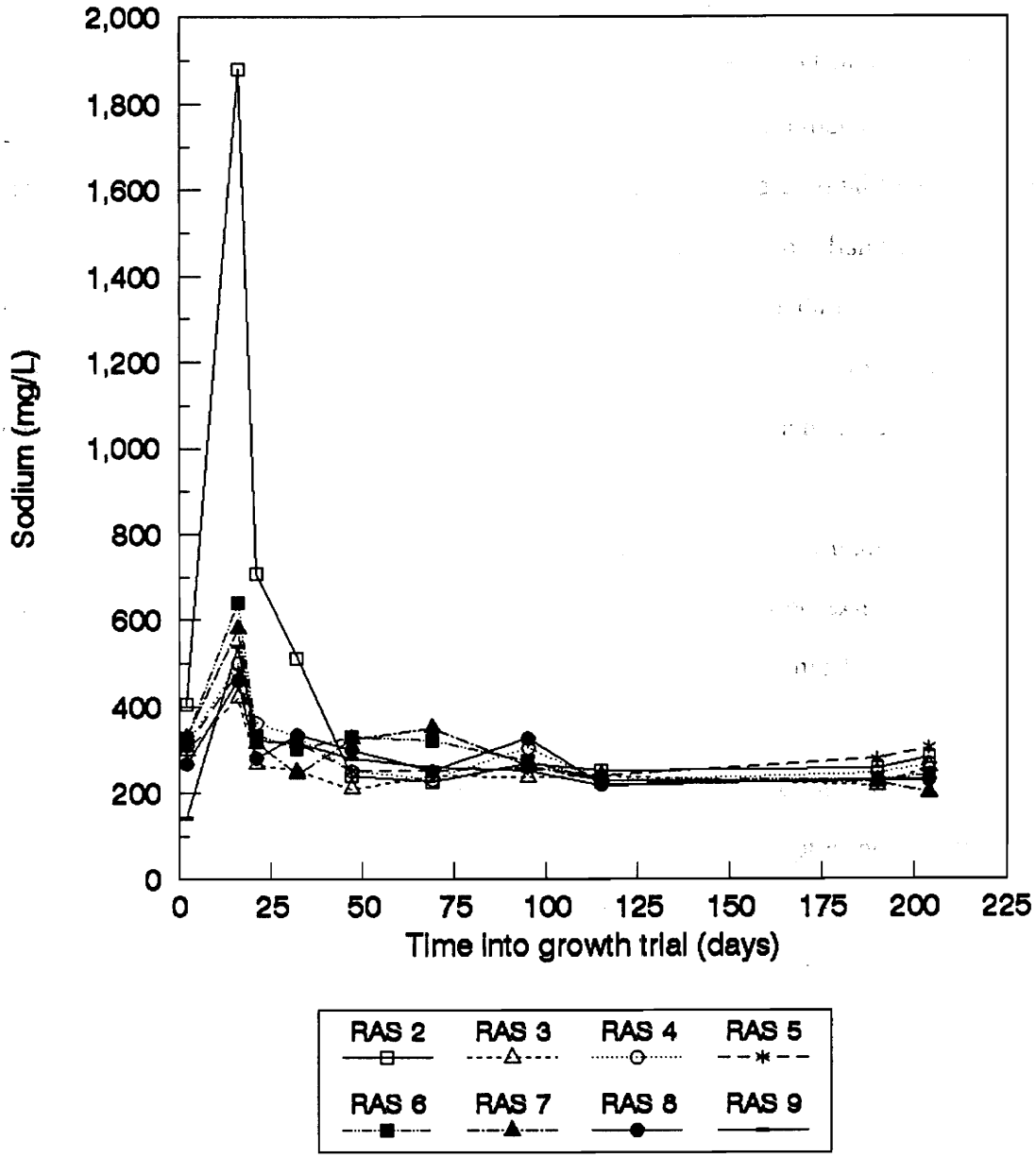


Figure 4. System sodium levels as a function of days into the growth trial.

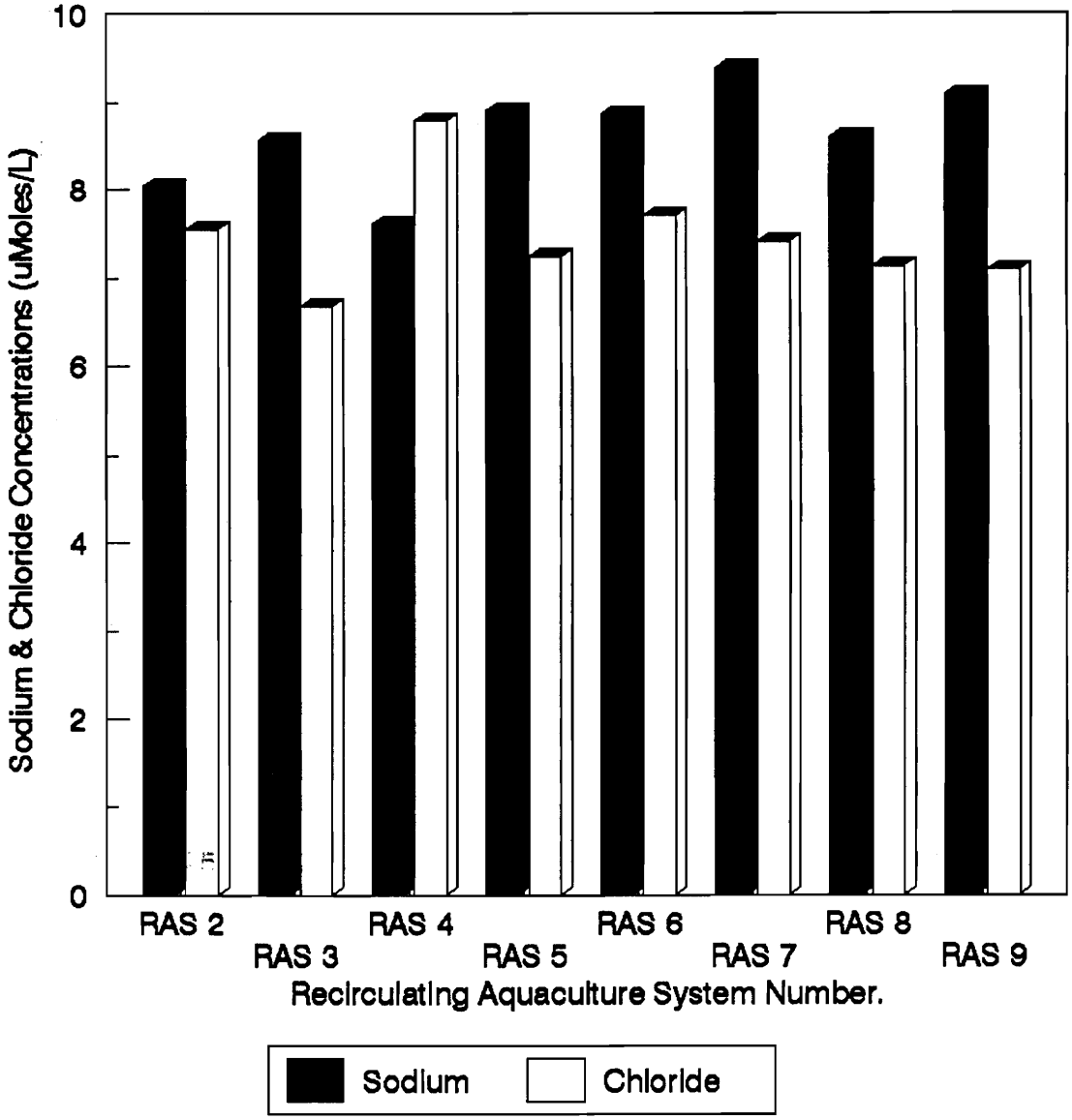
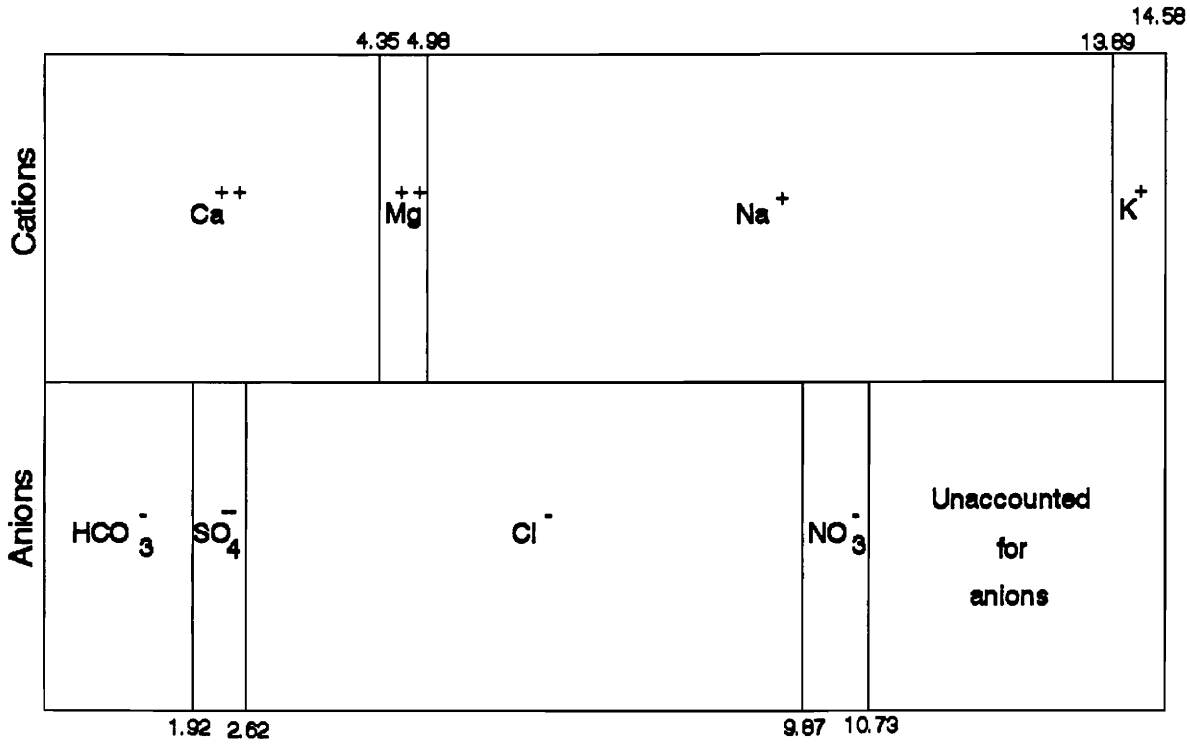


Figure 5. Comparison of representative sodium and chloride concentrations observed in recirculating aquaculture systems (19 April 1991).



Ion concentration (Meq/Liter)
RAS No. 5 19, April 1991

Figure 6. Ion balance bar graph analysis of representative RAS water.

ORGANICS AND SUSPENDED SOLIDS

The $CBOD_5$, COD, DOC and TSS observations are summarized in Appendices C through E. Figures 7 through 10 are plots of these water quality parameters as functions of time into the growth trial. Each plot shows the average water quality parameter concentration observed for each population density (33 fish/m^3, 66 fish/m^3 and 132 fish/m^3). Note that COD and DOC generally increased with time as daily feeding levels were also rising. $CBOD_5$ and TSS achieved stable levels after about 50 days with higher population tanks showing higher average water parameter values than lower population levels.

Figures 11 through 14 demonstrate the dependency of these organic and solids water quality indices on RAS feeding rate. Although some scatter exist in the data accumulated from the eight independent recirculating systems, organic levels increased with time and with increasing feeding levels.

The DOC molecular weight distribution (Table 2) observed at the end of the growth trial (when DOC levels were at their highest) indicates the presence of organic compounds across the entire range of molecular weights being evaluated.

The suspended solids particle size distribution data is summarized in Appendix F. On average the majority (67%) of the suspended solids were between 1.5 and 30 microns in size (Figure 15). Approximately 75% of the TSS was volatile.

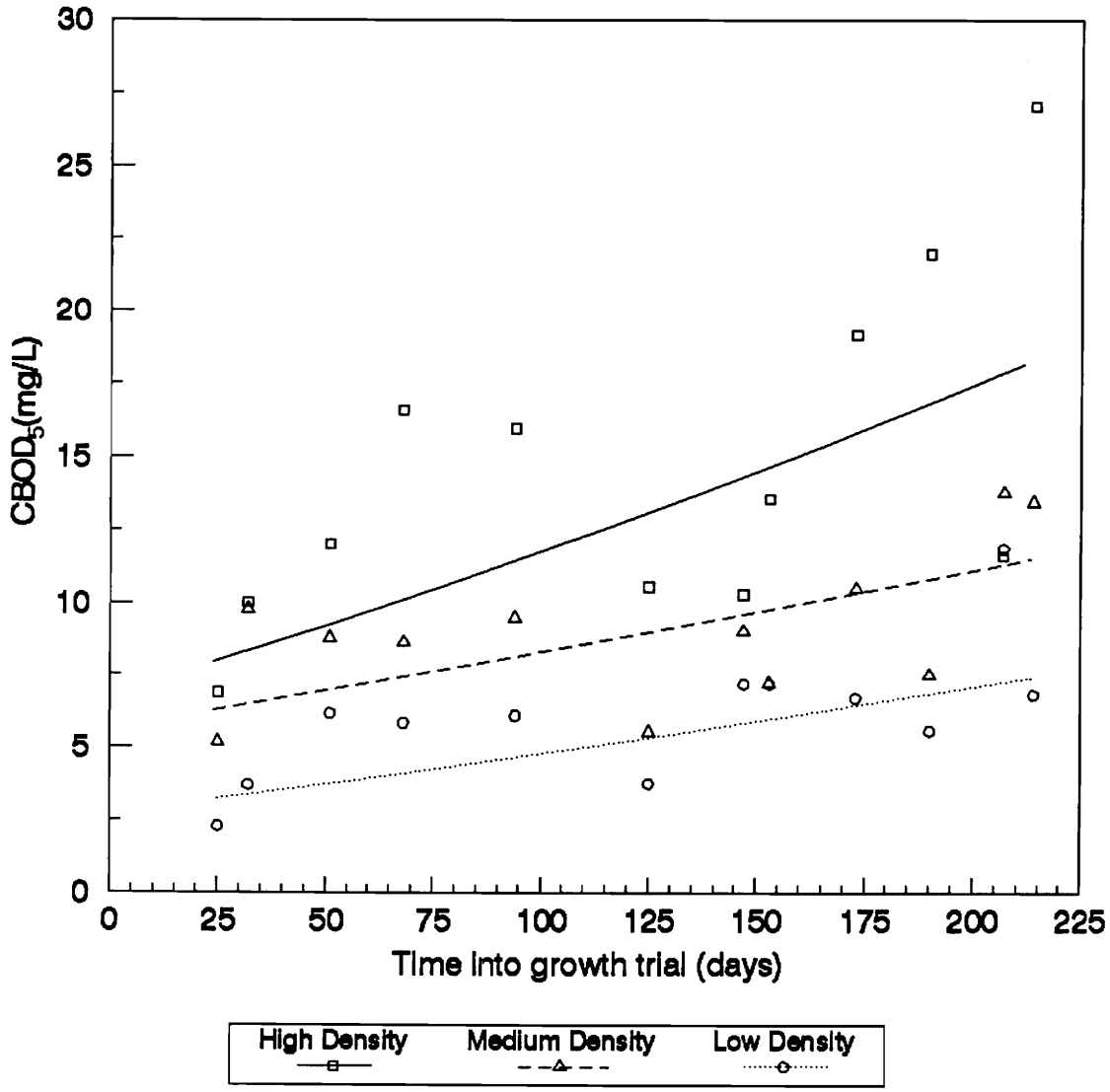


Figure 7. Average 5 day carbonaceous biochemical oxygen demand of each stocking density vs days into the growth trial.

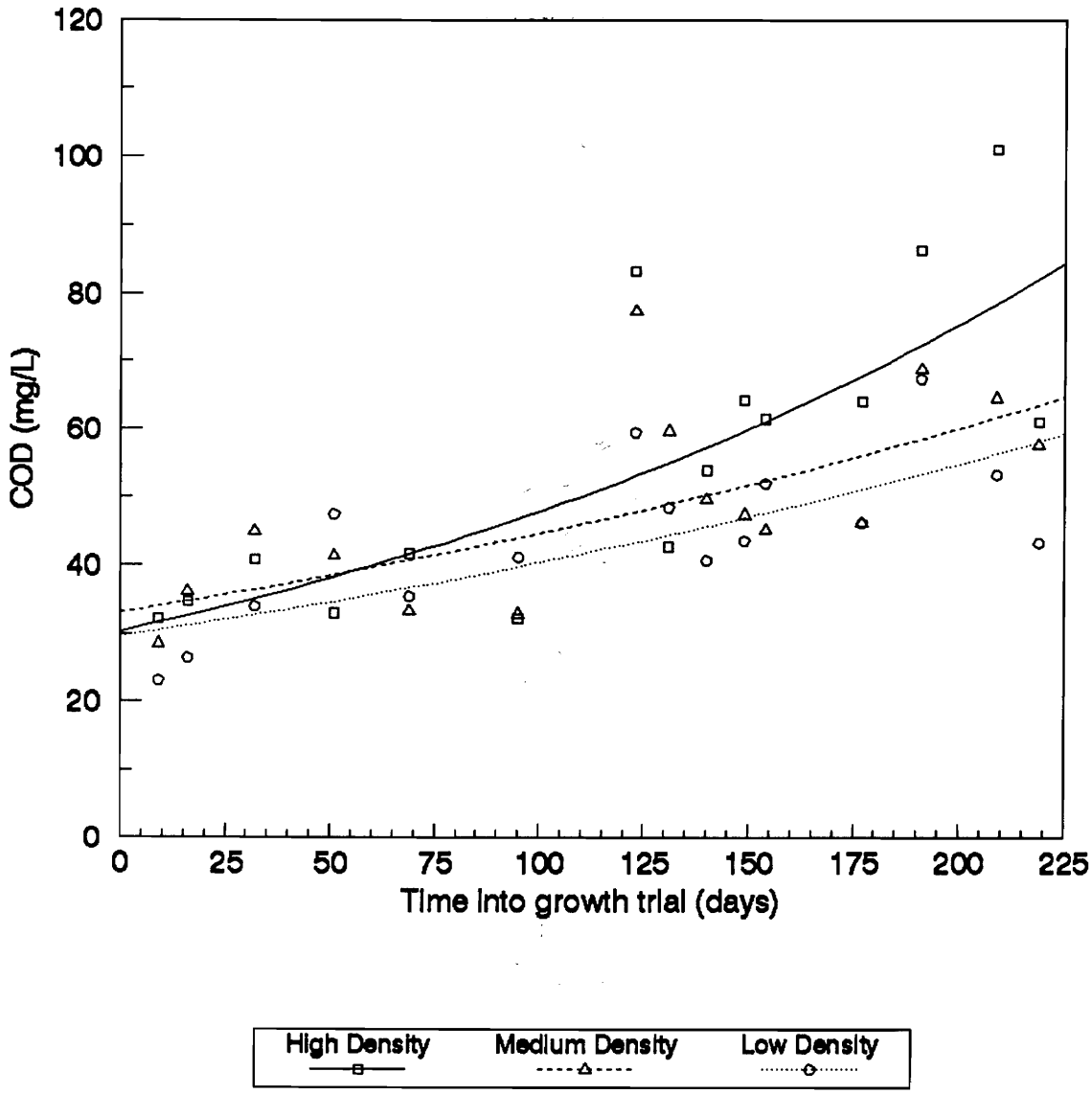


Figure 8. Average chemical oxygen demand of each stocking density plotted against days into the growth trial.

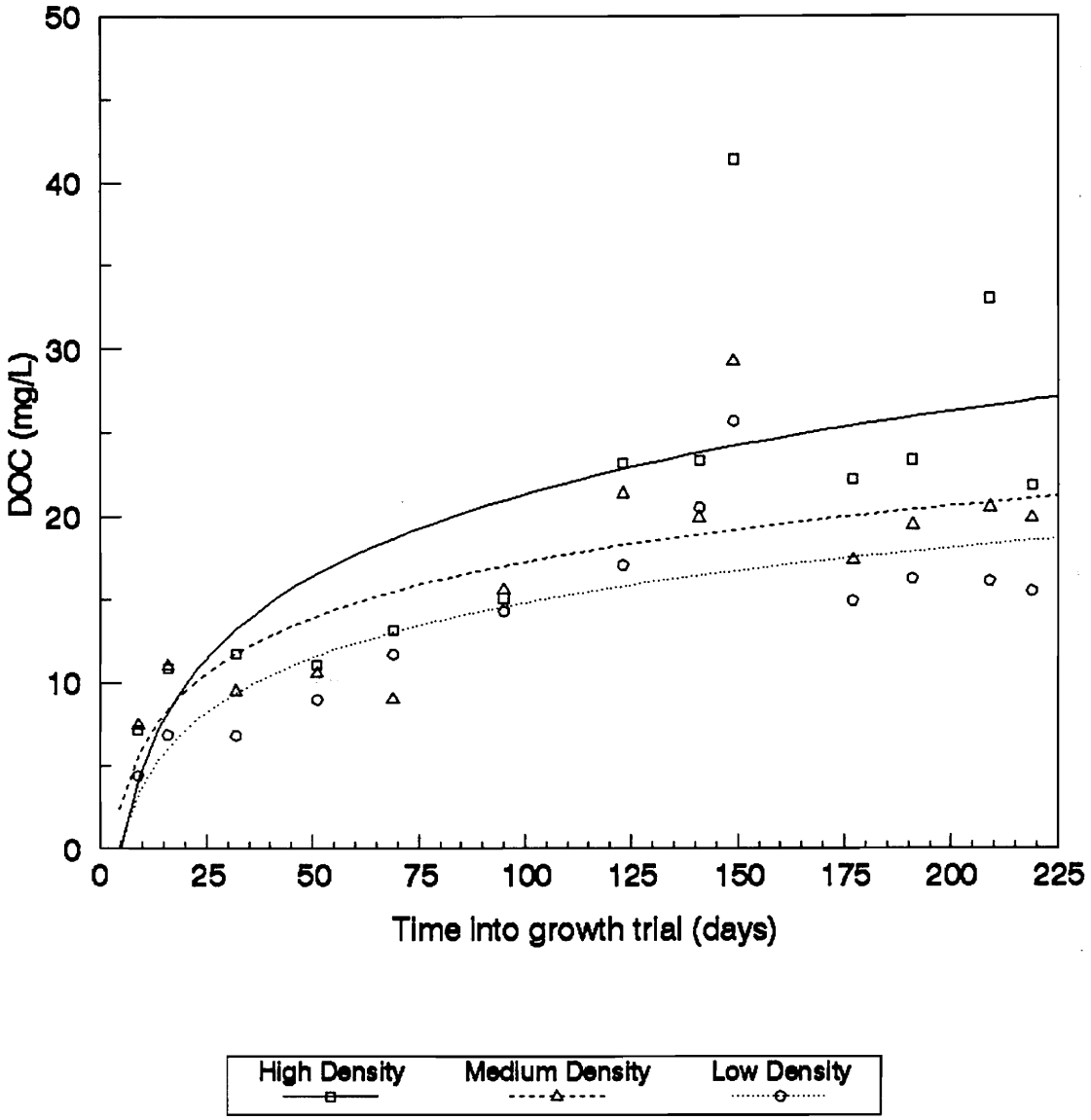


Figure 9. Average dissolved organic carbon of each stocking desnsity plotted against days into the growth trial.

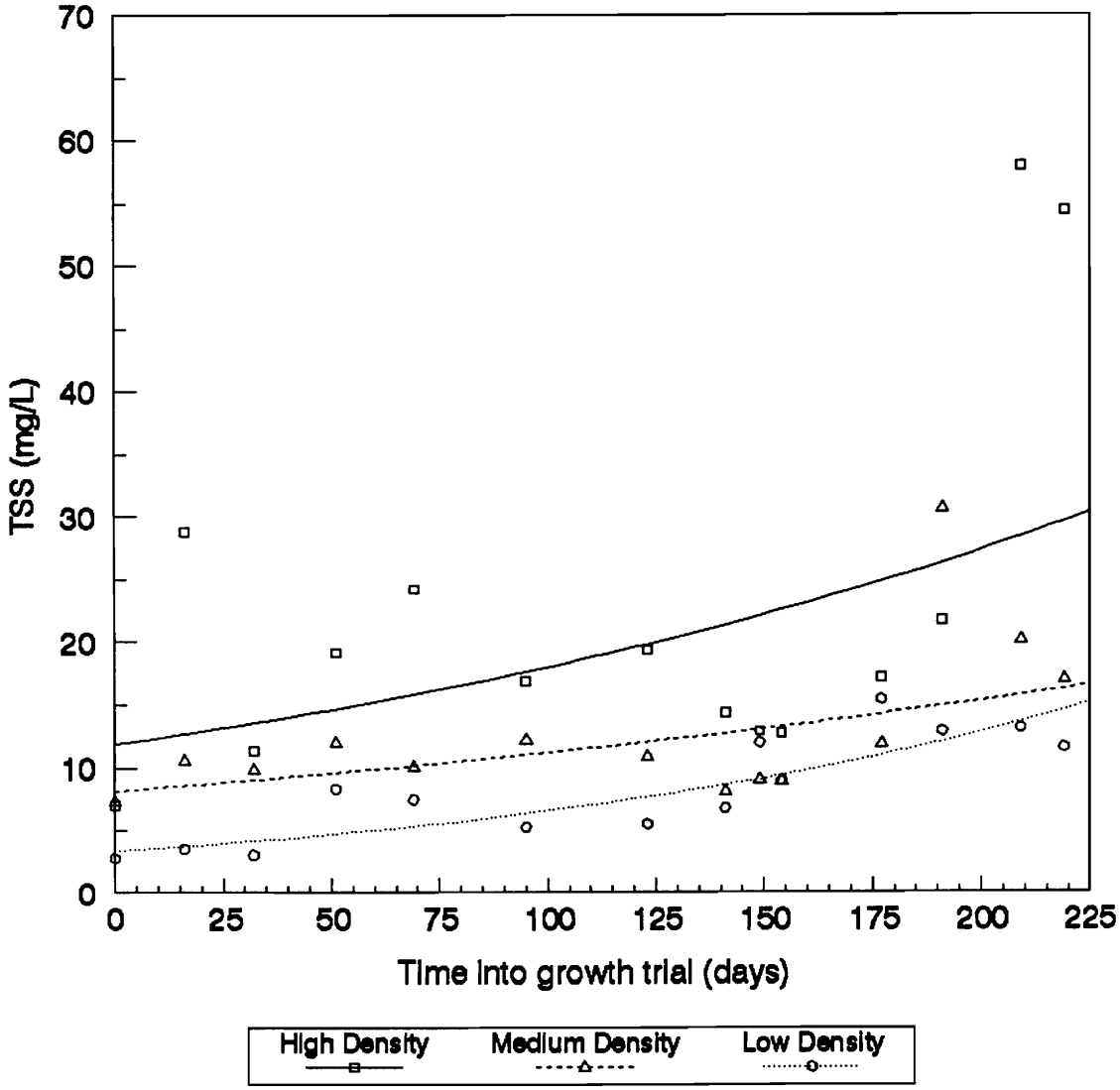


Figure 10. Average total suspended solids of each stocking density plotted against days into the growth trial.

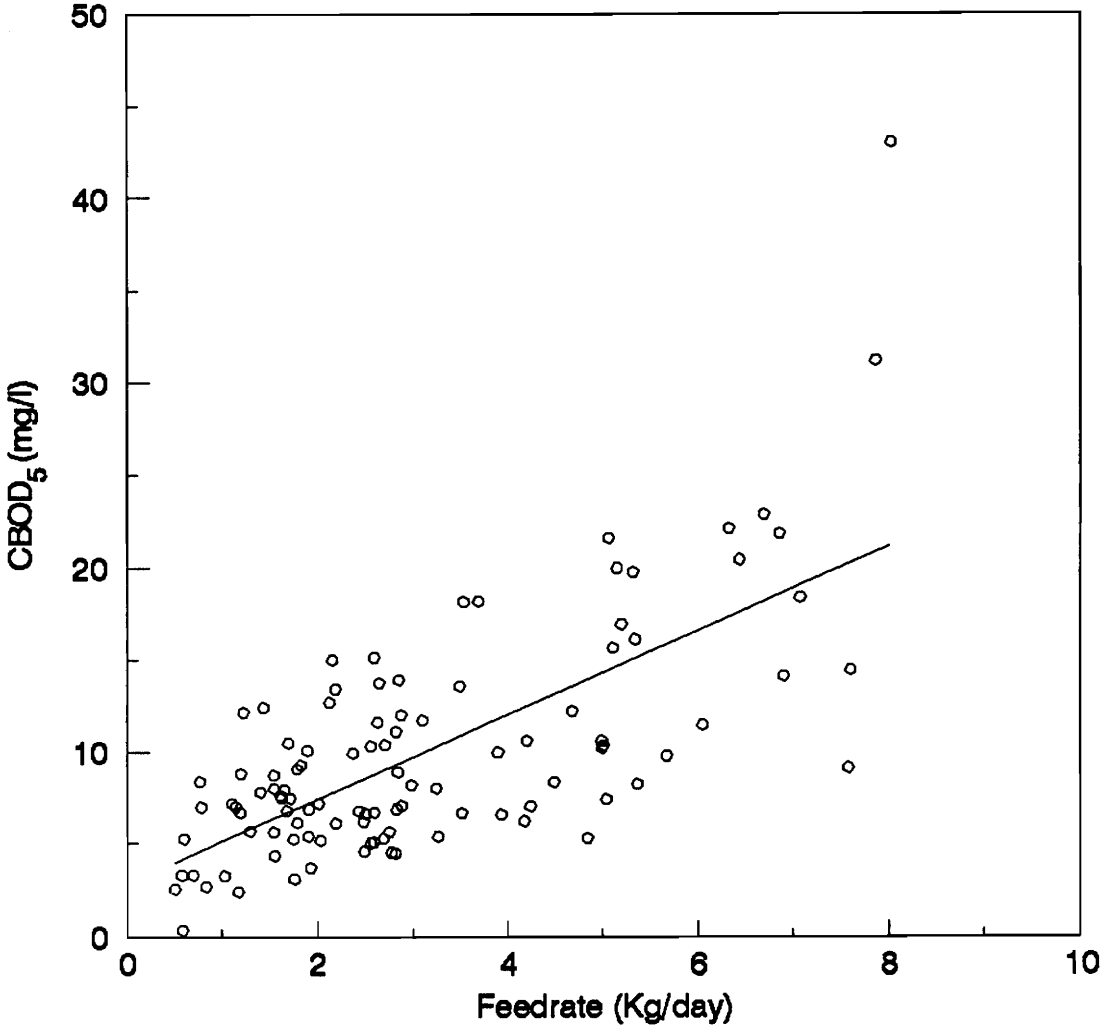


Figure 11. Five day carbonaceous biochemical oxygen demand plotted against feed rate for all population densities.

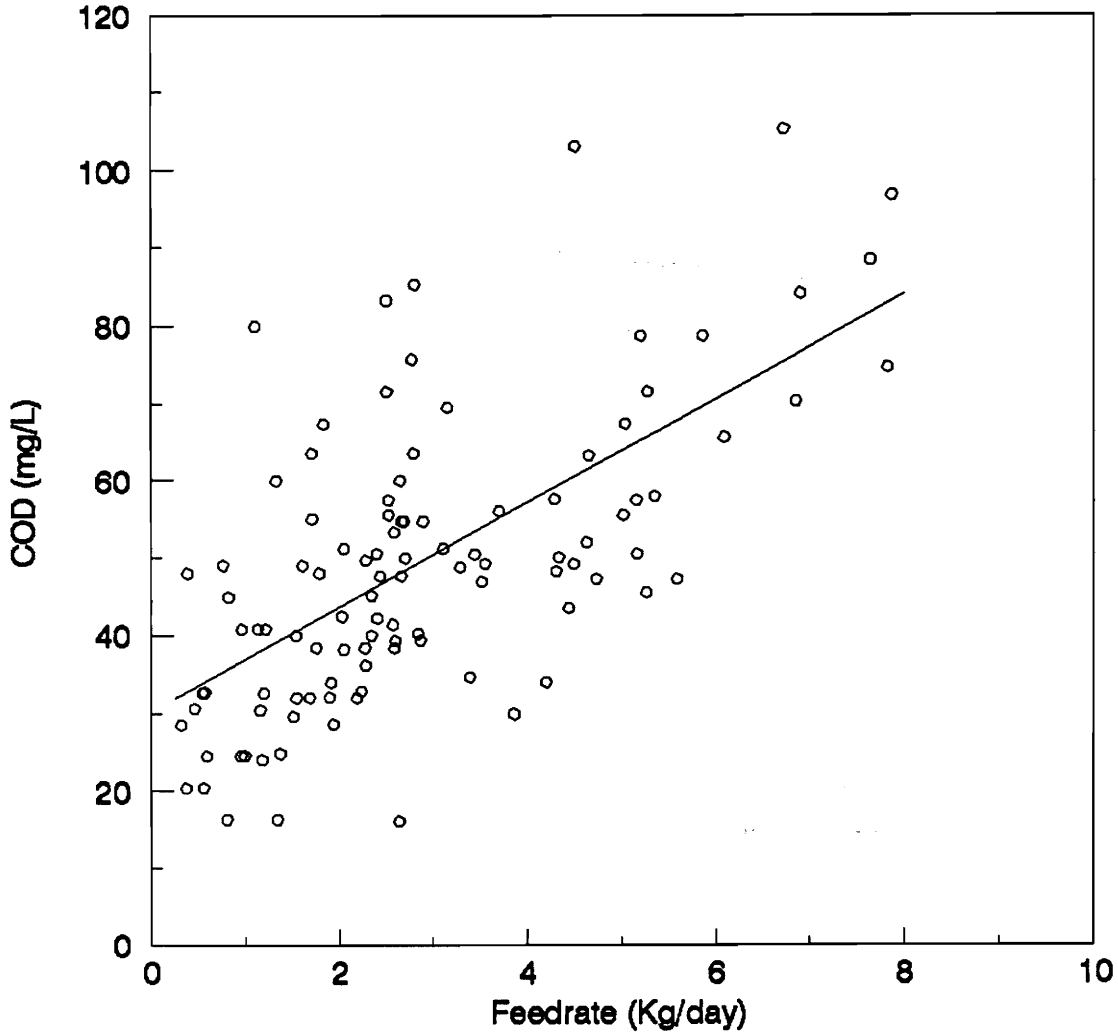


Figure 12. Chemical oxygen demand plotted against feed rate for all population densities.

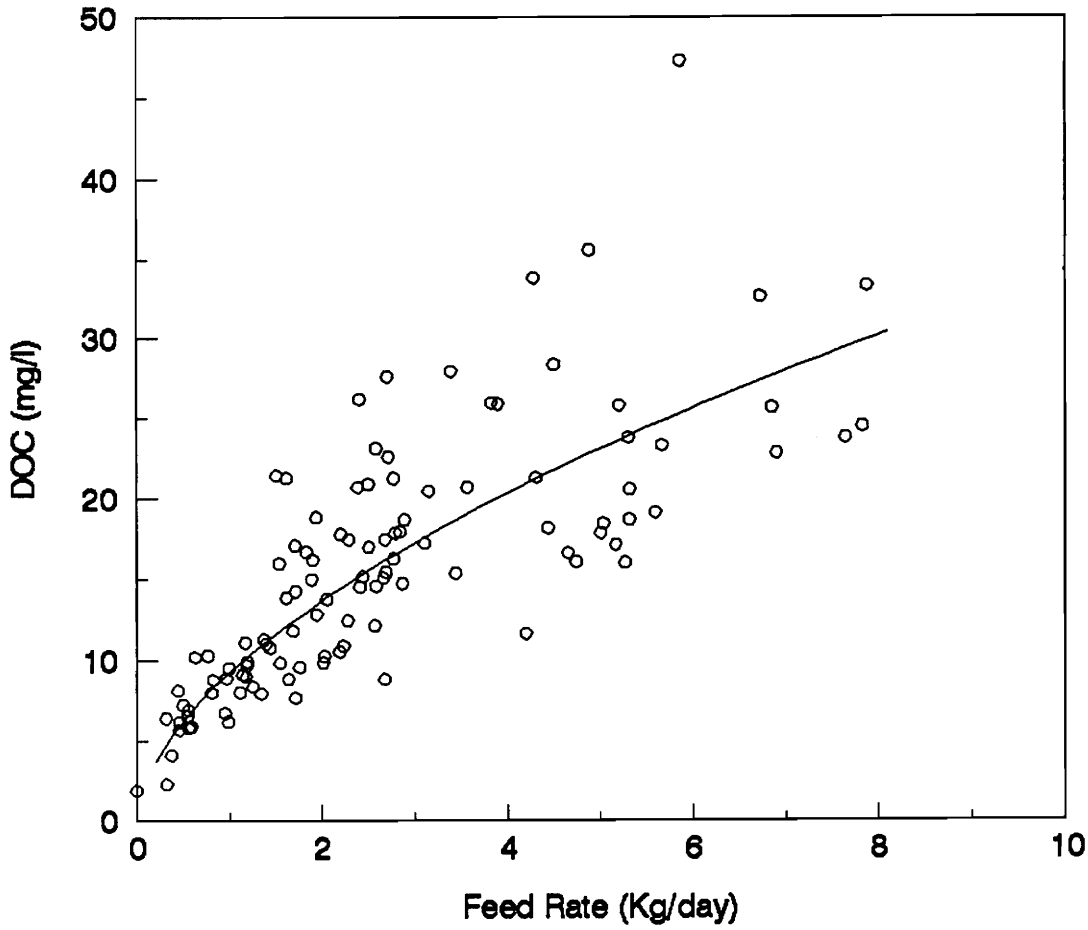


Figure 13. Dissolved organic carbon plotted against feed rate for all population densities.

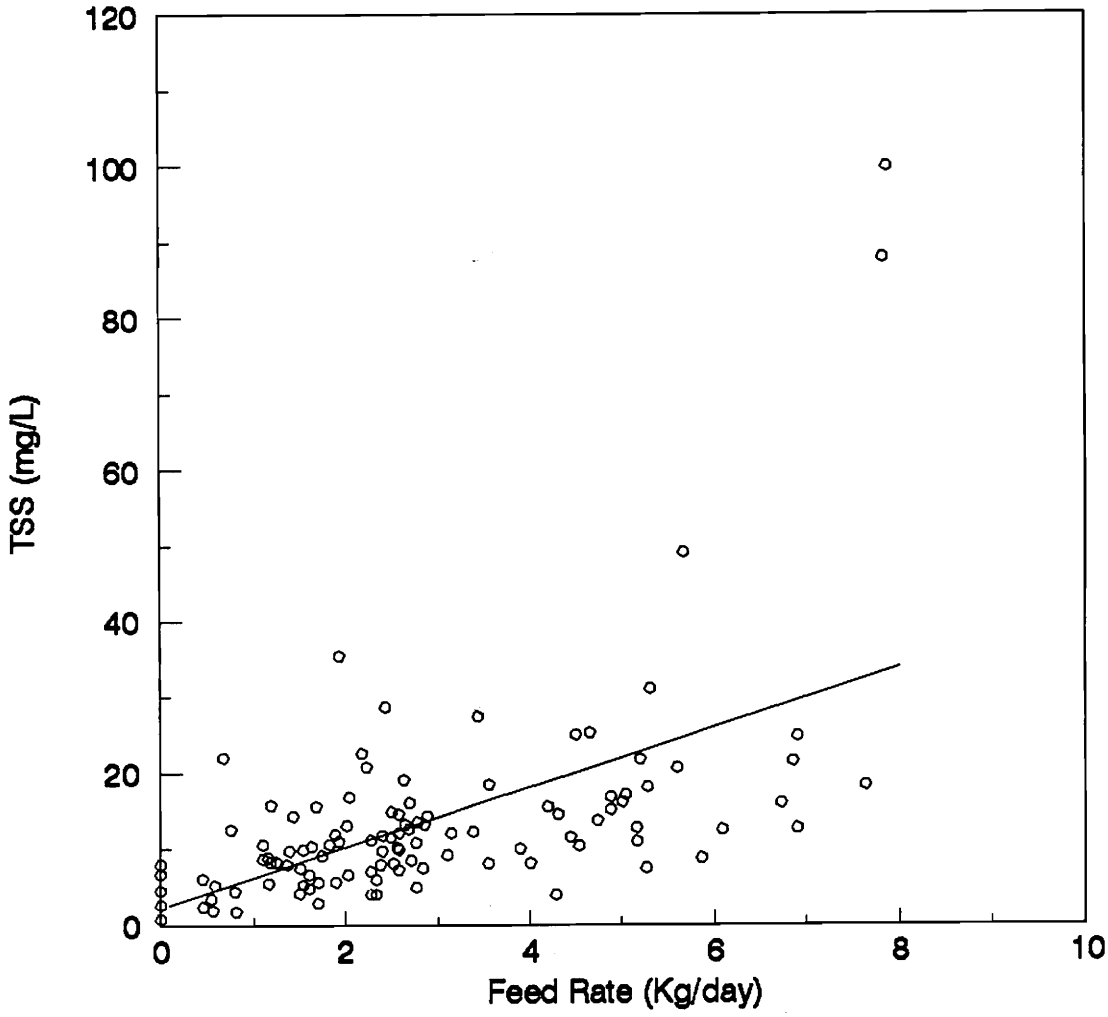


Figure 14. Total suspended solids plotted against feed rate for all population densities.

Table 2. Molecular weight distribution of dissolved organic carbon.

Molecular Weight Range	Percent Distribution (High density)	(Medium density)	(Low density)
>30,000	28.6	26.5	27.4
<30,000 >10,000	9.8	7.9	16.8
<10,000 >3,000	12.3	20.8	8.2
<3,000 >1,000	28.6	16.5	18.1
<1,000	20.7	28.3	29.5

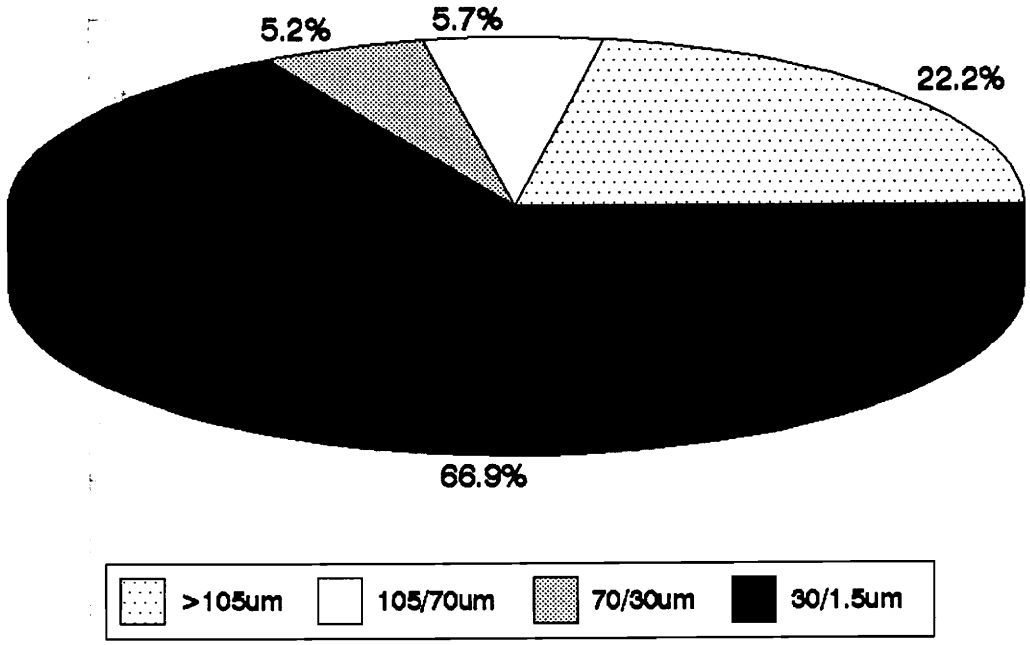


Figure 15. Average suspended solids particle size distribution observed in populated culture tanks.

DIURNAL CYCLES

Data collected over the 24 hour period from 9AM 22 August to 9AM 23 August 1991 is summarized in Appendix H. Decreasing oxygen levels were observed (Figure 16) as fish activity increased in the early morning with a second slump in DO in the mid afternoon after the second feeding for that day. The high, medium and low density systems show decreasing drops in DO of 3.3, 2.3 and 1.7 mg/L respectively. In all three systems DO levels increased after lights out around 6 to 7 PM returning slowly back to maximum levels as fish activity dropped during the night.

Figure 17 shows total ammonia-nitrogen concentrations peaking around 1 pm approximately four hours after lights were turned on and morning feeding was completed. Figure 18 demonstrates the alkalinity destruction occurring on a daily cyclic basis as TAN is oxidized to nitrate. The large increases in system alkalinity from 1 to 5 PM are due to water exchanges during washing and refilling of multi-tube clarifiers. Of particular interest are the slopes of the alkalinity curves for all three systems from 5 pm to 9 am the next morning. These slopes indicate alkalinity destruction rates of 3.13, 2.38 and 1.44 milligrams per liter per hour respectively for the high, medium and low density systems.

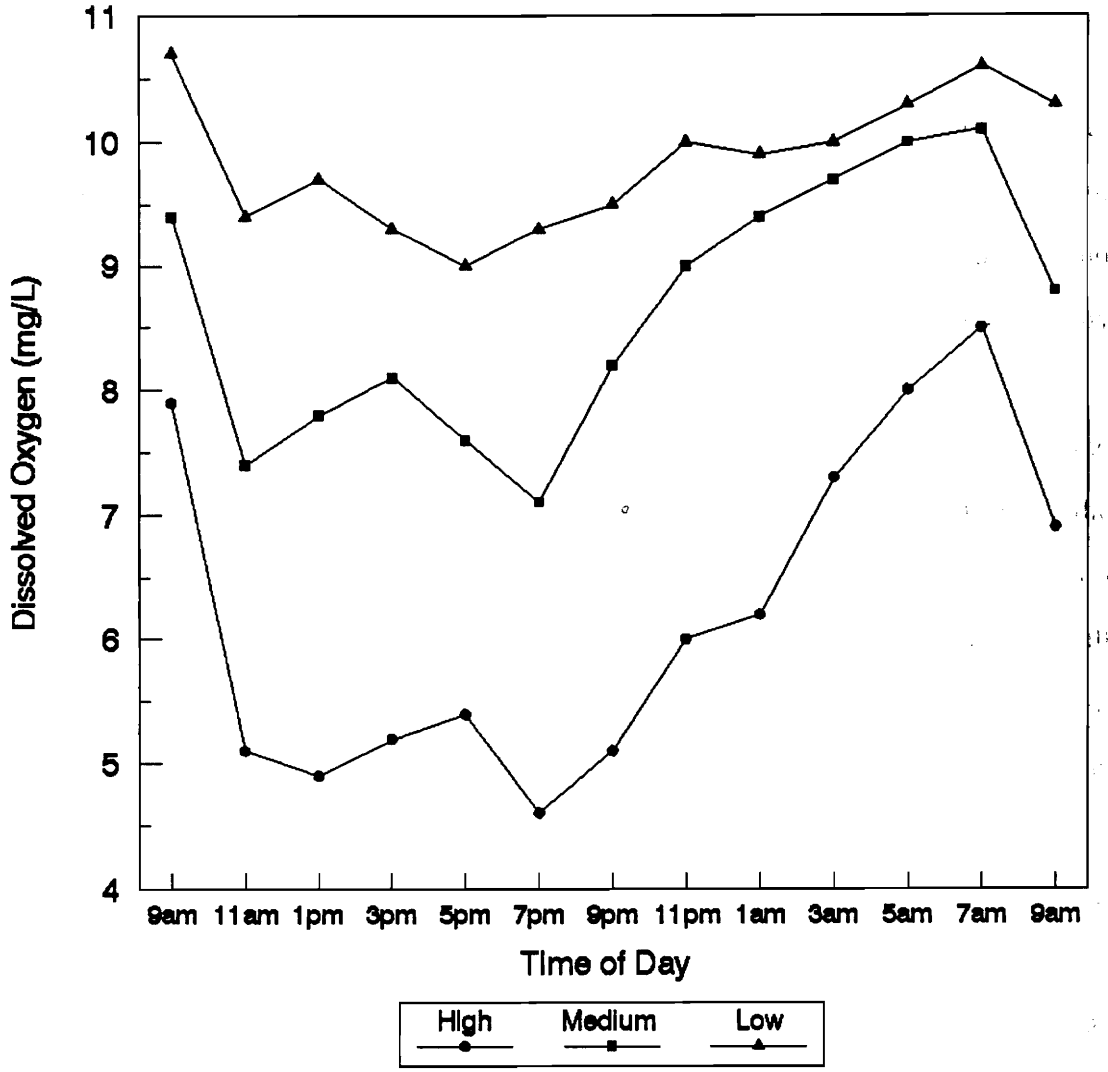


Figure 16. Dissolved oxygen diurnal cycle for one recirculating aquaculture system at each population density.

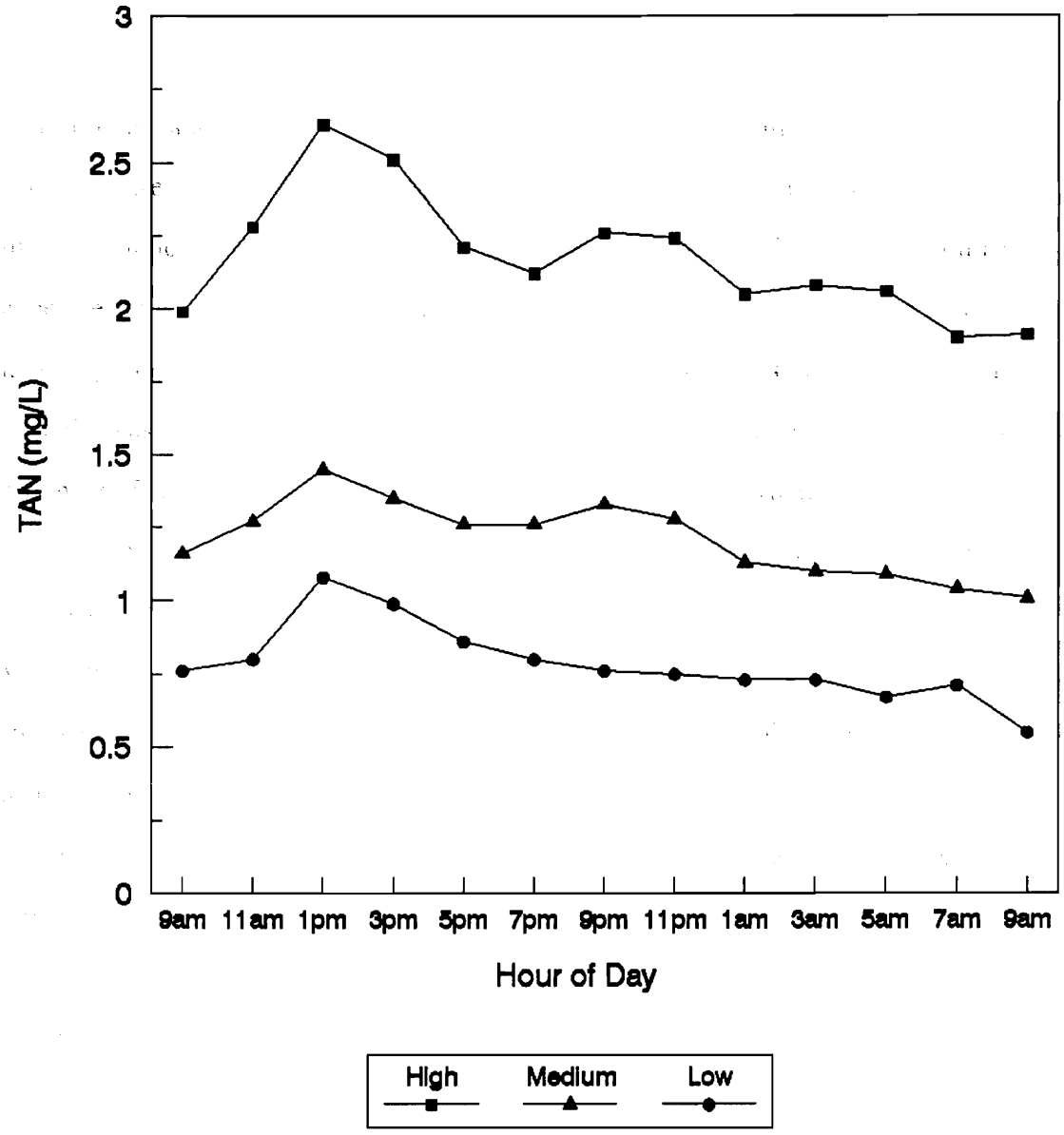


Figure 17. Total ammonia-nitrogen concentration observed during a diurnal cycle for each population density.

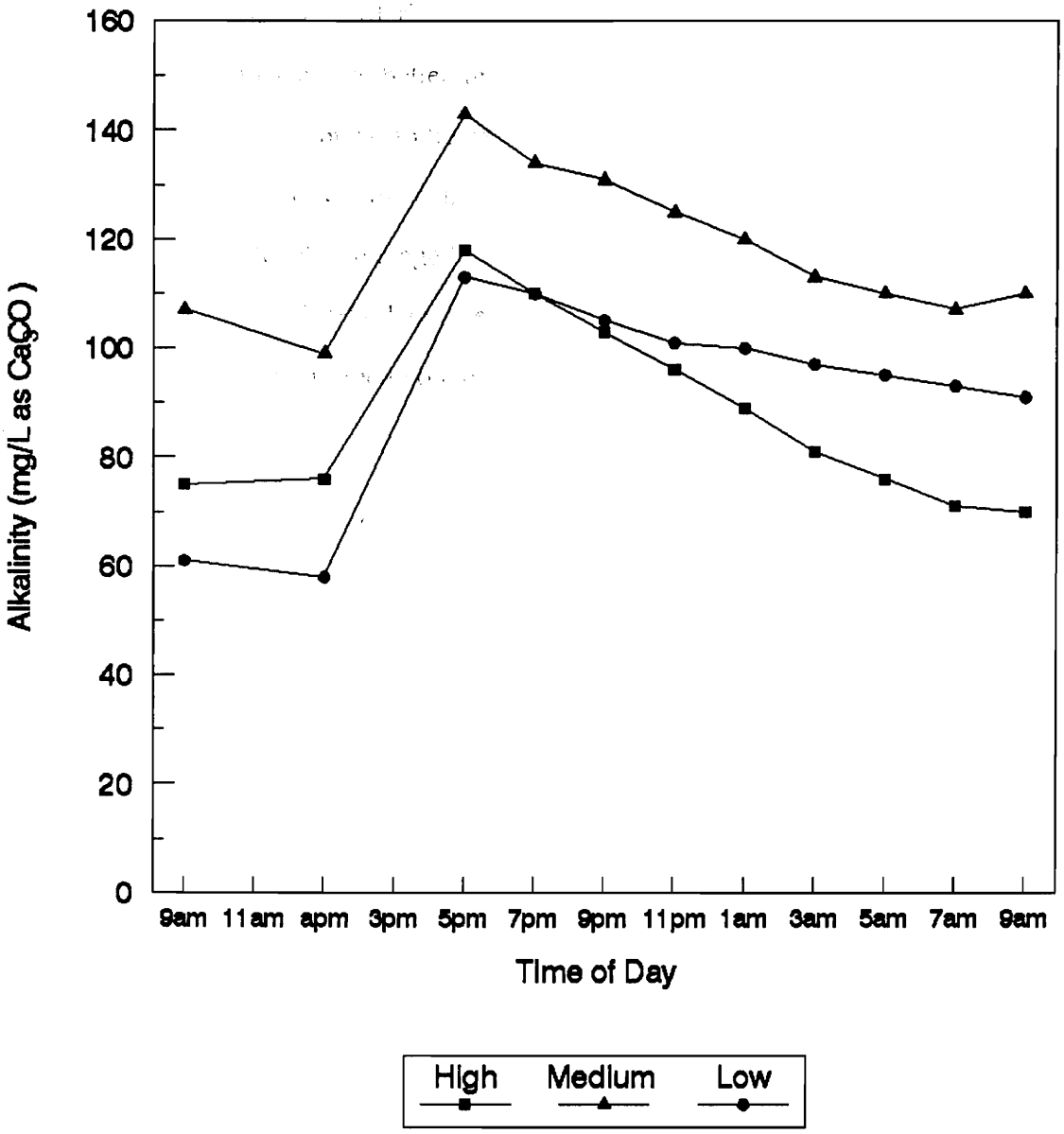


Figure 18. Alkalinity levels observed during a diurnal cycle for examples of each population density.

MICROTOX RESULTS

The results of the Microtox evaluations are summarized in Appendix I. Out of 50 attempted evaluations 15 had to be discarded because the blanks were outside the 2% tolerance range established by the Beckman procedure. Of the 35 evaluations completed within the strictly defined boundaries of the Beckman procedure only 4 produced 20 percent light reduction responses within 15 minutes and 7 within a 30 minute period. Reductions in bioluminescence at the 50% level were never observed. Thirty minute EC20's ranged from 28 to 45% aquaculture water while fifteen minute EC20's ranged slightly higher from 36 to 45%.

No significant fish mortality or dramatic off-feed episodes occurred that could be correlated to these mild responses by the Microtox bioassay. Correlation of Microtox results to poor fish performance was not possible because all eight systems exhibited good to excellent fish growth and very low fish mortality.

DISCUSSION

IONS AND ION BALANCE

Ion levels and balances are a concern because of the potential for osmotic stress to the fish culture which will have to do more work transporting ions across the gill membranes under poor ion balance conditions. To the degree that we can control and balance these ion levels we can minimize stress to the fish and the general biosystem by maintaining optimal levels and minimizing sudden dramatic shifts in water chemistry.

This was particularly true in our case regarding sodium, calcium, chloride and bicarbonate. The managed approach to chemical addition described earlier allowed the sodium and chloride levels to reach a reasonable balance near a targeted 1:1 ratio on a molar basis (Figure 6). Sodium chloride levels by the end of the growth trial were all very similar between systems ranging between 0.06 and 0.07 % (Figures 2 and 4). Calcium levels near the end of the trial ranged from 112 to 167 mg/L constituting the majority of the water hardness.

Other ions are not as easy to control. These include those ions that result from metabolic activity excreted as fish waste and those leaching into the system from the uneaten feed. Magnesium, potassium, nitrate, sulfate and phosphate are considered to be in this category. The levels observed for these ions were not considered harmful in any way to the fish population although phosphate might become an issue when considering effluent disposal.

Nitrate built over time as the RBC system oxidized the ammonia-nitrogen produced from the increasing feed rates. Based on literature data, our systems were always well within the margins of safety for acute or chronic nitrate toxicity. Nitrate, like phosphate, may be important when considering effluent disposal.

Figure 6 indicates that the ion balance did not close. Differences between the total milliequivalents of anions and cations must either be due to sample handling, analysis error or incomplete consideration of all possible charge contributing ions. Some degree of difference is likely because the anion and cation water samples were not taken at the same exact moment or handled in the same way. Anion samples were filtered and measured at whatever pH resulted when dilution's were made. Cation samples were not filtered and were fixed and stored at or below pH 2 . Acidification could oxidize organics or solubilize solids releasing cations. It is also plausible that unconsidered anions in the form of soluble organics contribute to the charge balance scheme. For all of these reasons the error margin observed in Figure 6 is not surprising.

AMMONIA AND NITRITE NITROGEN

The trends observed for total ammonia-nitrogen and nitrite-nitrogen are fully discussed by Nunley (1992). The more pertinent findings will be summarized here in the interest of completeness. TAN concentrations averaged 1.01, 0.70 and 0.50 mg/L for the high, medium and low density tanks. Mean TAN concentrations rose throughout the growth trial with significant differences being observed between high and low density populations. This correlation is not surprising since TAN production is feed related and higher population systems received more total feed. Unionized ammonia (the toxic form) remained below 0.035 mg/L. This is above the very strict 0.0125 mg/L set by Colt and Orwicz (1991). Further, the TAN water samples used to calculate unionized ammonia levels were taken early in the morning when diurnal TAN levels were at their lowest levels as indicated in Figure 17. Peak TAN periods occurred between 11am and 3pm. During this portion of each day fish were actually exposed to slightly higher levels of unionized ammonia than those considered by Nunley (1992). Although no obvious ammonia toxicity response was observed during the growth trial, it appears likely that

systems were operating at borderline TAN levels in terms of chronic exposure to unionized ammonia-nitrogen. A better understanding of the chronic effects on growth and feed conversion would appear to be desirable since it will be impossible to operate at a no effect level because of system economic limitations.

Nitrite-nitrogen levels averaged 1.05, remaining well below toxicity levels identified by Mazik *et al.* (1991). However, once again a more in depth understanding of chronic effects on growth and feed conversion will help in designing and operating these systems at an optimal level.

ORGANICS AND SUSPENDED SOLIDS

Levels of organic indicator parameters increased as the growth trial progressed (Figures 7 through 10). The soluble organic fraction observable as a brownish yellow color and measured by COD and DOC, increased gradually over the entire 228 day growth trial (Figures 8 and 9). Soluble organics appear to exist across the full range of molecular weight with fractions being observed ranging from molecular weights of below 1000 to above 30,000. Organic compounds falling in these ranges would indicate the likely presence of humic and fulvic acids as well as large proteins. $CBOD_5$ and TSS levels quickly reached more or less steady state levels with higher density tanks averaging higher levels of $CBOD_5$ and TSS. Further, all four water quality parameters appear correlated to feeding rates as shown in Figures 11 through 14.

No correlation was observed between fish growth or mortality rates and the organic parameter levels experienced during the growth trial. In addition the lack of response by the Microtox bioassay at the EC50 level would appear to support the contention that acutely toxic conditions were never reached.

The Microtox bioassay is reportedly very sensitive to complex toxicants. Indeed, it was selected for use in this study to investigate its usefulness as a diagnostic tool to help identify toxic events and their causes. That is, having once identified the presence of a toxicity problem Microtox would be used on water samples treated by various methods such as activated carbon. Removal of the toxicity problem as observed by Microtox might help identify the nature of the problem and the appropriate remedy. The lack of significant response by Microtox implies generally good water quality and the lack of significant toxicants.

A methodical management approach appears to be possible and indeed key to improved performance. In this study, feeding rates were incrementally increased based

on a planned schedule. Water exchange and chemical addition were based on feed inputs and the resulting alkalinity consumption considerations already discussed. This methodical approach was credited with an increased ability to maintain higher rates of feeding and improved fish growth in this study as opposed to the earlier growth trial (Nunley 1992). Because of this management approach, systems at the highest population levels did not experience the severe problems expected indicating that water qualities representative of those severe situations never existed.

DIURNAL CYCLES

Diurnal variation was observed for system DO, TAN and alkalinity levels while system temperatures and pH were nearly constant. The DO concentrations varied in each of the different density systems with higher drops in DO in the higher density tanks (Figure 16). These shifts in DO are caused by increased activity by the fish population in response to lighting changes and feeding. Total ammonia-nitrogen levels peaked approximately 4 to 5 hours after the morning feeding (Figure 17). Fish were also exposed to constantly changing alkalinity levels with sudden increases each time a clarifier was cleaned and emptied. Higher density tanks were exposed to more rapid changes in alkalinity than lower density tanks although system pH's remained stable to 0.1 pH units. These diurnal cycles might be contributing to stress for the fish population with higher density populations being exposed to more dramatic cycles and potentially higher levels of stress. Management of these cycles would seem reasonable. This might be accomplished by increasing the number of daily feedings or by feeding on a slow but constant basis during the light hours. This would spread the impacts of feeding over a wider period and tend to flatten the cycles. Hardware changes in the system could also help minimize DO cycling by creating timed or feed back controlled increases in oxygen injection during peak demand periods (Wood 1991).

With this 24 hour analysis, we were also able to confirm and refine our basic assumption used to set the planned water exchange and chemical management approaches. Knowing the initial and final TAN concentrations and the rate of TAN removal at two hour intervals, TAN production was estimated for that 24 hour period. Knowing the feeding rate for that 24 hours an estimated TAN production per kilogram of feed input was calculated. TAN production was calculated by two methods. First, the bihourly TAN removals were calculated and averaged. Using average values over the 24

hours and the known flow rates across the RBC's TAN removed in 24 hours was calculated. Similarly, the bihourly TAN removals can be expressed as TAN removed per unit of time and plotted against time. An area under the curve analysis yields a more accurate estimate of TAN removal for that 24 hour period with the average method providing a check for the values obtained by the area under the curve approach.

The alkalinity destroyed per kilogram of feed was also estimated from this 24 hour data. Alkalinity destruction rates calculated from slopes of curves in Figure 18 were 3.13, 2.38 and 1.44 mg alkalinity as $CaCO_3$ per liter per hour for the high, medium and low density systems. Using these rates an estimate of the alkalinity destroyed per kilogram of feed input into the system was calculated. Table 3 provides a summary of the observed TAN production and alkalinity destruction per unit of feed input into these systems. These observed values averaged 41.6 grams TAN produced per kilogram of feed falling close to the assumed range of 30 to 40 grams of TAN per kilogram of 44% crude protein feed. A more useful expression of this finding is based on the protein input into the system as protein is the primary source of the total ammonia-nitrogen within the feed and different feeds will assay at different levels of protein. For the 44% crude protein feed the average 41.6 value can be used to calculate that 94.5 grams of TAN will result from each kilogram of crude protein consumed. Knowledge of this type is useful in designing and managing recirculating systems.

Table 3. Total ammonia nitrogen production and alkalinity destruction per kilogram of feed input for three different system densities.

Parameter	RAS 2 (High density)	RAS 6 (Medium density)	RAS 9 (Low Density)
Feed rate (Kg/day)	3.99	2.88	1.97
TAN generated (mg/day) average method	165,015	114,122	63,763
TAN generated (mg/day) area under curve method	187,554	117,607	70,406
Grams of TAN produce per Kilogram of feed	47.3	40.8	35.7
Grams of Alkalinity destroyed per kilogram of Feed	264 as $CaCO_3$	278 as $CaCO_3$	264 as $CaCO_3$

CONCLUSIONS

The following conclusions are drawn from the data presented and discussed in this chapter.

1. Total ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen levels increased over time because of increasing system feed rates, but never reached levels toxic to the fish population.
2. Soluble and suspended organic concentrations increased over time, increasing as a function of increasing feed rates.
3. On average, 67% of the suspended solids particles present in the culture tanks were between 1.5 and 30 microns in size.
4. Diurnal cycles existed for dissolved oxygen, total ammonia-nitrogen and alkalinity destruction. These cycles are feeding rate dependent and can and should be minimized to help prevent induced stress to the fish population.

CHAPTER III

MULTI-TUBE CLARIFIER PERFORMANCE AND SYSTEM EFFLUENT CHARACTERIZATION

INTRODUCTION

Suspended solids accumulation in recirculating aquaculture systems can cause damage to fish gill tissues and has been credited with fish mortalities in recirculating systems. Additionally, suspended solids can cause clogging of biofiltration systems and lead to increased system loading in terms of biochemical oxygen demand and system contamination due to the decay of the organic fraction. Rapid removal of these solids from the culture system is dictated in the interest of maintaining optimal water qualities. Multi-tube clarifier sedimentation basins were chosen for the Virginia Tech recirculating systems because they have low maintenance requirements and produce very little head loss. Proper handling of the effluents generated from recirculating aquaculture systems will require knowledge of the effluent characteristics particular to these unique systems.

OBJECTIVE

The objective of this chapter is to characterize the performance of the multi-tube clarifiers in removing suspended solids and to characterize the effluent generated by the multi-tube clarifier systems.

LITERATURE REVIEW

NATURE OF THE SUSPENDED SOLIDS PROBLEM

Limited information exists regarding the nature of wastewater effluents generated in high density recirculating aquaculture systems. Much of this literature is concerned with clogging of biofilters or the efficiency of ultra violet light or ozonation disinfection systems (Luchetti and Gray 1988). Perhaps more importantly, suspended solids in recirculating aquaculture systems can impact fish health directly by causing gill abrasion or indirectly by degrading water qualities (Chen and Malone 1991).

Chen and Malone report typical TSS production levels in finfish aquaculture systems ranging from 30 to 60 % of the total feeding rate with volatile suspended solids (VSS) accounting for the major portion of this TSS (88% on average (Wimberly 1990)). This high organic content tends to lower the density difference between the TSS and water making it difficult to settle. The organic fraction represented by VSS can decay if not quickly removed from the culture system adding to the biochemical oxygen demand and producing additional unwanted contaminants.

It is clear that suspended solids have the potential to negatively impact recirculating aquaculture systems by either directly impacting fish health or by degrading system performance and water quality. Despite this, standards for allowable suspended solid levels in aquaculture systems have not been clearly defined. Chen and Malone (1991) report a recommendation by Muir (1982) of a maximum allowed range of 20 to

40 mg/L of TSS for recirculating systems. Colt and Orwicz (1991) quote work by Wickins (1980) suggesting a limit of 15 mg/L . Chapman *et al.* (1987) reported toxicity to rainbow trout credited to suspended solids particulates in the range of 5 to 10 microns in diameter. Recent work by Chen (1991) demonstrated that particles smaller than 30 microns in size dominate in aquaculture systems. It is indicated that aquaculture systems are dominated by fine particles and that these fine particles are potentially harmful to the fish population. Therefore, the particle size distribution as well as maximum TSS levels must be considered.

TREATMENT METHODS

Wheaton (1987) discussed possible technologies for suspended solids removal in aquaculture. These include an assortment of screening devices, sand filters, diatomaceous earth filters, sedimentation basins, centrifuges and hydroclones. Most of these technologies were originally developed for single pass water and wastewater treatment applications. In recirculating aquaculture systems the application of these traditional technologies can lead to problems. Recirculating aquaculture systems are unique in that the effluent of the water treatment device is the influent water to the fish culture. This continuous recycling combined with even small single pass treatment inefficiencies, can induce a cumulative contamination of the fish culture water. Further, the high soluble organic and VSS levels present in aquaculture systems "frustrates technologies dependent on density difference and contributes to biofouling problems that can quickly clog filters" (Chen and Malone 1991).

Economic considerations demand that aquaculture treatment systems have low energy requirements and low water use. Sedimentation technology has the advantages of being relatively simple, inexpensive and characterized by low head loss. Plain sedimentation is however inefficient in removing particles below 100 microns, (Chen and

Malone 1991), especially when these particles have specific gravity approaching 1.0 because they are high in organic content. These observations can be explained using Stoke's law which will control for this type of particle system where settling is similar to dilute suspensions observed in water and wastewater treatment (Reynolds 1982).

Stoke's Law

$$V_S = (g/18\nu)(S_S-1)d^2$$

where: V_S=settling velocity
g=acceleration due to gravity
ν= kinematic viscosity
S_S=specific gravity of the particles
d=particle diameter

Most dramatic is the squared effect of decreasing particle size. The fine nature (<30 microns) of particles in aquaculture systems ensures that the settling velocity will be characteristically very low. Combine this with the fact that the largely organic suspended solids present in aquaculture systems have specific gravity's near 1.0 and the result is a poorly settling suspension of solids.

Tube settlers or multi-tube clarifier media can help overcome these settling problems and thereby allow higher loading rates. However, treatment will largely be limited to particles larger than 75 microns (Chen and Malone 1991). Multi-tube clarifier sedimentation basins were chosen for the Virginia Tech aquaculture systems because of their simplicity, low head losses and low maintenance requirements..

Suspended solids treatment in future aquaculture systems may require multistage treatment configurations with primary treatment, such as multi-tube clarifiers, followed

by polishing treatment by diatomaceous earth filtration , pressure sand filtration or foam fractionation to remove particles below 30 microns.

MATERIALS AND METHODS

Each of the eight RAS's was plumbed with its own multi-tube clarifier (Figure 1). Figure 19 shows a schematic of the multi-tube clarifier system. Clarifier (sump) basin dimensions were 1.52 meters wide by 1.52 meters long by 1.22 meters tall. The fiberglass clarifier tanks had two valved 7.62 cm diameter inlet pipes. The clarifier bottoms were V-shaped sloping toward a valved effluent drain ensuring evacuation of all solids during a cleaning cycle. Multi-tube clarifier media (BIODECK 19060, .305m thick) was suspended on fiberglass lips attached 0.305 meters above the base of the clarifier. Water contaminated with suspended solids from the fish culture migrated into the bottom of the sump slowing down as it spread and slowly migrated through the multi-tube clarifier media. Clarified water was removed from the sump by two submerged pumps supported on the top of the clarifier media. The clarifier was loaded at a rate of 0.123 cubic meters per square meter of cross section surface area per minute (3 gallons/ft^2/minute).

SAMPLING

Periodically during the growth trial, water samples were taken for systems at all population densities from the clarifier influents and effluents. Samples were taken from inside one of the clarifier influent pipes and, after waiting one sump detention time (8 minutes), from above the clarifier media next to the effluent pumps. These samples were sequentially filtered through 105, 70, 30 and 1.5 micron filters as described in Chapter II. This procedure allowed for an analysis of the single pass solids removal performance of the multi-tube clarifiers on a particle size basis.

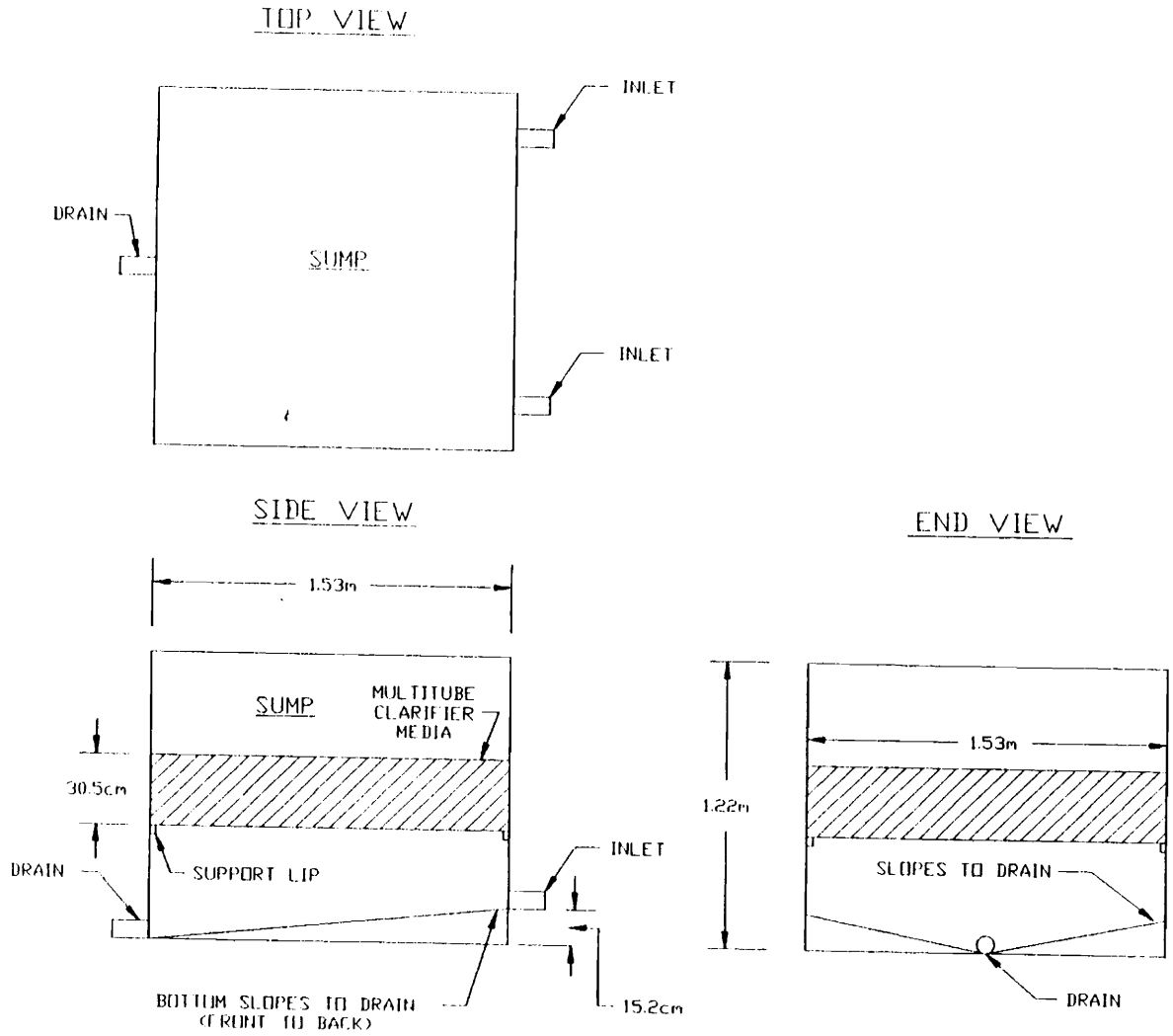


Figure 19. Schematic diagram of the multi-tube clarifier system.

Samples of the system effluent were also collected on a periodic basis. This was accomplished by closing the influent valves and shutting down the two submerged pumps thereby isolating the clarifier. Once a clarifier was isolated, the multi-tube clarifier media was lifted out of the water and suspended on boards above the clarifier basin. Water from the basin was used to wash the clarifier media clean of the attached solids. This was accomplished manually using a 170 liter per minute pump and by bucketing water over the clarifier media. Next, the cleaned clarifier media was placed to the side and the water and solids now in the clarifier basin were thoroughly mixed. Once the basin appeared as homogeneously mixed as possible, a 4 liter plastic bucket was used to collect a sample from the center of the clarifier basin. These samples were considered representative of the system effluent discharged each time a system clarifier was isolated and cleaned out.

System effluent water samples were analyzed for Total, volatile and fixed suspended solids , $CBOD_5$ and unfiltered COD as described in Chapter II. Total Kjeldahl nitrogen (TKN) analysis was done following the Semi-Micro Kjeldahl Method outlined as 4500-N in Standard Methods. Dissolved TAN was analyzed following the HACH procedures already described in Chapter II. Total and dissolved phosphates were obtained by following the HACH kit Total Phosphorus organic and acid hydrolyzable procedure (also called the Acid persulfate digestion method, EPA approved.) followed by analysis using HACH procedure 490 with 3 powder pillows.

RESULTS

The clarifier particle removal performance data is summarized in Appendix J. Figure 20 shows the average suspended solids removal performance observed on a particle size basis. Average removal efficiencies were above 81% for particles 70 microns and larger with a decrease in efficiency to 56 percent for finer particles below 30 microns in size.

System effluent analysis results (Appendix K) are summarized in Table 4. Total suspended solids averaged 371 mg/L with 277mg/L (75%) being volatile. COD and $CBOD_5$ averaged 320 and 125mg/L respectively. TKN levels averaged 25 mg/L, while dissolved TAN levels averaged 0.80 mg/L, indicating that most of the TKN was bound in soluble or solid organics. Nitrate levels as discussed in Chapter II, increased with time to a maximum observed level of 170 mg/L. Average total phosphate was a high 85 mg/L while dissolved phosphates averaged 25 mg/L.

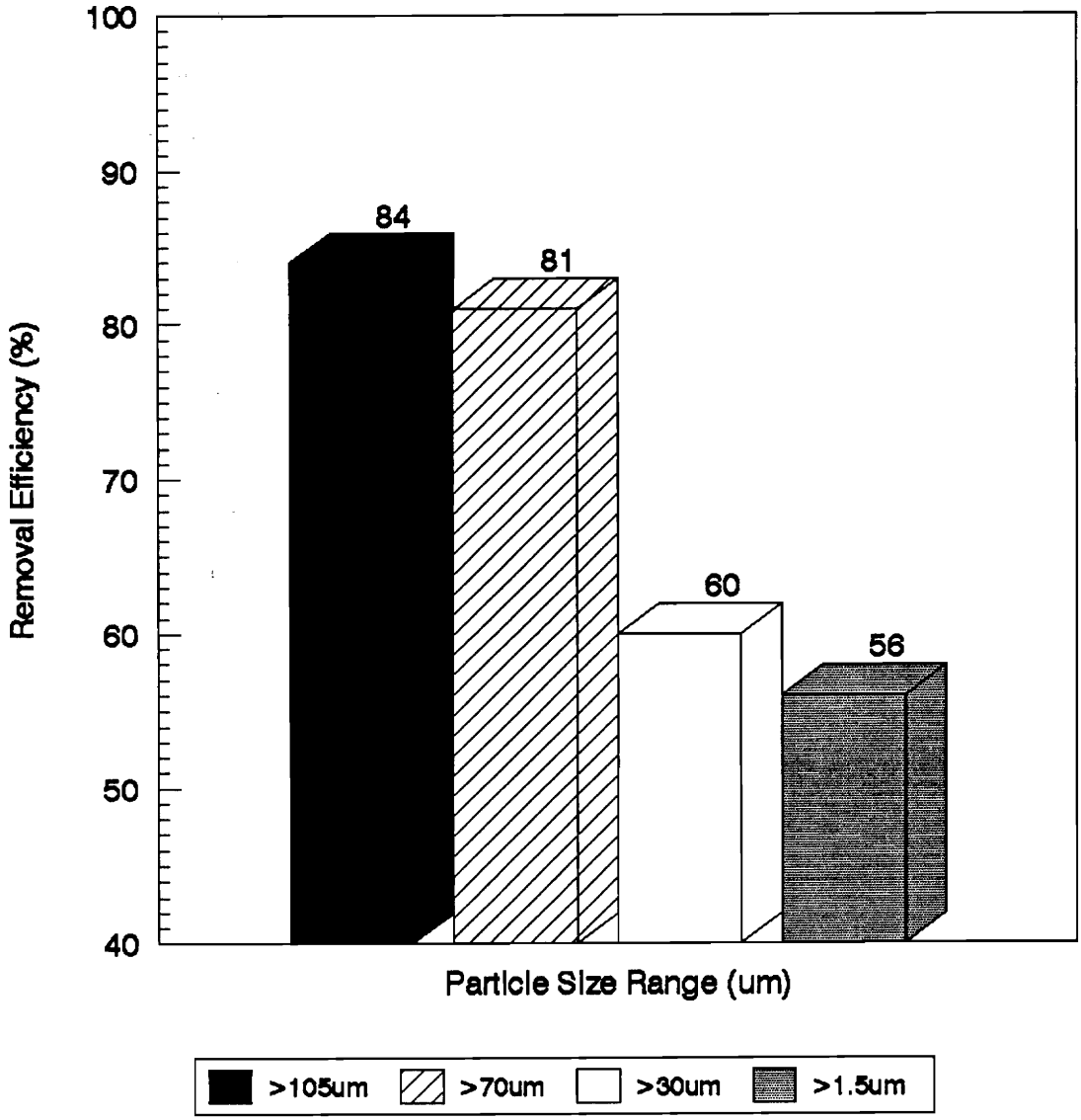


Figure 20. Average Multi-tube clarifier particle removal efficiencies for particles larger than 105, 70, 30 and 1.5 microns.

Table 4. Representative values of various water quality indicators observed for recirculating aquaculture system effluents from well established recirculating culture systems.

Parameter	Average (mg/L)	Standard Deviation (mg/L)	Number of observations	Waste strength rating *
Total Suspended Solids	371	180	24	Strong
Volatile Suspended Solids	277	131	24	Strong
Fixed Suspended Solids	94	56	24	Strong
Chemical oxygen demand (unfiltered samples)	320	102	24	Weak to Medium
Carbonaceous biological oxygen demand ($CBOD_5$)	125	46	29	Weak to Medium
Total Kjeldahl Nitrogen as N	25	11	17	Strong
Total ammonia-nitrogen as N	.80	.52	17	Very weak
Nitrite-nitrogen as N	.52	.32	17	Strong
Nitrate as N	170**		1	Strong plus
Total Phosphate as PO_4	85	15	6	Strong
Soluble Phosphate as PO_4	25	6	6	Strong

* Rating based on criteria from Metcalf and Eddy (1991) for domestic waste.

**Nitrate value is a maximum observation for the entire growth trial.

DISCUSSION

Clarifier Performance

The multi-tube clarifiers performed their tasks well, removing suspended solids at an average overall efficiency of 56%. A high removal efficiency for particles 70 microns and larger was expected and agrees with data presented by Chen and Malone (1991). Good removal of particles between 30 and 1.5 microns in size was surprising based on performance values reported in the literature.

It was noted in Chapter II that 67% of the suspended solids present in the culture tanks was smaller than 30 microns. This earlier observation can now be explained. On average nearly 50 percent of the suspended solids smaller than 30 microns is not removed by the multi-tube clarifier. Further, all of the particles passing through the clarifier are immediately exposed to the high shear environment of the submerged pumps as they are pumped out of the clarifier basin. The effect of this removal difference followed by particle shearing is to increase the portion of smaller particles present in the culture system. The multi-tube clarifiers do the job they were installed to accomplish but leave behind a continuously increasing level of very fine, largely organic, particles below 30 microns in size. Because these fine particles are potentially harmful to the fish population, additional technologies should be considered to remove them from the culture system.

SYSTEM EFFLUENT

Metcalf and Eddy (1991) offer their classification of a typical composition of "weak, medium and strong domestic waste water". The average aquaculture effluent TSS and VSS values place our aquaculture water in the strong category. The average aquaculture effluent COD would be classified as a weak to medium strength waste (Table 4). Direct comparison of $CBOD_5$ values to the BOD_5 values listed in Metcalf and Eddy

indicates that the aquaculture water falls in the weak to medium strength category. With the exception of TAN and nitrite-nitrogen, the aquaculture effluent water is well above levels listed as strong waste water for nitrogenous compounds. The same is true for the observed phosphate levels.

To summarize, the average aquaculture effluent has nutrient characteristics very similar to domestic waste water in terms of those solids and organics parameters measured in this study. Particular attention should be drawn to the nitrogen (TKN and nitrate) and phosphate levels present. Algal blooms will tend to occur in waters with inorganic nitrogen and phosphate levels above 0.3mg/L and 0.01mg/L respectively (Metcalf and Eddy 1991). The combination of organic nutrients, nitrogen species and phosphates indicates that aquacultural effluents will cause eutrophication in receiving waters. The use of this effluent in hydroponics deserves consideration. Lewis *et al.* (1978) successfully used a very similar aquaculture water to grow a cash crop of tomatoes while simultaneously lowering nitrate and phosphate levels in the aquaculture system.

CONCLUSIONS

The following conclusions are drawn from the data and discussion presented in this chapter.

1. The multi-tube clarifiers performed well, removing on average 56 percent of the total suspended solids on a single pass basis.
2. Multi-tube clarifier performance was best for particles over 70 microns in size with an average efficiency observed at 81%.
3. Continuous recycling combined with the relatively poor removal of smaller particles by the multi-tube clarifier creates a situation where the fish culture water is dominated by fine particles (<30 microns).
4. Effluent from aquaculture systems is similar in nutrient strength to domestic waste water but much higher in both nitrogen and phosphate.

CHAPTER IV

ROTATING BIOLOGICAL CONTACTOR PERFORMANCE

INTRODUCTION

Ammonia-nitrogen is the single most critical parameter in aquaculture systems once adequate dissolved oxygen levels are reached (Colt and Armstrong 1981). One of the most effective processes for controlling ammonia-nitrogen in the Recirculating Aquaculture System (RAS) is the Rotating Biological Contactor. The RBC has been shown to outperform other fixed film configurations applied to fish culture systems (Miller and Libey 1984,1985; Rogers and Klemetson 1985; VanGorder and Fritch 1980).

RBC's have inherent advantages for aquaculture (Libey 1992). Among these are:

1. the RBC is self aerating, providing oxygen to the attached biofilm.
2. the RBC is a low head device minimizing pumping energy needs.
3. the RBC is non-clogging due to shearing of loose biofilm caused by the rotation of the media through the water. This leads to self maintenance of an active biofilm.
4. once established, the RBC performance is reliable and resistant to sudden failures.

RBC's were selected for the Virginia Tech recirculating aquaculture systems because of their proven performance and inherent advantages.

OBJECTIVE

The objective of this portion of the overall study was to characterize the nitrification performance of the rotating biological contactors used in support of hybrid striped bass production in the recirculating aquaculture systems.

LITERATURE REVIEW

THE AQUACULTURE AMMONIA-NITROGEN CHALLENGE

Ammonia-nitrogen is the principal excretory byproduct of fish affecting water reuse systems in aquaculture (Luchetti and Gray 1988). Colt and Orwicz (1991) set an allowable design limit for unionized ammonia at 12.5ug/liter NH_3-N. Using this criterion, at normal pH (7-7.5) and temperature (24-25 ^oC) ranges for Hybrid striped bass culture, allowable TAN levels could range between 0.7 and 2.2 mg/liter based on equilibrium relationships for unionized ammonia-nitrogen (NH_3-N) and ionized ammonium (NH_4^+-N) (Emerson et al. 1975). In fact, TAN values in Recirculating Aquaculture Systems are dependent on water exchange rates, degree of water treatment and fish feeding levels. Table 5 provides a summary of representative TAN ranges experienced in Recirculating Culture Systems over the last 16 years. Mean TAN values experienced in these fish culture systems ranged from 0.67 to 2.46 mg/l. It is obvious that recirculating systems operate at low levels of TAN as compared to waste water treatment applications which often range well in excess of 5 mg/liter TAN (Brune and Gunther 1981).

Table 5. Comparison of Mean TAN values experienced in fish culture systems.

Cultured Fish	Fish Density (Kg/m^3)	Mean TAN (mg/liter)	Percent Daily Water Exchange	Literature Source
Hybrid Striped Bass	59.7	1.01	15	Nunley 1992
Tilapia	6.4	1.5	4	Provenzano & Winfield 1987
Channel Catfish	227	0.46	123	Miller & Libey 1985
Hybrid Striped Bass	260	2.2	141	Woods et al. 1985
Channel Catfish	18.1	0.67	unknown	Van Gorder & Fitch 1982
Tilapia	217	1	0.7	Otte & Rosenthal 1979
Channel Catfish	137.9	2.46	unknown	Broussard & Simco 1976

RECIRCULATING AQUACULTURE SOLUTIONS TO AMMONIA

Three potential ammonia removal methods have been investigated for application in water reuse systems for aquaculture. They are; air stripping, ion exchange and biological oxidation.

Volatilization of gaseous ammonia by aggressive aeration techniques can remove ammonia-nitrogen from waste water (Metcalf and Eddy 1991). However, efficient removal of ammonia-nitrogen by air stripping requires pH levels in excess of 10 and large stripping towers to attain the needed air:water ratios (Luchetti and Gray 1988). Miller and Libey (1983) tested a trickling filter and an RBC and concluded them to be inefficient for stripping ammonia under standard fish culture conditions. The physical and chemical manipulations required to attain high removal efficiencies of ammonia by air stripping makes it a poor choice for aquaculture endeavors.

Ion exchange with clinoptilolite has been used to remove ammonia from fish culture systems (Spotte 1979). Inherent problems exist in applying ion exchange technology to aquaculture. Once this resin becomes saturated it must be purged of the adsorbed ammonia. Regeneration of the clinoptilolite requires flushing with brine solutions using as much as 20 to 30 filter bed volumes (Lucchetti and Gray 1988). In addition to the operation and maintenance burdens of ion exchange systems, storage and disposal of regenerant can represent a significant problem.

" One of the most useful techniques in treating ammonia-laden water (particularly at low levels of ammonia) has been the biological fixed film process" (Brune and Gunther 1981). In fixed film biofiltration systems, ammonia-laden water is passed across the surface of a support media. Biological growth occurs on the surface of the media and the attached film of microorganisms oxidizes the dissolved ammonia. Early biofilter configurations in aquaculture included submerged bed and trickling filters (Wheaton

1987). Commonly used support media included gravel, expanded shale, polystyrene beads, plastic rings and oyster shells (Luchetti and Gray 1988). Paller and Lewis (1982) investigated a gravel "reciprocating" biofilter that was automatically filled and emptied on a regular cycle thereby alternately purging the filter of water and allowing atmospheric oxygen to infiltrate the biofilter media. They credited superior performance over ordinary submerged bed filter operation to resistance to filter clogging due to suspended solids and improved aeration within the filter.

More recently RBC systems have been applied in aquaculture. In an early study of a rotating plate biological contactor, Lewis and Buynak (1976) suggested that this type of filter had "features particularly attractive for use in recirculated fish production units and holding facilities" and concluded that further investigation of this type of system was warranted. Rogers and Klemetson (1985) used a synthetic fish culture water to compare four biological filters (RBC, biodrum, trickling filter and submerged filter) finding that the RBC provided the best ammonia removal. Van Gorder and Fritch (1982) compared an RBC, a submerged gravel filter and a submerged plastic media filter supporting channel catfish culture. Running duplicated systems until maximum fish loading capacities were reached, they found the largest standing fish crop, best fish survival and best feed conversion rates were produced in the systems with RBC's. Miller and Libey (1985) compared the performance of an RBC, a fluidized sand bed and a packed tower supporting a single tank populated with channel catfish at three population densities. The RBC provided the highest nitrification efficiency at all densities.

NITRIFICATION

In fish culture systems the primary purpose of the biofilm is the conversion of highly toxic ammonia-nitrogen into nitrate. The primary microorganisms responsible for this are two genera of chemolythotrophic bacteria in the family Nitrobacteraceae. *Nitrosomonas*, which oxidizes ammonia to nitrite and *Nitrobacter*, which oxidizes nitrite to nitrate are the most common and well-studied genera (Gaudy and Gaudy 1988).

In addition to *Nitrosomonas*, other genera are known to oxidize ammonia including *Nitrosospira, Nitrosogloea, Nitrosocystis and Nitrosococcus* (O'Shaughnessy et al. 1982). *Nitrosomonas* and *Nitrobactor* are considered to be of primary importance in waste water fixed film nitrification. These two genera are primarily autotrophic in that the only required source of carbon is inorganic CO_2 while they are capable of using "at least certain organic compounds" if available (Gaudy and Gaudy 1988). They are chemotrophic in that the energy source is a chemical reduction/oxidation (redox) reaction and lithotrophic in that the electron donor in the redox reaction is an inorganic compound (nitrogen).

Table 6 summarizes the energy providing redox reactions and the proposed cell synthesis reactions for *Nitrosomonas* and *Nitrobactor*. The redox reaction represented by equations (1) and (2) provide these bacteria with their energy by way of the electron transport system generating adenosine triphosphate (ATP). *Nitrosomonas* and *Nitrobactor* require large amounts of substrate for their energy needs (Hochheimer and Wheaton 1991) . Equations (3), (4) and (5) from Table 6 show that ammonia, nitrite, oxygen or carbonate alkalinity might well become limiting to the overall conversion of ammonia to nitrate. Equations (4) and (5) are based on "reasonable" values for cell yields in terms of cell mass produced per mass of substrate consumed. Equations (6) and

Table 6. Oxidation and cell synthesis reactions for biological ammonia treatment.

Reaction	Equation	Equation number	Comments
Oxidation by *Nitrosomonas*	$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NO_2^- + H_2CO_3 + H_2O$	1	58-84 kcal energy released /mole of ammonia
Oxidation by *Nitrobacter*	$NO_2^- + .5O_2 \rightarrow NO_3^-$	2	15.4-20.9 kcal energy released/mole of nitrite
Overall oxidation	$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 2H_2CO_3 + H_2O$	3	Oxygen demand= 4.6mg/l O_2 / mg NH_4-N
Synthesis by *Nitrosomonas*	$13NH_4^+ + 23HCO_3^- \rightarrow 8H_7CO_3 + 10NO_2^- + 3C_5H_7NO_2 + 19H_2O$	4	stoichiometry based on cell yield of 0.15 mg/mg NH_4-N
Synthesis by *Nitrobacter*	$NH_4^+ + 10NO_2^- + 4H_2CO_3 \rightarrow 10NO_3^- + C_5H_7NO_2 + 3H_2O$	5	stoichiometry based on cell yield of 0.02mg/mg NO_2^--N
Oxidation and Synthesis by *Nitrosomonas*	$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3$	6	*
Oxidation and Synthesis by *Nitrobacter*	$400NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195O_2 \rightarrow C_5H_7NO_2 + 3H_2O + 400NO_3^-$	7	*

Equations based on Kaiser and Wheaton (1983).

*Note. complete oxidation of 1 mg ammonia to nitrate destroys 7.14 mg alkalinity as $CaCO_3$

(7) are the summation of the oxidation and synthesis reaction equations providing an overall stoichiometric relationship for microbiological nitrification including cell growth and metabolism.

ENVIRONMENTAL EFFECTS ON NITRIFICATION

WATER QUALITY PARAMETERS

DISSOLVED OXYGEN

Haug and McCarty (1972) studying a submerged bed filter, showed that as long as the stoichiometric oxygen requirement was met, that nitrification was independent of Dissolve Oxygen (DO) concentration. However, Kaiser and Wheaton (1983) suggest that increasing DO will benefit the nitrifiers more than the organic consuming heterotrophs present in aquaculture biofilters and by enhancing nitrifier biomass improve nitrification performance. Kaiser and Wheaton offer data based on waste water treatment systems at different DO levels to support this contention. Hoichheimer and Wheaton (1991) suggest an absolute lower DO limit of 2.0 mg/L.

pH EFFECTS

Wheaton (1991) summarizes 9 different studies where optimum pH ranges for *Nitrosomonas* and *Nitrobacter* were investigated. *Nitrosomonas* optimum pH range was reported between 6 and 9, while *Nitrobacter* optimum pH range was 6.3 to 9.4. Wheaton suggests that Nitrification filters can probably operate from as low as a pH 5 to as high as 10. This is in slight conflict with limitations reported by Haug and McCarty (1972). They observed dramatic reduction of nitrification in a fixed film system below pH 6 and cessation of nitrification at pH 5.5.

The fraction of ammonia which is unionized (the toxic species) is pH dependent , with increasing percentages of total ammonia being in the unionized state at higher pH's. Therefore, Lewis and Buynak (1976) suggest that recirculating fish culture systems be maintained at the lower end of the optimum pH for the cultured species but high enough not to dramatically impact the nitrification system.

Rapid pH shifts in excess of 0.5 will greatly reduce the filtering efficiency of the nitrifiers until they have had a chance to adapt (Wheaton 1991).

MINERAL AND CHEMICAL EFFECTS

As noted in Table 6, 7.14 mg alkalinity as $CaCO_3$ is destroyed for each mg of ammonia completely oxidized to nitrate. Gujer and Boller (1986) indicated that 75 mg/l alkalinity as $CaCO_3$ was sufficient to maintain maximum nitrification rates. Addition of high alkalinity water (Miller and Libey 1985) or sodium bicarbonate (Otte and Rosenthal 1979) can maintain pH levels optimum for the cultured species and in support of the alkalinity consuming nitrification system (Nunley 1992).

Biofilters will adapt to salinity's ranging from fresh water to 40 parts per thousand given time to acclimate. However, Wheaton (1991) reporting on an unpublished Doctoral Dissertation by Hochheimer at the University of Maryland suggest that sudden changes of salinity in excess of 5 parts per thousand will significantly decrease nitrification rates.

High calcium levels are reportedly a necessity for *Nitrosomonas* metabolism while high magnesium concentrations are essential for *Nitrobacter.* Sharma and Ahlert (1977) summarize findings from a spectrum of early (pre 1970) literature. They list phosphate, magnesium, molybdenum, iron, calcium, copper (at less than 0.06 mg/l) and sodium as "stimulatory" to nitrification.

Sharma and Ahlert (1977) provide an extensive list summarizing substances inhibitory to nitrification. These include heavy metals such as copper (over .56 mg/l), chromium, nickel and a wide range of organic compounds including vitamins, amino acids, alcohol's and others. Painter (1970) lists zinc, copper, mercury, chromium, nickel, silver and cyanic acid as inhibitory inorganics of concern.

Treatment of fish culture systems with therapeutic levels of formalin, copper sulfate, potassium permanganate and sodium chloride did not effect nitrification (Kaiser

and Wheaton 1983). System treatment with antibiotics such as erythromycen stop nitrification dictating that biofilters not be exposed to such treatment.

The literature on mineral nutrients that might become growth limiting and chemical species that are reported as inhibitory to nitrification is extensive, somewhat contradictory and confusing. Analysis for the presence and effects of all inhibiting species was beyond the scope of this study.

DISSOLVED ORGANICS EFFECTS

Pano and Middlebrooks (1983) studied RBC's in waste water treatment and found that ammonia nitrogen removal was influenced by organic loading with ammonia removal rates decreasing as organic loading increased. Four stage RBC's were used in this study. Inhibition of ammonia removal in the first stages was found to be proportional to the organic loading rate. This concept is consistent with data presented by Grady and Lim (1980) based on work from a Doctoral Dissertation by Huaug at the University of New York at Buffalo in 1973. They observed that nitrification rates were maximized approaching a BOD_5/TKN ratio of approximately 0.25 and that at higher BOD_5/TKN ratios nitrification rates were reduced. The true meaning of this ratio effect to aquaculture is obscured because the specific makeup of the TKN was not reported (i.e. How much was ammonia-nitrogen). In studies of staged RBC's treating carbonaceous BOD loads, Antonie (1976) found that in the later stages ,once BOD fell below 30 mg/l, nitrifiers began to establish themselves. He suggested that at higher BOD's the nitrifying organisms cannot compete with the faster growing carbon-oxidizing organisms, and that they are "diluted out of the process through population dynamics."

MASS LOADING EFFECTS

Brune and Gunther (1981) agreed that most aquaculture systems will operate at TAN levels at or below one mg/l. It is suggested that aquaculture filter units tend to operate at low total biomass levels due to diffusion barriers caused when transporting relatively low ammonia across a bacterial film (Brune and Gunther 1981). This problem results in reduced efficiency of aquaculture filter systems as compared to those observed in conventional waste water treatment. Based on the work by Brune and Gunther (1981), Wheaton (1983) observed that for these lower concentration ranges of ammonia that shorter filter detention times (higher flow rates) produce higher ammonia mass removal rates. Wheaton states,"this experiment supports the contention that the nitrifier growth rate and the nitrification rate are not a function of the concentration of the limiting substrate, but are a function of the mass load of the limiting substrate. " A similar principle was reported by Cook and Kincannon (1971). In their study of a trickling filter configuration, they determined that COD removal efficiency depended on the total COD applied and not its concentration or volumetric flow rate.

PHYSICAL PARAMETERS

TEMPERATURE EFFECTS

Temperature requirements in fish culture will be dictated by the requirements of the cultured species. Nitrifiers have been shown to acclimate over a very wide range of temperatures. However, the ammonia consumption rates are effected. Haug and McCarty (1972) report the following relationship between the ammonia removal rate and concentration S (mg/liter) at Temperature T in ^{o}C for a submerged bed filter.

$$\text{Ammonia consumption Rate(mg/l/min.)} = (0.11T - 0.2)(S/10)$$

Wheaton (1991) reports unpublished masters thesis work by Wortman(1990) at the University of Maryland where temperature effects on a biodrum's nitrification performance were investigated. In this study, nitrification occurred over a temperature range of 7 to 35^{o}C. The following linear relationship is reported between Ammonia removal rate and temperature.

$$AMR = 140 + 8.5T$$

where:AMR=ammonia removal rate (mg/liter of filter media/day)

T=temperature in Celsius

Hochheimer and Wheaton (1991) summarize reported optimum temperature ranges for *Nitrosomonas* between 30 and 36 ^{o}C and *Nitrobacter* between 28 and 42 ^{o}C. They also suggest that these bacteria can acclimate to diverse temperature ranges.

Temperature effects on the kinetics of biological systems are well established. This will be discussed in further depth in the following pages. Generally speaking, decreasing temperatures reduces nitrification rates.

LIGHT EFFECTS

Olson (1981) reported inhibition to nitrifying bacteria by light less than one percent of sunlight intensity. Light is believed to oxidize cytochrome C, which is part of the electron transport system needed for generating ATP. *Nitrobacter* is reported to be more sensitive to light because it contains less cytochrome C than *Nitrosomonas* (Olson 1981).

RBC STAGING EFFECTS

Grady and Lim (1980) suggest that the performance of staged Rotating-disc reactors and RBC's can be modeled as a series of Continuously Stirred Tank Reactor's (CSTR's) containing biofilms. Therefore, the benefits of sequencing CSTR's in series apply to staged RBC systems. Grady and Lim show that placing infinite numbers of CSTR's in series is equivalent to creating a plug flow reactor (PFR) of equal volume. The larger the number of CSTR's in series (stages) the closer we get to PFR performance characteristics. Grady and Lim state that a larger number of small CSTR's in series will outperform a lesser number of larger CSTR's in series , of the same total volume. "In practice, the greatest improvement in performance occurs in going from a one CSTR to a two CSTR system, and the improvement becomes less significant as more and more CSTR's are added". This is because the first stages of the series see the highest substrate concentrations resulting in the highest removal rates and biggest reductions in substrate. It has also been noted that staging of RBC's can help separate the different groups of microorganisms (heterotrophic vs autotrophic) helping to establish desired treatment effects (Antonie 1976).

RBC ROTATIONAL SPEED AND DISC SUBMERGENCE EFFECTS

RBC rotational speed impacts the level of contact between the biofilm and the substrate and the degree of shearing force to which the biofilm is exposed to. In studies presented by Antonie (1976), BOD and ammonia-nitrogen removal were enhanced by increasing rotational speeds up to a point where the peripheral velocity of the RBC disc relative to the water was .305 m/second (1 ft/sec.). Increasing speeds above this level did not improve treatment.

Disc submergence levels for RBC's are generally near 50 percent (Grady and Lim 1980). Grady and Lim present a graphical relationship between treatment efficiency and submergence indicating that a range between 35 and 50 percent submergence is optimal.

FILTER STARTUP

Nitrifiers have relatively long cell generation times. Haug and McCarty (1972) reporting on earlier literature suggest 10 to 30 hours. Development of initial active biofilms in aquaculture nitrification systems has required 26 to 59 days of filter conditioning time. Van Gorder and Fitch (1982) slowly increased stocking levels of channel catfish in order to provide incremental ammonia-nitrogen loading during filter activation. They found that 59 days were needed before filter acclimation was complete. Filter acclimation was defined by a decline in high nitrite levels that were causing fish mortalities. Collins *et al.* (1975) experienced the same nitrite spikes in the culture of channel catfish while establishing a new nitrification system. They attributed the loss of all the catfish in the system to nitrite levels in excess of 2.5 mg/l.

During filter startup, it is normal to see high levels of ammonia until the *Nitrosomonas* population gets established. This is the first critical phase of system startup where toxic ammonia levels can be fatal to the cultured species. Once *Nitrosomonas* is

well established the nitrite levels will peak, awaiting the establishment of *Nitrobacter*. During this second critical phase nitrite toxicity is a concern until the entire nitrifier population has become well established (Wheaton 1987). Manthe and Malone (1987) studied methods for accelerating biofilter acclimation in closed blue crab shedding systems. A summary of their findings follows:

1- Commercial additives of nitrifying bacteria did not accelerate filter acclimation.

2- No significant effect of media size was observed on acclimation time.

3- Ammonia addition as ammonia chloride , instead of a crab population, induced filter acclimation but did not reduce the acclimation period.

4- Simultaneous addition of both ammonia chloride and sodium nitrite reduced filter startup to 26 days vice the 35 days required for filter acclimation induced by incrementally populating the system with crabs.

THE BIOFILM

Biofilms are communities of microorganisms that generally produce a gelatinous matrix (slime). Nutrients and oxygen pass into the film matrix by diffusion. A generally accepted concept of the development of a mature biofilm is summarized below based on work presented by Hoehn and Ray (1973).

-In the presence of adequate nutrients and favorable environmental conditions biofilm organisms begin to colonize solid surfaces.

-Biofilm growth continues covering the entire solid surface and then begins to grow outward covering the initial layers of biofilm.

-Nutrients and oxygen diffuse into the growing film until the film thickness will not allow diffusion to the basal layers of the film.

-Lack of oxygen supply to the basal layers of the biofilm cause anoxic or anaerobic conditions leading to death of the aerobic biofilms previously populating the basal layer.

-Death and subsequent cell lysing in these now anaerobic layers causes detachment of portions of the biofilm from the solid surface.

-The newly exposed surface is now available for repopulation by the aerobic microorganisms.

Biofilms exposed to high organic levels are reported to range from .07 to 4.0mm (Grady 1982). However, Grady suggests that those films subjected to mechanical or hydrodynamic shear are generally less than 0.2 mm thick.

CONCEPTUAL BIOFILM MODEL

A biological film model was proposed and verified by Williamson and McCarty (1976a and 1976b). Figure 21 is a diagram taken from this model that provides valuable insight into the limiting mechanisms of fixed film water treatment. Williamson and McCarty's biofilm model considers uptake of a substrate from waste water to be controlled by a combination of the physical diffusion of a rate limiting substrate into the biofilm and the biological utilization kinetics of that substrate by the biofilm. Diffusion from the waste water into the biofilm is modeled using Fick's law.

$$J_o=-D_W A_c(ds/dz)=-D_W A_c(S_o-S_s)/(L_1+L_2)$$

where: J_o=substrate surface flux (mg/day)
D_W=diffusion coefficient of limiting substrate in water (cm^2/day)
A_c=biofilm area (cm^2)
S_o=substrate concentration in bulk liquid (mg/L)
S_s=substrate concentration at the biofilm surface (mg/L)
L_1+L_2=stagnant liquid layer depth

Biological uptake of the substrate within the biofilm is modeled using Monod kinetics that will be reviewed later in this chapter.

Follow up work by Rittman and McCarty (1980a,1980b and 1981) expanded the original model to include considerations of biofilm growth and decay rates.

The substrate profile proposed in figure 21 shows a bulk liquid concentration (S_o) some distance away from the biofilm surface. Incomplete mixing near the biofilm and diffusion limitations from the liquid film surface into the biofilm create a "stagnant liquid layer", causing a reduction in the effective substrate concentration exposed to the biofilm surface. This stagnant liquid layer is represented by the distance (L_1+L_2) in figure 21. The stagnant layer is broken down into a layer effected primarily by mixing turbulence (L_1) and (L_2) which is believed to be a function of the physical characteristics of the biofilm surface. With adequate mixing (L_1) can be reduced to zero while (L_2) is

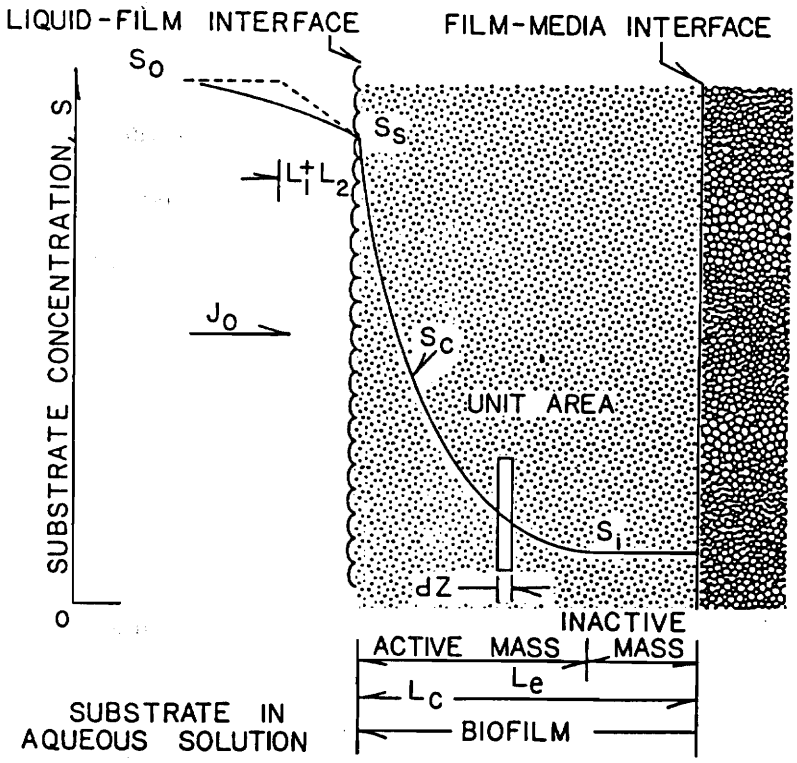


Figure 21. Potential substrate profiles within a biofilm. From Williamson and McCarty (1976).

considered to be a constant (Grady 1982). Diffusion limitations and biofilm metabolism of the substrate causes a decrease in the substrate concentration as we penetrate deeper into the biofilm. The depth at which the substrate concentration profile levels off is the effective biofilm depth (L_e) where the rate of substrate diffusion and substrate biofilm metabolism have reduced the substrate concentration to the limiting value (S_i). The concept of an active biofilm limiting depth is supported by several studies. Hoehn and Ray (1973) demonstrated that substrate consumption's increased with increasing biofilm depth up to 70-100um, beyond which, consumption rates were independent of film thickness. Bungay (1969) found that measured respiration ceased at depths of 50-150um. "It is now generally accepted that the active thickness is a result of transport limitations within the biofilm" (Grady 1982).

This model allows that any required nutrient would have its own biofilm concentration profile and might become the limiting nutrient. For example in nitrification the electron donor (ammonia or nitrite) or the electron acceptor (oxygen) might become rate limiting according to this model. Williamson and McCarty (1976a) point out that for nitrification, considering the stoichiometric oxygen needed for complete oxidation of ammonia to nitrate and the differences between the diffusion coefficients for ammonia and oxygen that 2.7 times as much oxygen would be needed as ammonia to avoid an oxygen limiting condition.

This biofilm model approach presented in Figure 21 is very useful for understanding the conceptual mechanisms involved. However, this type of modeling has application limitations and requires measured or assumed values for biofilm thickness, biofilm density and the use of empirically determined or literature derived coefficients.

NITRIFICATION REACTION KINETICS

In reviewing the nitrification literature, a diversity of opinion was observed concerning nitrification reaction rate kinetics. However, three major reaction rate equations repeatedly surfaced. The equations were the Monod model, zero order and first order reaction equations.

Grady and Lim (1980) state that the type of application of Monod kinetics applied to heterotrophic bacteria can be applied to nitrifying bacteria. The Williamson and McCarty film model discussed earlier uses Monod kinetic expressions to model the biological uptake side of their overall fixed film model. Pano and Middlebrooks (1983) concluded that Monod growth kinetics can be used to describe ammonia-nitrogen removal in a study using RBC's.

Huang and Hopson (1974) stated that nitrification followed zero order kinetics for ammonia-nitrogen levels between 2.5 and 673 mg/l. Shieh (1977) concludes that the "observed rate in a zero order RBC is first order if external mass transfer is rate limiting".

These apparently contradictory observations can be reconciled. The Monod equation has its basis in cell production and biological metabolism kinetics. It is an empirical fit to data modeling these enzyme driven reactions.

Monod Equation

$$u=u_m S/(K_s + S)$$

where: u=specific cell growth rate
K_s=substrate concentration where $u=0.5u_m$
u_m=maximum growth rate
S=substrate concentration for growth the growth limiting nutrient

Considering the Monod equation, should the substrate concentration (S) be much larger than the constant (K_s), then the reaction rate (u) becomes a constant very nearly

equal to the maximum reaction rate (u_m). This is a pseudo-zero order reaction resulting in a constant substrate removal rate for higher substrate concentrations. If the substrate concentrations are very small compared to the constants (K_m or K_s) then the equations effectively become first order. For the Monod equation this condition yields the effective rate expression ($u=(u_m/K_s)S$).

Therefore, at low concentrations of the limiting nutrient the monod expression reduces to a first-order expression and at high concentrations it becomes a zero-order reaction (O'Shaughnessy et al. 1982). O'Shaughnessy and his colleges concluded that nitrification in fixed film systems is best described by first order kinetics for ammonia-nitrogen concentrations less than 4 mg/l TAN and zero-order kinetics for higher ammonia-nitrogen concentrations. As was shown earlier (Table 5), aquaculture systems have historically operated at TAN ranges between 0.67 and 2.46 mg/l. Therefore, pseudo-first order reaction kinetics will apply.

TEMPERATURE EFFECTS ON MONOD CONSTANTS

K_s an u_m are temperature dependent. Knowles *et al.* (1965) indicated the following regression relationship for $u_m$and K_s for *Nitrosomonas* at varied temperature (T) in ^oC.

$$\log_{10}(u_m)=0.0413T-0.944 \quad ;u_m=days^{-1}$$

$$\log_{10}(K_s)=0.051T-1.158 \quad ;K_s=mg\ NH_4^+\text{-}N/liter$$

Lawrence and McCarty (1970) presented an empirical equation re expressing the Monod equation in the following form.

$$(ds/dt)/X = U= (kS)/(K_s+S)$$

The values of u and u_m describe the microorganism specific and maximum growth rates when applied to the original form of the Monod equation. Lawrence and McCarty (1970) describe U, the specific substrate utilization rate, expressed in units of substrate uptake per unit biomass. In this equation u_m is replaced by k.

Novak (1974) showed that k and K_s were similarly effected by temperature. He reported that θ for aerobic systems ranged from 1.0 for low substrate concentrations to 1.18 for higher substrate concentrations, depending on the substrate being metabolized. These θ values can be applied to the following simplifications of the Arrhenius equation.

$$k_1/k_2=\theta^{(T_2-T_1)} \quad \text{or} \quad K_{s1}/K_{s2}=\theta^{(T_2-T_1)}$$

where: θ=temperature coefficient =(1.0 to 1.18)
T_i=temperature ^oC for constants k or K_s evaluated at the ith temperature.

The value θ is usually taken at 1.035 (Metcalf and Eddy 1991) for trickling filter systems.

A summary of Monod constants found in the literature is offered in Table 7

Table 7. Summary of representative Monod constants for the overall oxidation of ammonia to nitrate by *Nitrosomonas* and *Nitrobacter.*

Temperature (^oC)	Source	K_s (mg NH_4-N/ liter)	u_m ($days^{-1}$)
20	Williamson and McCarty 1976	0.5	2.0
23.2	Knowles *et al.* 1965	1.3	1.3
22.2	Knowles *et al.* 1965	1.0	1.02
18.8	Knowles *et. al* 1965	0.6	0.7
20	*Grady and Lim 1980	1.72	0.48
20	Metcalf and Eddy 1991	0.6	0.7

* Note. Average of six values reported by Grady and Lim for other researches.

RBC THEORETICAL MODELS

A more complicated fixed film model developed for rotating disc reactors is presented by Grady and Lim (1980). This model approach makes the following assumptions.

1. Steady state conditions exist between biofilm growth and sloughing due to shear.
2. Stage turbulence is sufficient to suspend the detaching organisms.
3. Both attached and detached organisms contribute to substrate removal.
4. Oxygen and other nutrients are not rate limiting.
5. Biofilm thickness is uniform throughout the entire surface area.

In this model, contactor disc are separated into "submerged and aerated" sectors requiring considerations of disc submergence and rotational velocity. The model incorporates mass transfer for both sectors, and a Monod style kinetic expression very much like the Williamson and McCarty film model. This model is exceedingly complex and impractical in application.

Grady and Lim (1980) also offer a simplified analytical equation for RBC's. Here the only assumptions are:

1. The attached biomass is much larger than suspended biomass, and that the contribution of the suspended biomass is negligible.
2. If pilot plant data is available for configurations similar to the full scale system then the rate can be considered to be a function of effluent substrate concentration only.

This means, the RBC can be treated as a CSTR with biofilm. Writing a mass balance around the RBC (CSTR) yields the following relationship assuming steady state.

$$QS_o - QS + r_s A_s = 0$$

where: Q=volumetric flow rate
S_o=influent substrate concentration
S= effluent substrate concentration
r_s=rate of substrate removal per unit of media surface area
A_s=media surface area

The rate (r_s) can be expressed using a form of the Monod equation. Substitution into the mass balance for r_s yields the following analytical expression.

$$Q/A_s = q_m S/[(K_s+S)(S_o-S)]$$

where: q_m=maximum utilization constant by the fixed film biomass.
K_s= half saturation Monod constant.

This equation can be simplified even further if the assumption of pseudo-first order kinetics is applied. The Monod expression collapses to a first order form where rate is equal to the product of a constant and the substrate concentration yielding the following expression when combined with the RBC (CSTR) mass balance equation.

$$Q/A_s = K_1 S/(S_o - S) \qquad \text{[equation 8]}$$

where: K_1=a constant combining q_m/K_s

We can take advantage of the concept of RBC stages acting as CSTR's in series. If each RBC stage held the same wetted surface area the simplified equation [8] can be used to generate the following expression adapted from Grady and Lim (1980) for n RBC stages.

$$Q/A_s = K_1(S_i)/(S_{i-1} - S_i) \qquad i=1,2,...,n \text{ stages}$$

This equation has the advantage of simplicity, requiring only a single constant K_1 determined by pilot study, and it can be iteratively solved for the required area (A_S) for an n stage RBC to reduce the influent substrate (S_o) to a desired effluent level (S_i).

EMPIRICAL FIXED FILM MODELS

TRICKLING FILTERS

Grady and Lim (1980) propose that the "first performance characteristic" that should be incorporated into a trickling filter model is the exponential decrease of substrate concentration as it flows down through a filter using the following equation.

$$S/S_0=e^{(-KD)}$$

where: S=influent concentration
S_0=effluent concentration
D=depth into the filter
K=empirical constant specific to a filter configuration

It is noteworthy that this equation would be identical to a first order decay reaction for a plug flow reactor if depth (D) were replaced by the "space time" through the PFR. Flow through a trickling filter can be though of as a wave front moving through the filter much like plug flow systems. This equation does not consider other factors effecting removal rates in trickling filters such as flow rate and substrate concentration.

Balakrishnan and Eckenfelder (1970) used the following " trickling filter equation" to study nitrification in a trickling filter applied to a modified activated sludge process. A stepwise procedure for analyzing the constants K and n is offered by Eckenfelder (1966) and by Veissman and Hammer (1985).

$$S/S_0=e^{(-KD/Q^n)}$$

where: S=effluent ammonia concentration
S_0=influent ammonia concentration
D=depth into the trickling filter
Q=volumetric flow rate
K and n=empirical constants

This equation is also offered as the design equation for trickling filters by Metcalf and Eddy (1991). It does attempt to consider volumetric loading characteristics but does not directly consider substrate concentration.

EMPIRICAL RBC MODELING

The complexity of empirically fitted RBC models found in research literature is demonstrated in the following equation presented by Wu et al.(1980) studying BOD removal.

$$F=(K/N)L^{a}T^{b}S^{c}B^{d}A^{e}D^{f}Q^{g}$$

where: F=fraction of influent loading remaining
K=treatability constant for the waste
N=Stage number
L=influent concentration of the waste
S=reactor residence time
T=waste water temperature
B=rotational speed
A=total effective disk surface area
D=submerged disk depth
Q=volumetric flow rate
a,b,c,d,e,f,g=partial regression constants

MODELING OPTION CHOICE

Although we see in the literature that Monod kinetics can be applied to fixed film nitrification, the traditional use of this approach will require measured or assumed knowledge of biofilm thickness and biofilm density to attain biomass numbers required in the analysis of constants K_s and u_m. These theoretical models also consider mass transfer by diffusion in submerged and aerated sectors of RBC systems and quickly become unmanageably complex and impractical in application. Clearly we must retain a degree of simplicity in our approach. Reaction coefficients must be simple to analyze, and the resulting equations simple to apply.

We have also seen that for the lower concentration levels of ammonia observed in aquaculture systems, we can expect first order kinetics to dictate based on past research.

The theoretically based equation [8] assumes first order kinetics and that the RBC stages are acting as CSTR's in series. By approaching the biomass in terms of surface area of media and assuming first order kinetics we have a relatively simple equation with a single constant to evaluate.

The equation used by Balakrishnan and Eckenfelder (1970) and supported by Veismann and Hammer (1985) and Metcalf and Eddy (1991) have a form involving the exponential decay that might be expected for a first order reaction. This trickling filter equation is also simple in form resulting in constants that are relatively easy to analyze. It also attempts to consider loading effects by including a volumetric loading variable (Q). However, based on the work by Brune and Gunther (1981) and latter interpretations by Kaiser and Wheaton (1983) it appears reasonable that mass loading not volumetric loading is the key to interpreting fixed film reactors in the unique environment of aquaculture.

It will be the intention of this study to pursue both the theoretical approach adapted from Grady and Lim (1980) as equation [8] and the empirical equation presented by Balakrishnan and Eckenfelder (1970) to attempt to model our observed RBC performance.

In the empirical equation mass loading (W) will be used rather than volumetric loading (Q) and the distance through the trickling filter (D) will be replace with the number of stages in the RBC. This reduces the smooth exponential decay concept expressed for trickling filters in terms of depth to a stepwise approximation of the same decay in substrate concentration as we move from the first to the last stages of the RBC. This rearrangement of the trickling filter equation results in the following proposed empirical RBC equation.

$$S/S_o = e^{(-K(\text{Stage Number})/W^n)}$$

where: S=effluent ammonia concentration
S_o=influent ammonia concentration
W=mass loading of substrate (mass/area of biofilter)
K and n=empirical constants

MATERIALS AND METHODS

EXPERIMENTAL METHOD

Eight well seasoned RBC's were used for nitrification treatment in near commercial scale recirculating aquaculture systems supporting hybrid striped bass. RBC influent and stage TAN, $CBOD_5$ and DOC concentrations were determined for systems supporting three different population levels of hybrid striped bass during a 228 day growth trial. At the end of the trial, one RAS was reconfigured splitting system flow between two RBC systems. This allowed further RBC analysis at six different flow rates ranging from 3.8 to 289 liters per minute.

RBC SYSTEM DESCRIPTION

Each of the eight near commercial scale recirculating aquaculture systems were equipped with a three stage RBC (Figure 1). Figure 24 provides a schematic of a single RBC. Each of the three stages of the RBC were constructed from triangular pieces of corrugated PVC media cut from 1' x 1'x 6' rectangular logs of media obtained from Munters Corporation. (BIOdek 12060, Munters Corp., Fort Meyers, Florida). These triangular pieces were laid out in circular molds and laminated on both sides with flat stock PVC by means of chemical welds using PVC glue. The media was rated at $223 m^2/m^3$ ($68 ft^2/ft^3$). Each stage was very nearly 1.83 meters (6 ft) in diameter and 0.31 meters (1 ft) thick. This resulted in a total media surface area for each three stage RBC of 536 m^2. The RBC disks were mounted on aluminum shafts and rotated at 3 RPM by means of a .186 kW Dayton electric gear motor through a sprocket and chain drive assembly. Although these RBC disks were "homemade" they appeared to retain excellent water infiltration characteristics. RBC flow rates during the growth trial were maintained

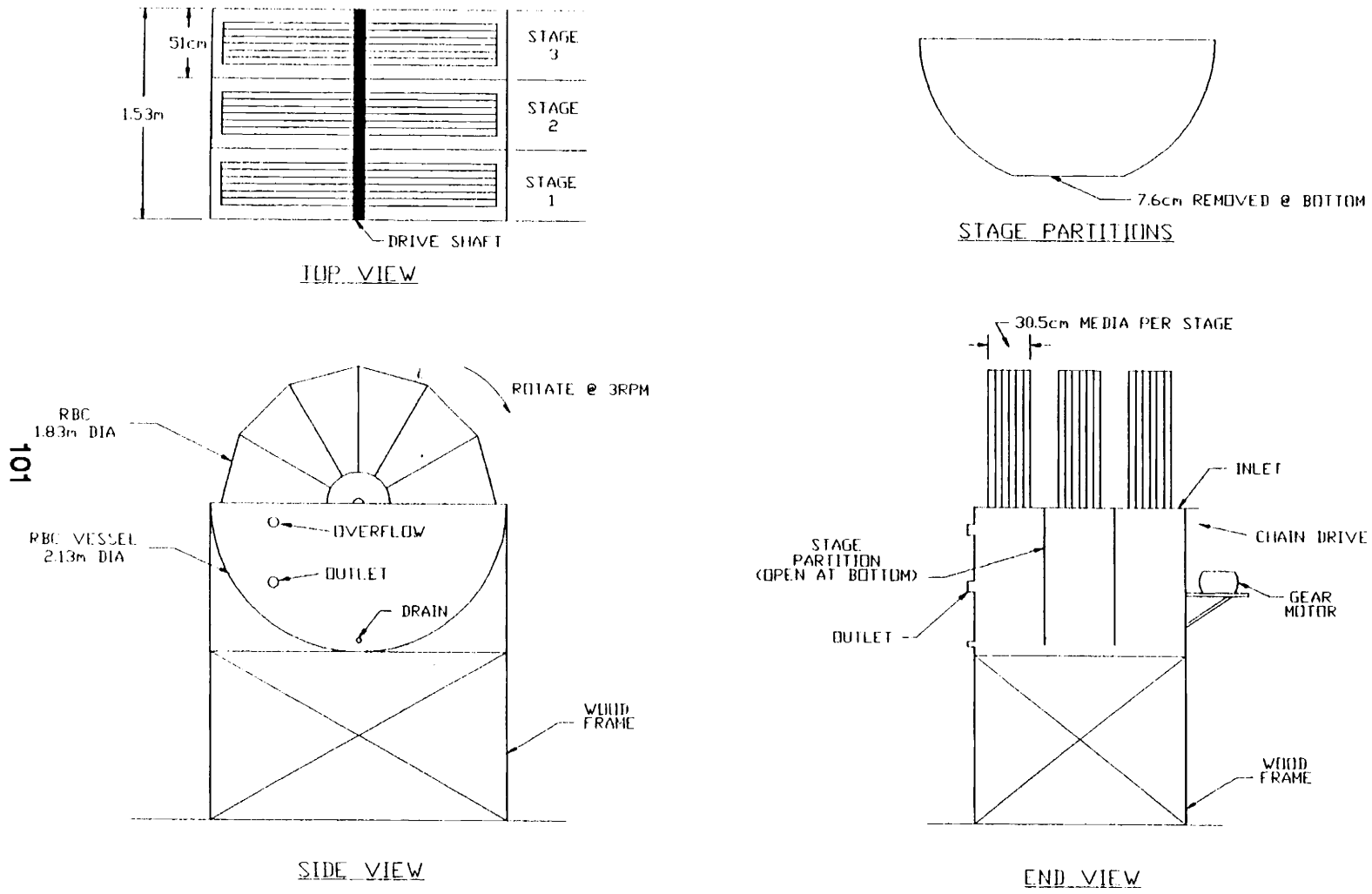


Figure 22. Schematic diagram of the Rotating Biological Contactor system.

at near 285 liters/min. by dual submerged pumps. Flow rates varied slightly from one system to the next. These differences were accounted for by individually calibrating each system as it was plumbed and operated. Flow calibration was done by timing the volumetric displacement of water being pumped out of the solids clarifier by the two submerged pumps.

The RBC disks were submerged approximately 35 to 40%, very nearly filling each of the three separate stages of the RBC tank. The total capacity of the three stage RBC tanks were 1,930 liters when filled to near the emergency overflow which vented back into the solids clarifier tank. Water levels in the RBC tanks were maintained in as much as possible at or near the overflow point by controlling flow with a PVC valve in the effluent line of the RBC. The RBC tanks were baffled into three sections of equal size resulting in stage detention times of approximately 2.3 minutes during the growth trial.

The RBC's were well seasoned for nitrification, having been used in a previous study, and required only a short startup acclimation period before the official beginning of the study on January 15, 1991. RAS dissolved oxygen levels were maintained using the U-tube oxygenation system. RBC DO levels were always over 5 ppm during the evaluation insuring an aerobic filter environment.

TRACER STUDY

On May 9,1991 a lithium carbonate tracer study was completed on RAS 9 in order to evaluate the flow characteristics of the RBC system. Lithium was chosen because it was deemed safe for the fish population and it was absent in the water allowing easy analysis by atomic absorption techniques. The first stage of the RBC was spiked with an impulse of lithium carbonate premixed into a 20 liter bucket. At initiation time (t_o) the upstream side of the first stage was impulse spiked at the inlet of the RBC from the solids

clarifier. Water samples were taken at t_0 and at one minute intervals from the downstream side of each RBC disk at a depth of 0.3 meters.

SAMPLING

Sampling was initiated at the end of May 1991. This allowed the systems to become well established with each receiving increasing feed levels as the fish biomass increased. RBC water samples were periodically taken from all eight RAS until harvesting of the fish crop or reconfiguration of a given system for other required research . At the end of the official growth trial, RAS 7 was reconfigured for further RBC study. The system flow was split between two RBC's allowing changes in the flow rates differing from that used during the growth trial. In total, six flow rates were evaluated by changing submerged pumps. Flows ranged from 3.8 to 289 liters/minute. This resulted in detentions times ranging from 6.9 minutes to 8.5 hours. Influent TAN ranges for the two RBC's supporting this system were maintained by adjusting fish feeding and stocking levels as required. RBC's were monitored for acclimation to shifts in flow rates before sampling was reiniated. Sampling was terminated on January 26, 1992. Daily sampling was completed between 11AM and 2PM on each sampling day. Water samples were taken of RBC influent (exiting the solids clarifier pumps) and on the downstream side of each of the three RBC stages approximately 0.3 meters below the water surface.

WATER QUALITY ANALYSIS

TAN and Nitrite-nitrogen were analyzed following the HACH kit procedures described earlier in Chapter II.

As often as possible, total $CBOD_5$ and DOC were simultaneously evaluated with ammonia and nitrite nitrogen. $CBOD_5$ and dissolved organic carbon levels were also

analyzed as described earlier in Chapter II. $CBOD_5$ and DOC evaluations were only conducted during the actual growth trial when flow rates were near 285 liters/minute.

RESULTS

LITHIUM TRACER STUDY

The raw data for the tracer study is summarized in Appendix L. Figure 23 compares the observed response of the three stage RBC system , to a lithium tracer impulse loading, with a theoretic response by a series of three ideal CSTR's. Note that the observed RBC response is in good agreement with the tracer response predicted for the ideal case and that the lithium concentrations are down to a theoretically predictable diluted background level by 9 stage detention times (3 RBC detention times). This evaluation was done on only one RBC, assuming that the evaluation results would apply similarly to the other 7 systems.

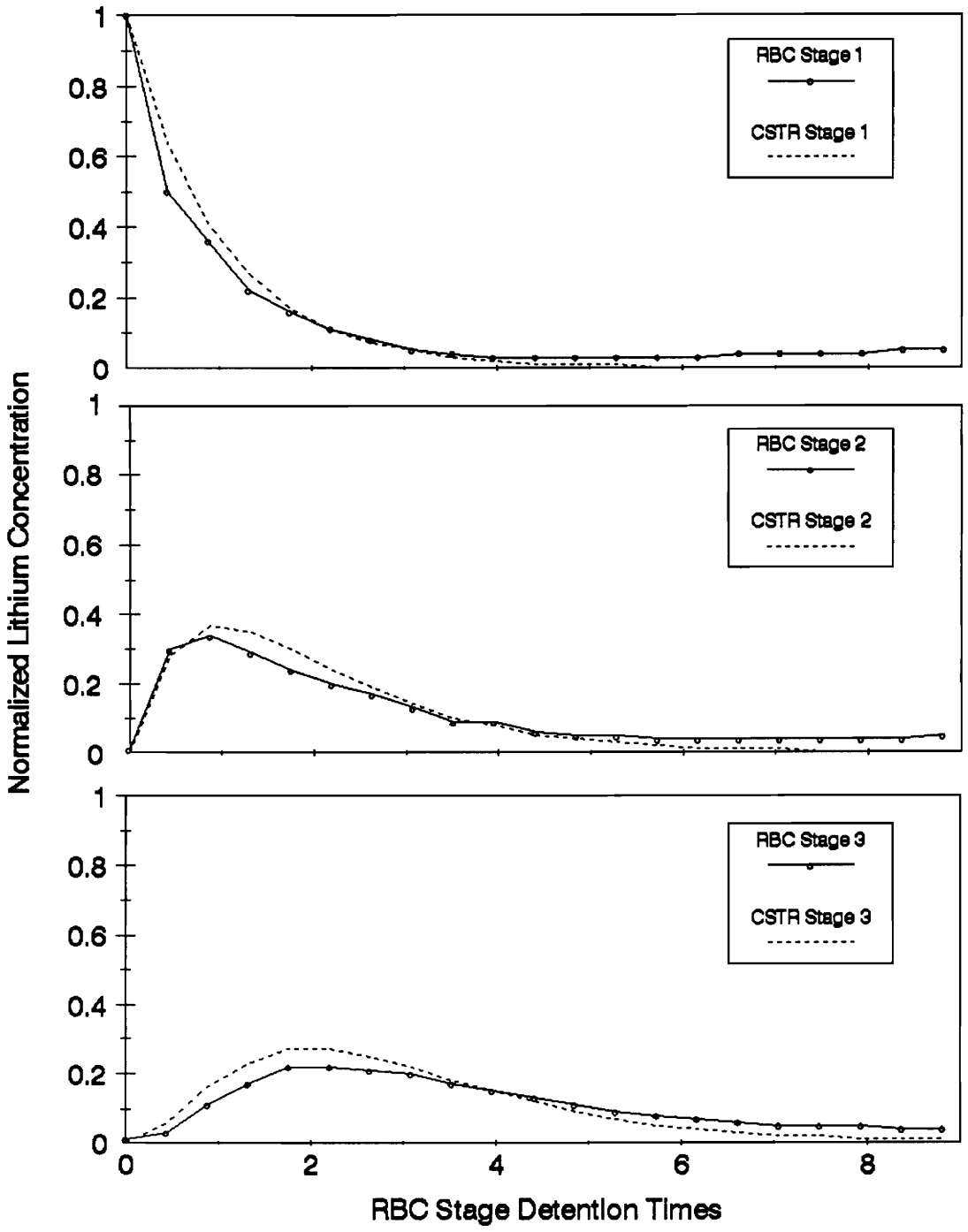


Figure 23. Comparison of the observed reponse of a three stage RBC with the theoretical response of three ideal continuous stirred tank reactors in series to a lithium tracer impulse.

Appendix M summarizes the results of the organic treatment performance of the RBC's including $CBOD_5$ and DOC. Figure 24 shows the observed relationship between observed $CBOD_5$ effluent and influent values across the three stage RBC's. Note that the slope is 0.985 (very nearly 1.0). This implies no observed reduction in $CBOD_5$ across the RBC.

Figure 25 presents similar data for DOC observations across the RBC's. However, in this case note that above DOC influent levels of approximately 20 mg/l, DOC removal is evident.

The RBC ammonia and nitrite-nitrogen data results are summarized in Appendix N. Six RBC flow rates were evaluated during this study. Figure 26 shows the linear relationship between RBC effluent and influent TAN levels observed for all RBC data taken during the growth trial study while RBC flow rates were above 252 liters/minute.

Figure 27 compares the TAN mass removal rate, percent TAN removed and resulting effluent TAN for the full range of mass loading into the RBC for all six flow rates studied over the 7 month RBC data collection. Mass removal versus loading rate is very nearly linear, implying that removal rate is a constant fraction of the mass loading. Percent TAN removed falls sharply to around 25 percent and holds constant above loading of 0.25 grams/meter^2 surface area of media/day. Effluent Tan rises abruptly until loading reaches around the 0.25 range where the impact of a constant 25 percent removal begins to control. Effluent TAN continues to rise more slowly over the remaining loading range.

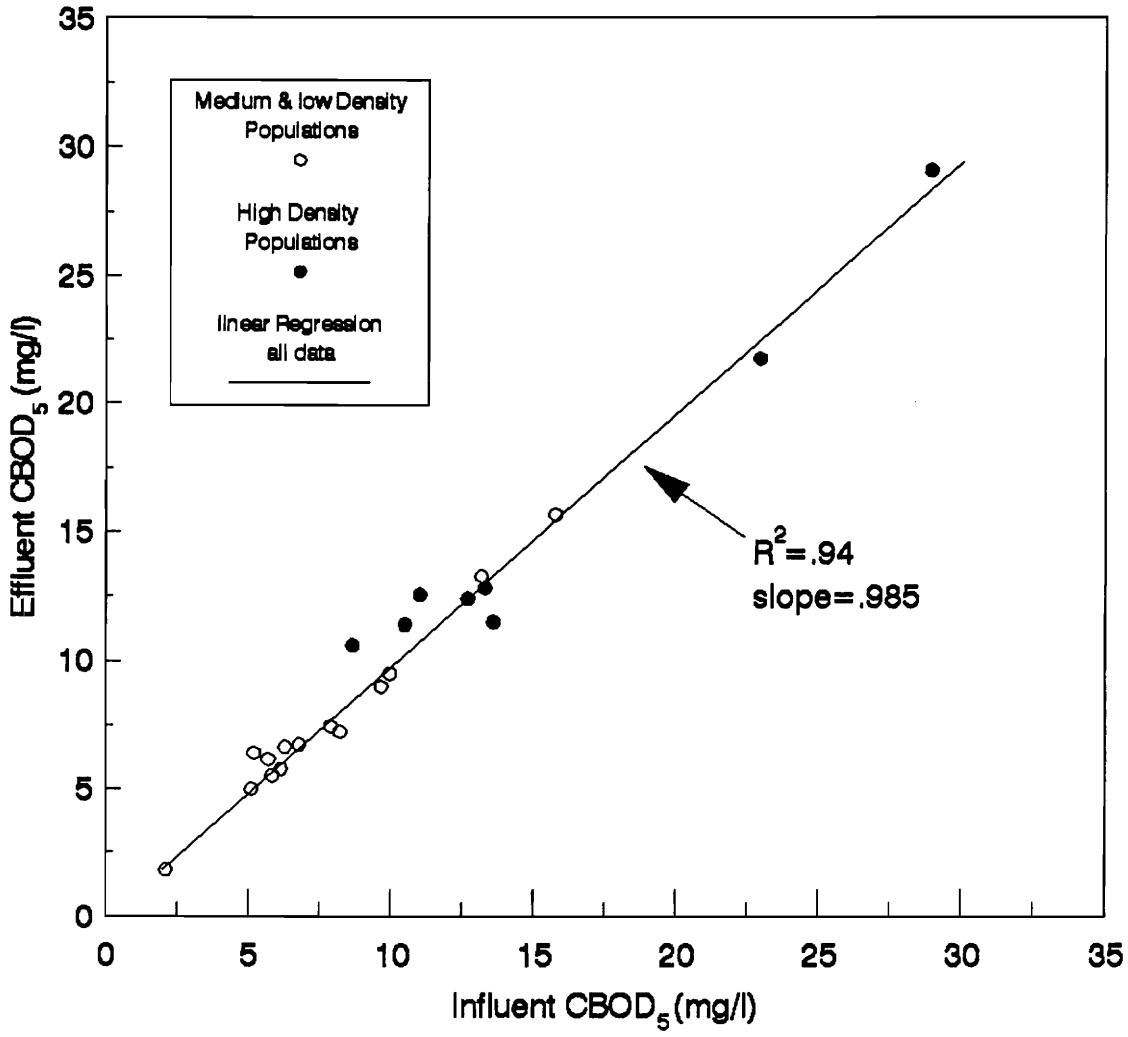


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Figure 24. Rotating Biological Contactor effluent vs influent 5 day carbonaceous biochemical oxygen demand.

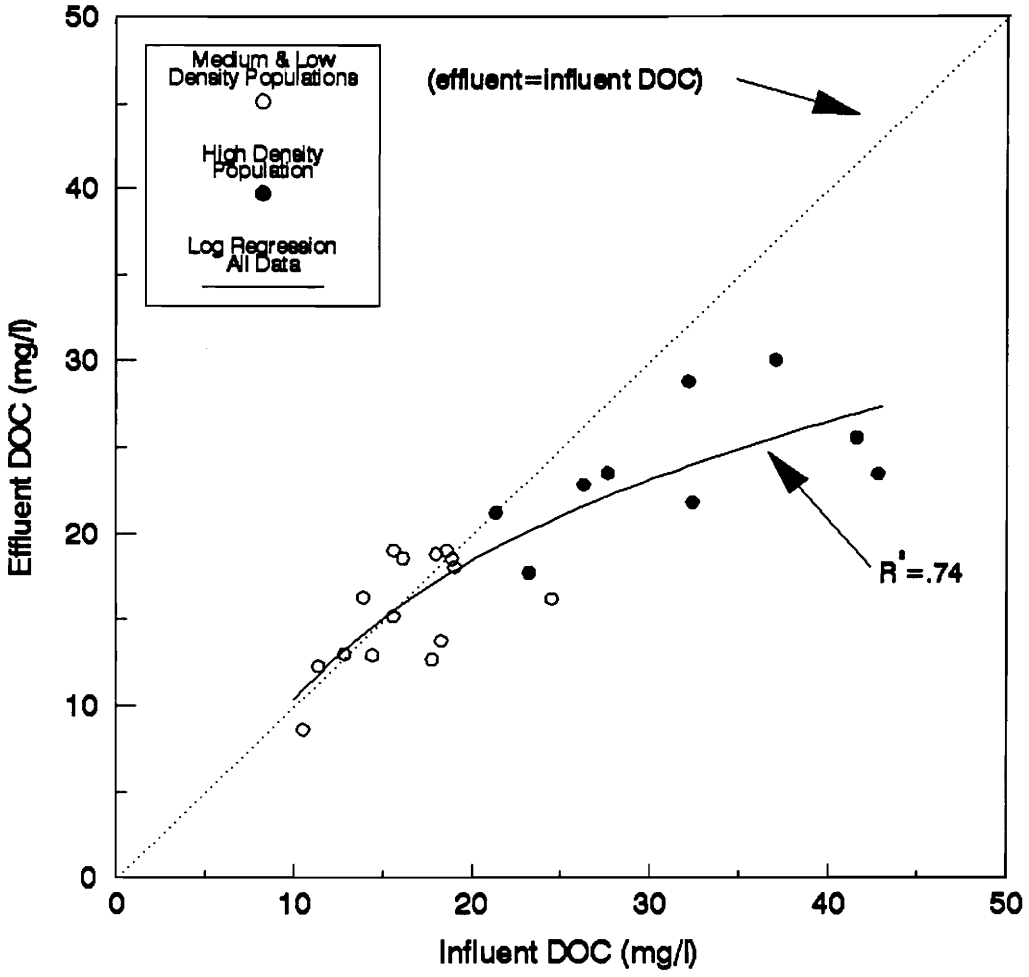
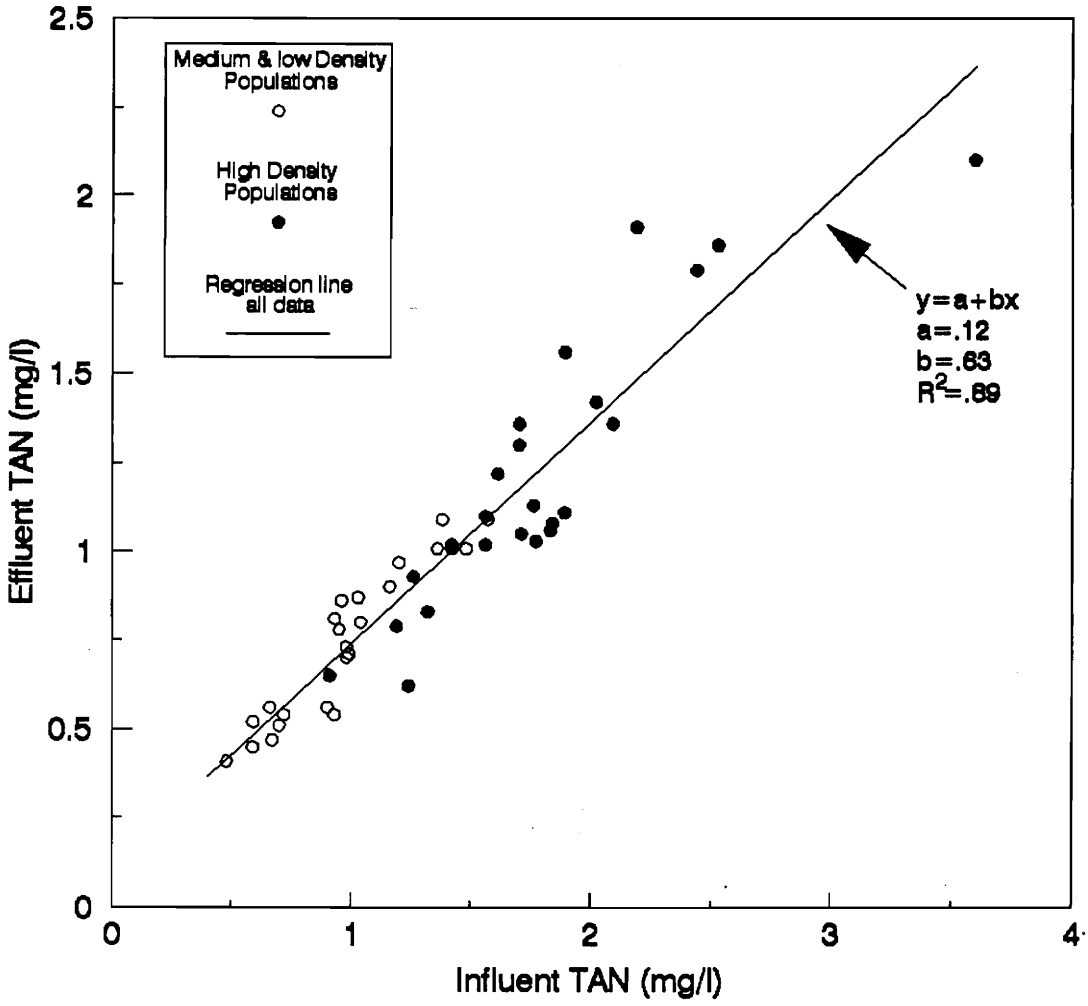


Figure 25. Rotating Biological Contactor effluent versus influent dissolved organic carbon.



Flow range was 252 to 269 liters/min.

Figure 26. Rotating Biological Contactor effluent vs influent Total Ammonia-nitrogen concentration.

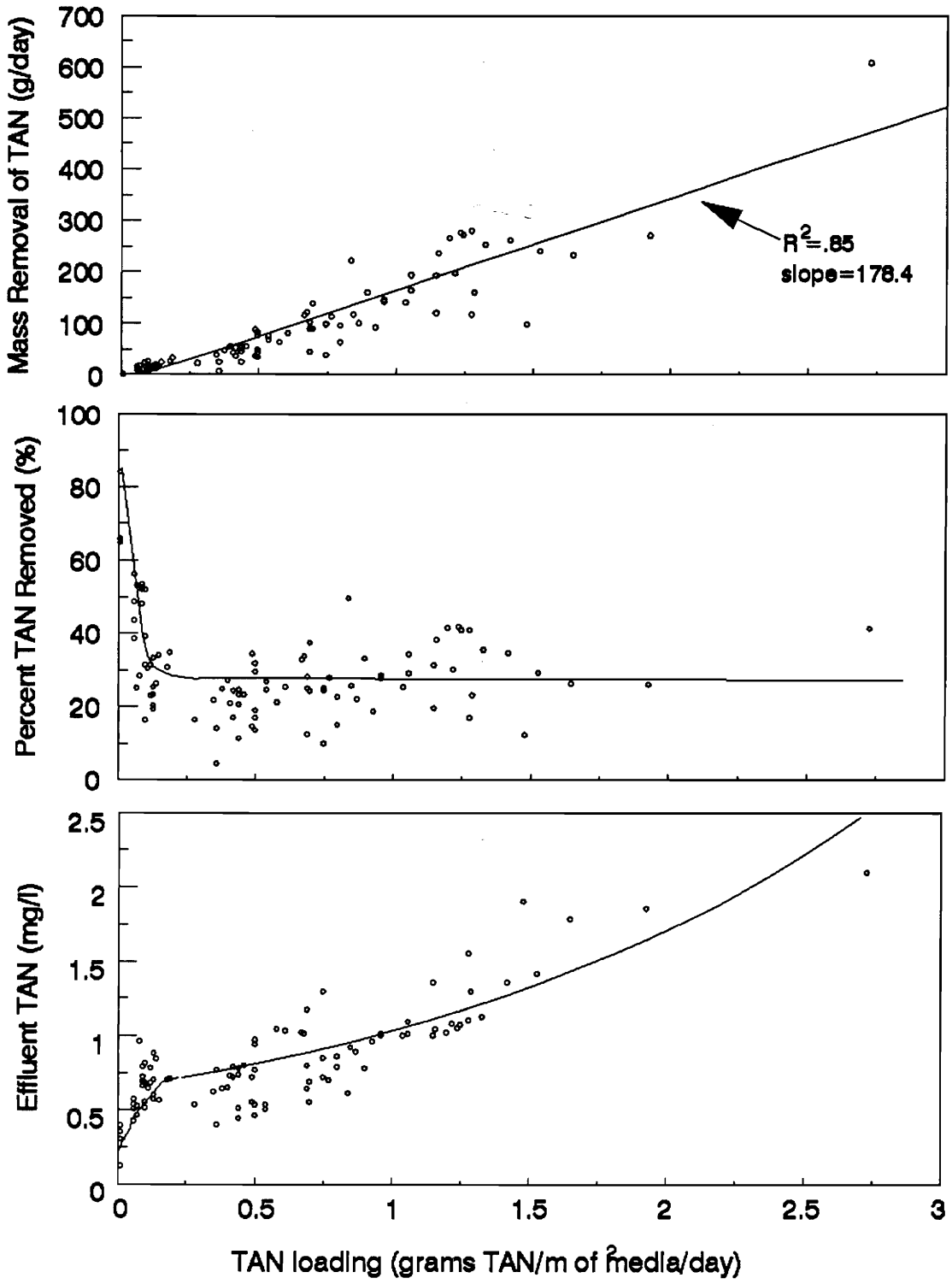


Figure 27. TAN Mass removal,percent removal and effluent vs mass loading rate in grams TAN/m of media/day.

RBC PERFORMANCE ANALYSIS

Attempts to use the theoretical equation approach derived from Grady and Lim (1982) and based on pseudo first order kinetics were unproductive. Assuming first order kinetics, individual RBC stage constants were determined using average rate and concentration values for each of the six flow rates studied (Table 8). If equation [8] were validated by this study's data all 18 calculated K_1 values would have been approximately equal. Since the K_1 values differ by more than an order of magnitude this study does not support the first order kinetics assumed to derive equation [8]. In fact no single ordered reaction rate or single constant K_1 describes the behavior of the observed data, which is a prerequisite for equation [8].

Performance evaluation of the RBC will therefore be solely based on the following proposed RBC equation derived from the trickling filter approach outlined by Balakrishnan and Eckenfelder (1970),Veismann and Hammer (1985) and Metcalf and Eddy (1991).

$$S/S_o = e^{(-K(\text{Stage Number})/W^n)}$$

where: S=effluent ammonia concentration (mg TAN/l)
S_o=influent ammonia concentration (mg TAN/l)
W=mass loading of substrate (mg TAN/m^2 of biofilter/day)
K and n=empirical constants

A procedure for analysis of the constants K and n is offered by Eckenfelder (1966) and by Veissman and Hammer (1985). The RBC equation is evaluated in the same manor except: depth (D)=Stage number and volumetric loading (Q)=mass loading (W).

Table 8. RBC stage reaction rate constants (K_1), calculated assuming first order kinetics and using average rates and concentrations for six different flow rates.

Flow Rate	Rate Constants K_1		
(Liters/minute)	($min.^{-1}$)	($min.^{-1}$)	($min.^{-1}$)
3.8	0.009	0.001	0.001
22.7	0.019	0.006	0.004
43.5	0.015	0.007	0.002
64.4	0.017	0.015	0.005
162.4	0.027	0.028	0.019
285	0.057	0.049	0.039

The analysis begins with a semi-log plot of the percent TAN remaining versus RBC stage number, offered as Figure 28. Several points can be noted from this figure. First, the response of the RBC is generally to loose efficiency with increasing flow rates up to around 43.5 liters/minute. Any further increase in flow rate seems to have little or no effect on the percent of TAN remaining as a function of RBC stage number. Secondly, the lines for each flow rate are not linear across all three RBC stages. Rather the first RBC stage appears to have a response that differs in slope from that of the second and third. This change in slope is interpreted to mean that the second and third stage level of treatment might be different from the first stage. This concept is reinforced by the widely varied theoretically based reaction constants listed in Table 8. Based on this interpretation, an analysis of K and n was completed for the overall RBC, using a best fit linear regression, along with each stage treated as an individual single stage reactor with an individual K and n constant. Development of individual stage constants required the reasonable assumption that the slopes for each line could be taken from lines passing through the 100 percent remaining point for" zero" RBC stages and the point defined by the percent remaining as we leave that individual single stage (a line defined by only two points).

The constants K and n were initially developed following the graphic procedures outlined by Eckenfelder (1966) and by Veissman and Hammer (1985). These graphically determined constants were used as the input to a nonlinear curve fitting software (SIGMAPLOT Vers. 4.1, JANDEL SCIENTIFIC , Corte Madera, California).

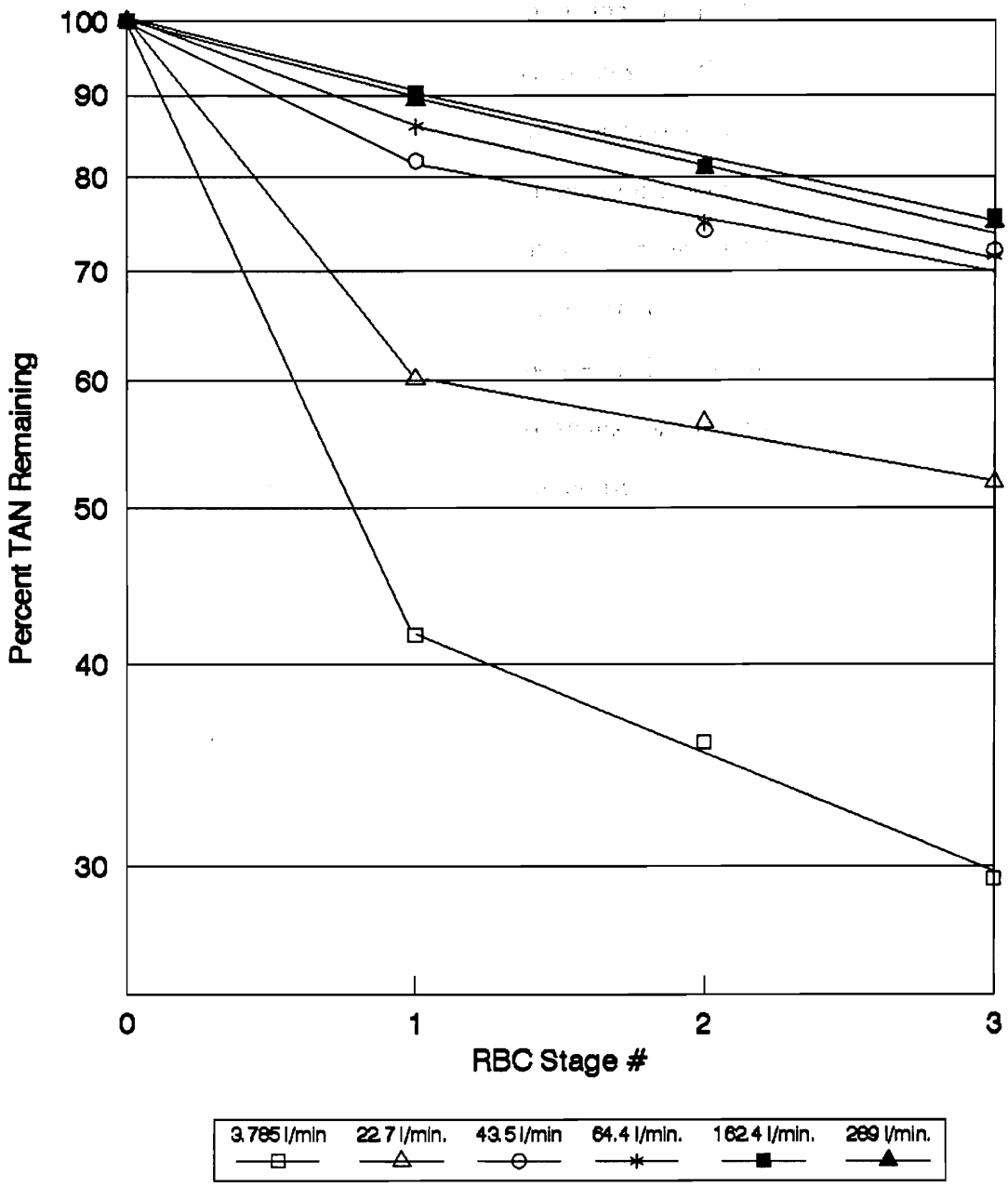


Figure 28. Percent TAN remaining versus RBC stage number.

This software uses a Marquardt-Levenberg algorithm, and iteratively finds the best fit of any entered equation to observed data. The iterations were started using the graphically determined constants allowing refinement by the software algorithm. Table 9 summarizes the refined constants. These constants were used to generate Figure 29, which compares the observed percent TAN remaining to that predicted by the. empirical equation .

Predicted percent TAN remaining in Figure 29 was calculated by two methods. First, the overall RBC constants K and n were used to directly obtain a predicted effluent TAN for a given influent concentration. This effluent concentration was used to calculate the percent TAN remaining based on the overall regressed constants. Secondly, each individual stage K and n were used to calculate an effluent TAN for that stage starting with an observed influent TAN to the RBC. Moving sequentially through the stages, using the predicted effluent TAN concentration for the previous stage, the effluent concentration for the current stage was calculated to obtain an overall RBC effluent TAN concentration. This effluent concentration determined by sequentially using the individual stage constants was used to calculate the percent TAN remaining based on the individual constants.

Table 9. Summary of empirical constants developed for a three stage RBC.

Filter configuration analyzed	n	K
RBC stage 1 only	0.55	0.14
RBC stage 2 only	0.14	0.11
RBC stage 3 only	0.18	0.06
All 3 stages	0.36	0.08

Equation form: $S/S_o = e^{(-K(\text{Stage Number})/W^n)}$

where: S=effluent ammonia concentration (mg TAN/l)
S_o=influent ammonia concentration (mg TAN/l)
W=mass loading (grams TAN/m^2 biofilter/day)
K and n=empirical constants

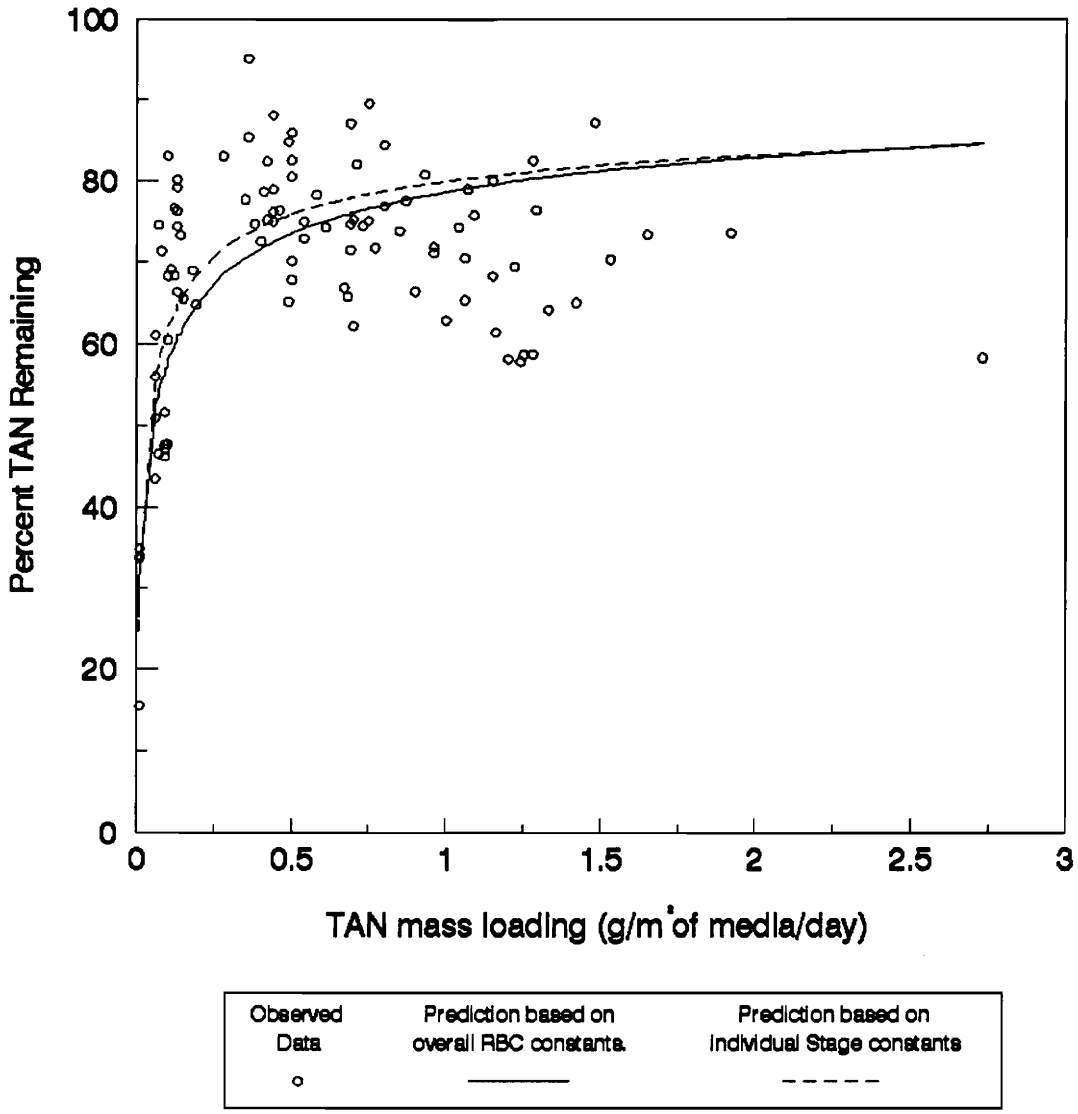


Figure 29. Comparison of observe RBC TAN removal as percent TAN remaining in the RBC effluent with two effluent predictions plotted against TAN mass loading.

Figure 29 clearly shows that either prediction line equally fits the observed data. The wide degree of scatter in the data is largely because of the very low TAN levels being treated. That is, even small analytical errors or variations in treatment between different RBC's and samples results in large differences on a percentile basis. Figure 30 provides an alternate way of viewing the ability of the empirical RBC equation to fit the observed RBC performance. Here the predicted effluent concentration is plotted against that which was observed. A perfect fit would be defined by a line of slope one. We again see that by using either an overall set of constants for the entire RBC or by using individual stage constants we have a nearly equal good fit.

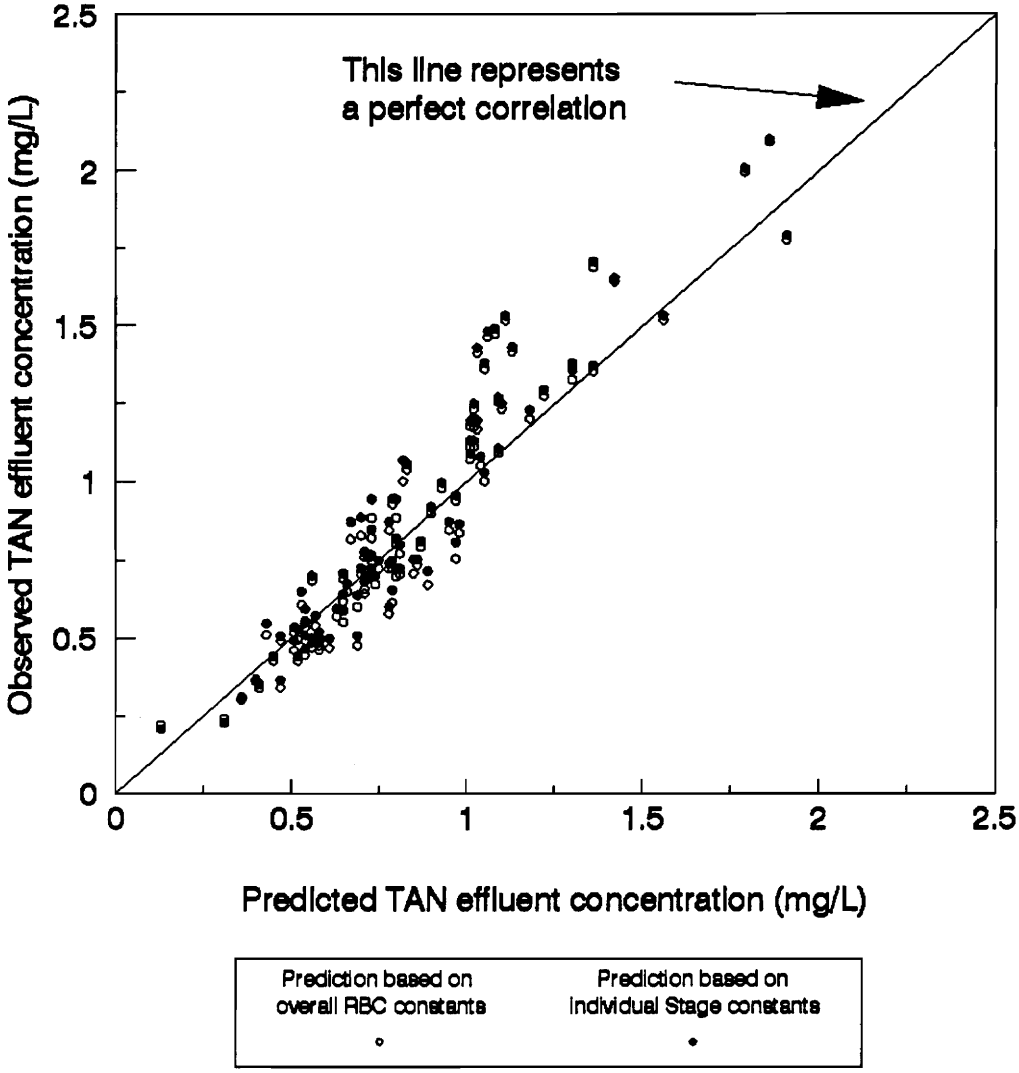


Figure 30. Demonstrated ability of the emperical equation to fit observed data. Two sets of overlapping points represent two approaches to using the emperical constants within the equation.

DISCUSSION

IMPORTANCE OF THE TRACER STUDY

The results of the lithium tracer impulse study indicate that it is reasonable and appropriate to consider our three stage RBC as closely simulating three ideal equal size CSTR's in series. Short circuiting was observed but only to a slight degree (Figure 23). The tracer response is important for both of the performance analysis approaches attempted in this study.

Accepting that this studies RBC stages closely approximate ideal CSTR's in series and that first order kinetics dictated, it would seem that the theoretical approach to the RBC as three CSTR's containing biofilm should have proven productive. The RBC data observed during this study does not support the theoretical first order CSTR approach. This indicates that the reaction kinetics in each RBC stage are following individual stage patterns dictated by the water conditions in that stage.

The logic of the empirical approach to analyzing RBC performance is also based on our tracer evaluation. The empirical trickling filter equation used to generate the proposed empirical RBC equation is an exponential decay much like the first order decay response that would be expected for a PFR. As mentioned earlier, a trickling filter produces a wave front of water carrying substrate through its depth much like a finite slice of water moving through a plug flow reactor. Extension of the trickling filter equation to a staged RBC system would be reasonable only if the stages of the RBC acted as CSTR's in series. This is a required condition if the stepwise movement from the first to the last stage of the RBC is to approximate the exponential decay observed down the length of a trickling filter.

ORGANICS

Based on literature review, it was expected that the RBC's would have a widely varied microbe population and that competition would be established between heterotrophic bacteria using organic food sources and the primarily autotrophic nitrifiers.

Analysis indicates essentially no observable removal of $CBOD_5$ across the entire RBC (Figure 24). It is hypothesized that the $CBOD_5$ represents an endogenous respiration by a microbial suspension building over time in the fish culture systems.

DOC analysis (Figure 27) does indicate organic treatment activity by the RBC ,but only after background DOC levels have reached 20 mg/l.

TAN

Figures 26 and 27 both indicate that ammonia-nitrogen removal is a linear function of mass loading. We have also noted (Figure 27) an approximately constant 25 percent removal of TAN above loadings of 0.25 mg TAN/m^2 of media/day.

RBC PERFORMANCE MODEL

Evaluation of RBC performance was based on rearrangement of the trickling filter equation resulting in the proposed RBC equation.

$$S/S_o = e^{(-K(\text{Stage Number})/W^n)}$$

Figures 29 and 30 show that the proposed RBC equation can fit the observed response of our eight RBC's over the entire flow, mass loading and influent TAN range experienced during this study.

The differences in TAN removal response of the first stage from the second and third stage of the RBC are graphically demonstrated in Figure 28 . The differences in slope before and after stage 1 were cause for suspicion that the reaction kinetics within the first stage were markedly different from the following stages. In fact, the resulting constants summarized in Table 9 do change as we pass from the first to the last. The failed attempt to use the theoretically based equation [8] reinforces the concept that each stage has its own individual kinetics as indicated by widely varied reaction rates listed in Table 8. No clear cut reason for the changes in the constants from stage to stage was determined. However several possibilities exist.

Effects of organics and organic uptake by the RBC are one possible explanation of this change in constants from stage to stage. For instance, if the DOC was being primarily taken up in the first stage then there could have been a difference in nitrification rate in latter stages depending on the ratio of organics to nitrogen compounds existing in each stage. Based on Figure 31 excerpted from Grady and Lim (1980) enhanced nitrification rates might have been expected at low ratios while higher ratios would inhibit. Unfortunately, exhaustive comparisons of the observed organics

levels and removal rates with the simultaneous TAN removal rates on a stage by stage basis does not provide support to this hypothesis.

The possibility of other rate limiting conditions such as oxygen in the second and third stage of the RBC might be postulated. Oxygen levels across the RBC's were maintained above 5 ppm as a result of the U-tube oxygenation system supporting the fish population and natural aeration by the RBC itself. Therefor oxygen is not considered to have been limiting.

A final possibility is that the types of microbe populations present in the sequential stages were just different in nature and that we have a resulting variation in treatment constants due to slightly different populations in each stage.

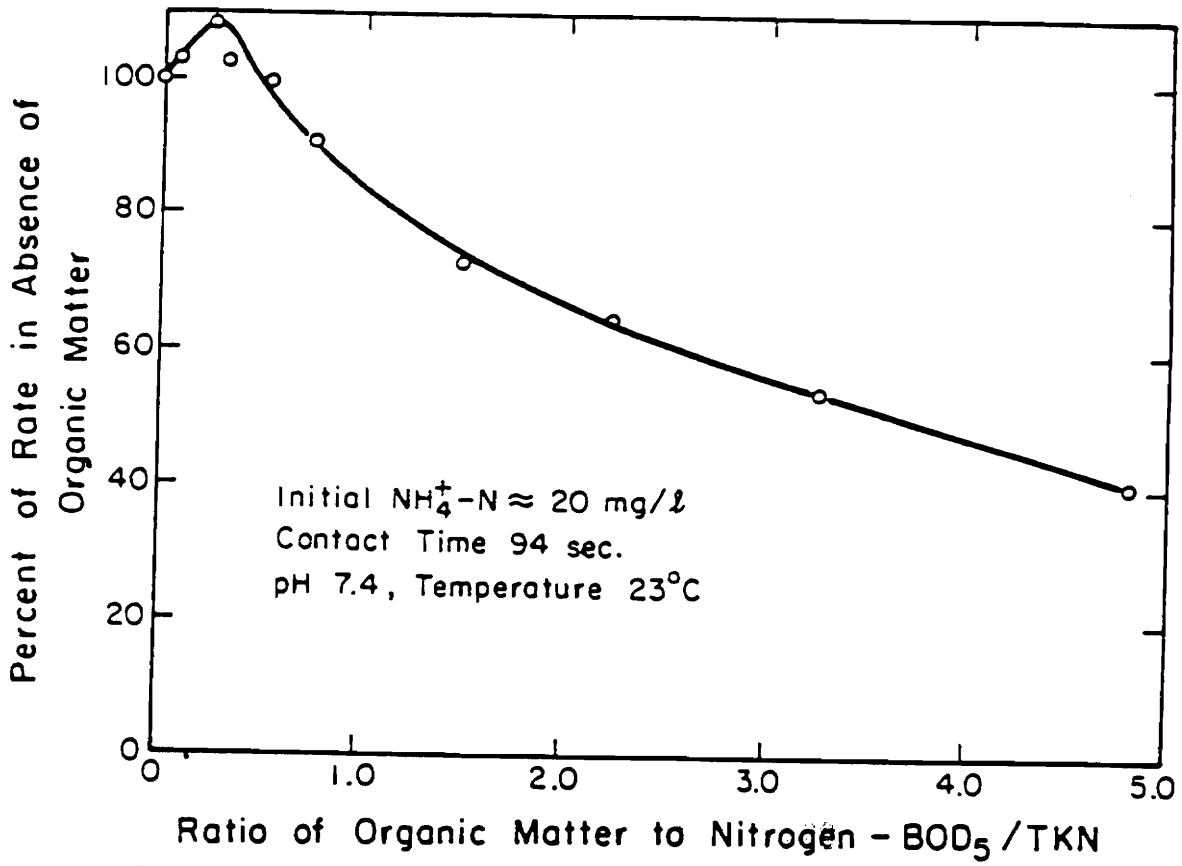


Figure 31. Effect of the ratio of organic matter to nitrogen on the rate of nitrification observed in fixed films. (Grady and Lim 1980).

The main goal was to analyze the RBC performance in hopes of yielding a design equation or procedure that proved useful to the design and optimization of other RBC systems applied to the unique aquaculture environment. It has already been shown that the proposed empirical RBC equation provides a reasonable fit of the data. The next questions might be:

1. How does this studies response compare to other nitrification systems based on past observations?
2. Can the empirical equation be successfully applied to RBC's in other aquaculture systems ?

It is enlightening to compare the observed nitrification performance to that expected of other nitrification systems as demonstrated in past research. Figures 32 and 33 offer such a comparison to activated sludge and trickling filter systems.

In order to make a comparison of the RBC performance to suspension systems, like an activated sludge, estimates of biofilm biomass are necessary. Monod expressions used in activated sludge modeling report substrate utilization rates in terms of mass of substrate removed per mass of microbes per day. Assuming an 80 micron effective biofilm depth and a biofilm density of 20 mg /cm^3 (Hoehn and Ray 1979) then an estimated biofilm mass for the entire RBC surface area can be calculated. Using this estimate of biomass to represent the mass of microbes, a specific utilization rate can be calculated for the RBC performance. Figure 32 provides a comparison of the estimated nitrification performance of this study's RBC to two predictions from representative literature sources for suspension activated sludge systems.

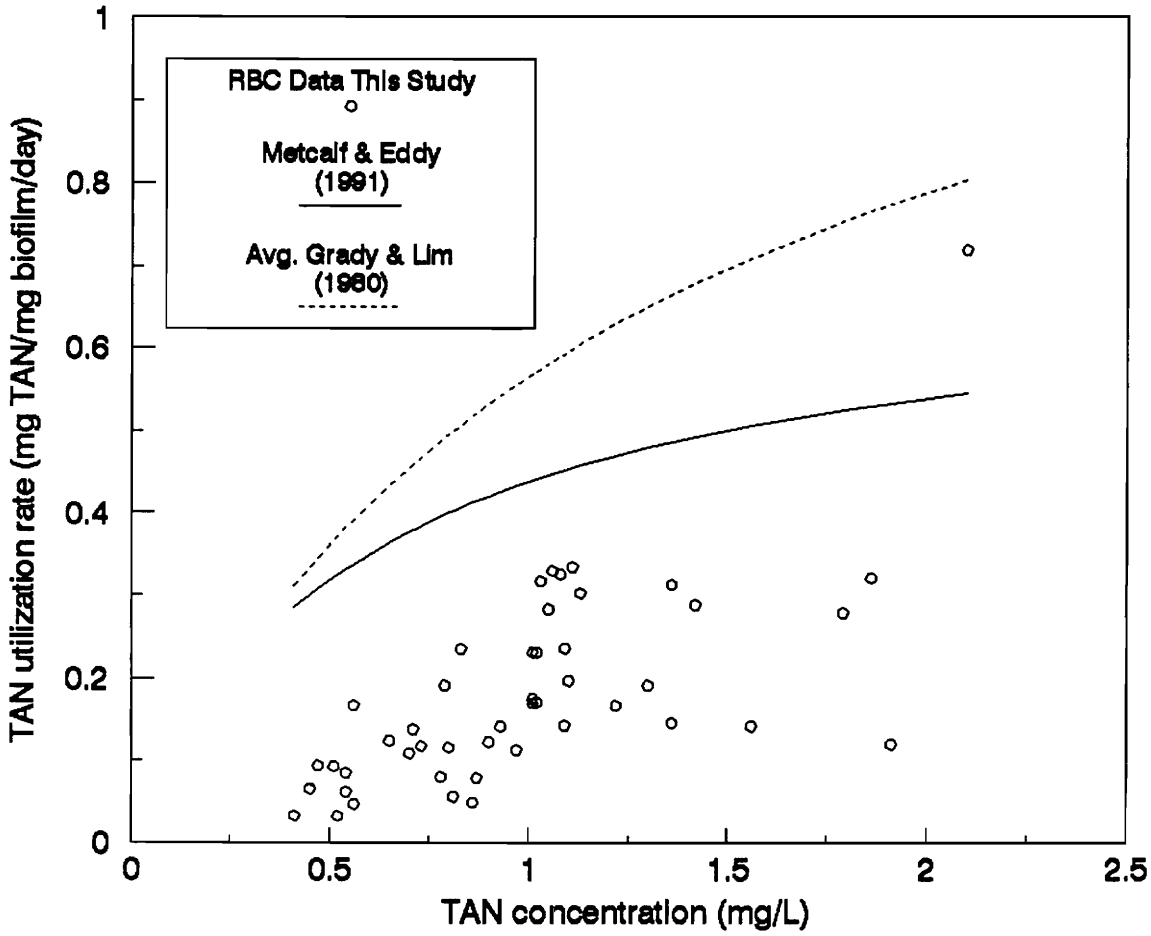


Figure 32. Comparison of calculated RBC utilization rates with those reported for representative suspension nitrification systems.

Figure 33 provides a similar comparison between the observed RBC nitrification performance and that predicted for trickling filters of equal media volume based on work presented by Balakrishnan and Eckenfelder (1970).

The purpose of these comparisons is primarily to see if the observed RBC performance is in reasonable agreement with that observed in other nitrification systems. It is interesting that the RBC data points fall generally below the performance predicted for the activated sludge while predicting superior performance to the trickling filter systems. However, care must be taken not to read to much into this overall comparison because of the assumptions required and the fact that the literature was waste water literature where high TAN levels were used to obtain the treatment constants. Reasonable agreement is really all that Figures 32 and 33 represent.

A direct comparison of the RBC performance observed in this study to other RBC's applied in aquaculture is needed. Very little complete data is available for this comparison. Figure 34 compares the TAN removal rates per m^2 of media surface area observed in an RBC study by Miller and Libey (1985) to that observed in this study. A dramatic difference exists between the nitrification performance observed by Miller and Libey and that observed in this study. The difference in slopes of the mass removal versus mass loading plots (Figure 34) was expected based on an average TAN removal efficiency of nearly a constant 77 percent observed by Miller and Libey (1985) versus 25 percent for this study. It is noteworthy however that in both aquaculture systems the RBC's developed constant mass removal rates expected at low TAN levels. Unfortunately no other RBC data is available for comparison. It should also be clearly noted that the system studied by Miller and Libey (1985) was essentially a flow through system with

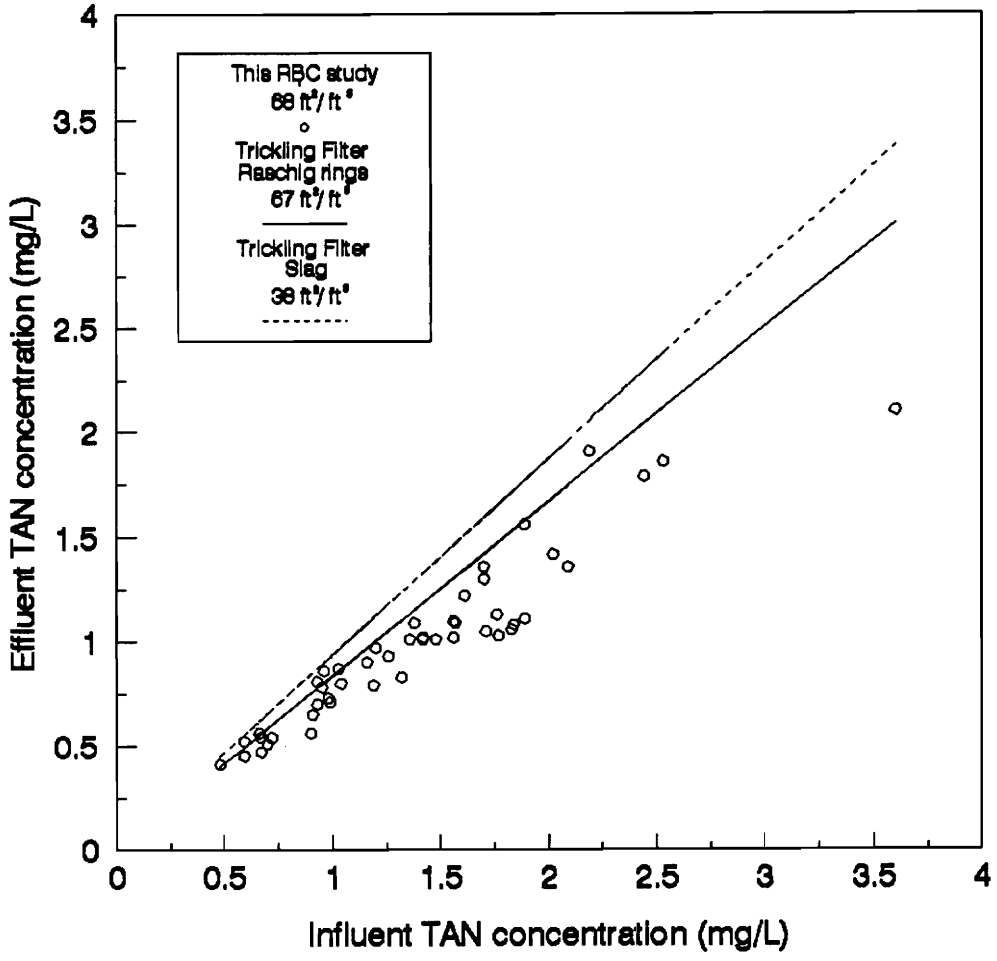


Figure 33. Comparison of observed RBC nitrification performance to that predicted by an equal volume trickling filter based on Balakrishnan and Eckenfelder (1970).

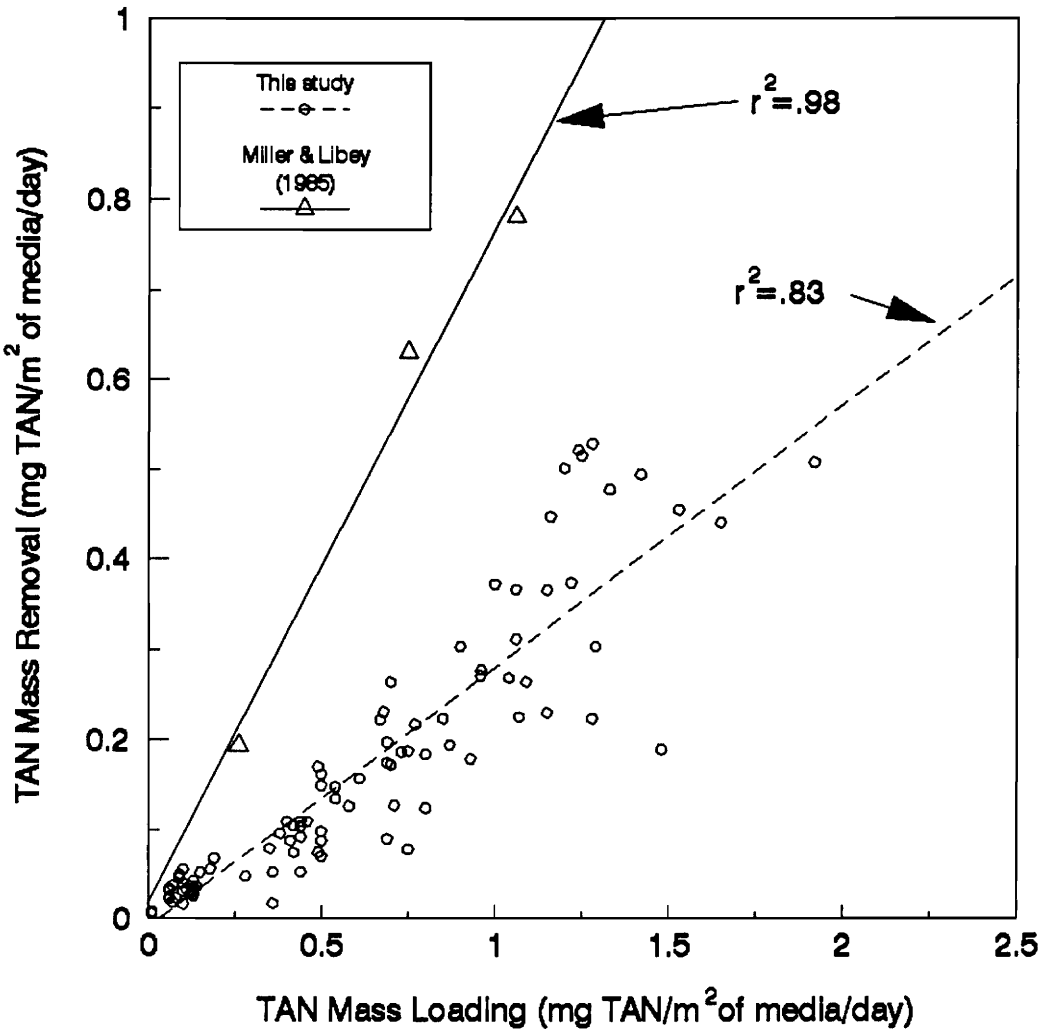


Figure 34. Comparison of observed RBC performance for this study and that of Miller and Libey (1985).

high rates of water exchange unlike in this study. The unique contamination levels and resulting water qualities existing in this study are more representative of production high density recirculating aquaculture systems. RBC performance must be evaluated under these conditions.

Finally, it is significant that a constant percent TAN removal occurred both in this study and that of Miller and Libey (1985). It appears that for mass loading rates resulting from normal flow rates experienced in aquaculture systems that RBC's will remove a nearly constant fraction of the TAN loading. Therefore, higher flow rates will result in more TAN removal. This directly supports the contentions of Wheaton (1991) and Brune and Gunther (1981) discussed earlier in this Chapter.

SUMMARY AND CONCLUSIONS

Fixed film biological treatment is the preferred approach to treating for ammonia-nitrogen in recirculating aquaculture systems. RBC's have a proven track record of outperforming other fixed film systems in side by side aquaculture studies.

Based on an extensive literature review, it seems reasonable to assume first order kinetics will exist for the low TAN levels experienced in aquaculture. Failed attempts to use a theoretical approach to analyzing the data from this study were based on an RBC acting as CSTR's in series following first order kinetics. The data recorded in this study did not support this approach.

A simple empirical RBC equation was proposed based on trickling filters analysis methods. The proposed equation fits the data generated in this study. The empirical equation predicts a nearly constant TAN removal efficiency for the TAN mass loading ranges experienced under normal operational RBC flow rates and detention times. This implies that for TAN mass loading rates representative of production aquaculture system configurations the empirical equation can be simplified to state that a constant percent TAN removal will occur for a given RBC and system configuration. This implies that higher flow rates will result in higher TAN removal on a mass basis.

. Design of RBC's for other aquaculture systems can be based on this empirical approach if and only if constants are developed in pilot studies done with similar media and water quality conditions. Great care should be exercised in using the constants developed from dissimilar situations. Future evaluations of the constants for an appropriate selection of RBC media would be indicated.

The following general conclusions are made based on this study.

1. Nitrification across RBC's in this study was empirically modeled by the following proposed RBC equation.

$$S/S_o = e^{(-K(\text{Stage Number})/W^n)}$$

where: S=effluent ammonia concentration
S_o=influent ammonia concentration
W=mass loading of substrate (mass/area of biofilter)
K and n=empirical constants

2. RBC TAN mass removal was nearly constant over the ranges of mass loading observed in this study. This indicates that higher RBC flow rates resulting in higher loading rates will yield higher TAN mass removals for a given RBC system.
3. The nitrification performance observed in this study was low as compare to the only other available RBC aquaculture study. Such system differences will dictate that pilot studies be completed to determine constants appropriate to an individual RBC media, system water quality and system configuration.

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APPENDIX A

Summary of ANION Data

Appendix A. Anions

Date	RAS	Population	Chloride	Nitrate	Phosphate	Sulfate
1991	Number	Density	(mg/L)	(mg/L)	(mg/L)	(mg/L)
01/23	2	High	3002.0	13.3	0.6	36.0
01/30	2	High	2195.5	21.7	1.3	39.0
03/24	2	High	185.0	34.6	1.4	25.9
04/19	2	High	267.7	36.7	0.0	31.5
05/17	2	High	292.0	58.7	1.9	39.0
06/17	2	High	266.0	76.3	2.8	34.5
07/24	2	High	322.5	134.6	7.8	45.5
08/12	2	High	338.7	129.9	7.1	42.0
01/23	3	Medium	473.0	24.8	0.6	33.3
01/30	3	Medium	454.6	37.8	0.7	36.6
03/24	3	Medium	207.0	44.9	1.4	28.9
04/19	3	Medium	237.1	60.9	1.4	36.7
05/17	3	Medium	286.7	85.0	2.7	42.7
06/17	3	Medium	260.6	83.7	3.2	36.0
07/24	3	Medium	292.6	110.1	6.2	42.6
08/12	3	Medium	343.0	103.2	4.6	41.6
01/23	4	Low	655.0	25.8	1.1	32.0
01/30	4	Low	519.1	28.7	0.7	28.5
03/24	4	Low	210.0	52.4	2.2	31.1
04/19	4	Low	303.1	54.8	0.0	37.4
05/17	4	Low	278.4	95.4	7.6	43.1
06/17	4	Low	240.9	91.0	3.6	37.7
07/24	4	Low	350.9	115.3	5.6	44.7
08/12	4	Low	337.2	111.8	3.5	40.1
01/23	5	High	749.0	52.0	3.6	35.1
01/30	5	High	478.2	47.5	1.8	33.0
03/24	5	High				
04/19	5	High	256.7	53.3	0.4	33.6
05/17	5	High	281.3	86.0	4.0	42.2
06/17	5	High	280.0	109.4	4.9	42.0
07/24	5	High	378.1	166.3	6.3	53.4
08/12	5	High	472.3	170.2	8.5	51.1

Appendix A.Continued

Date	RAS	Population	Chloride	Nitrate	Phosphate	Sulfate
1991	Number	Density	(mg/L)	(mg/L)	(mg/L)	(mg/L)
01/23	6	Medium	781.0	31.1	1.3	30.5
01/30	6	Medium	592.2	33.3	1.2	26.2
03/24	6	Medium	189.9	43.9	1.8	27.8
04/19	6	Medium	273.5	48.9	0.0	33.1
05/17	6	Medium	234.4	70.1	6.3	36.1
06/17	6	Medium	196.9	46.5	1.7	25.7
07/24	6	Medium	305.3	105.5	5.8	40.6
08/12	6	Medium	294.3	114.3	3.9	41.2
01/23	7	Low	910.0	40.9	0.2	43.6
01/30	7	Low	643.2	46.1	1.3	34.0
03/24	7	Low	206.2	44.8	1.5	27.5
04/19	7	Low	262.5	43.2	0.0	33.0
05/17	7	Low	281.2	87.9	0.3	38.7
06/17	7	Low	261.1	68.2	2.6	31.8
07/24	7	Low	305.6	118.0	7.0	44.4
08/12	7	Low	319.7	125.8	6.6	43.0
01/23	8	Low	571.0	6.9	0.5	27.2
01/30	8	Low	489.9	14.0	0.3	28.1
03/24	8	Low	212.8	41.4	0.8	28.4
04/19	8	Low	252.9	47.7	0.0	33.0
05/17	8	Low	253.9	67.5	1.1	33.6
06/17	8	Low	279.2	90.7	2.9	36.4
07/24	8	Low	270.6	98.6	4.7	40.0
08/12	8	Low	309.4	110.4	3.9	41.8
01/23	9	Low	714.0	4.9	0.3	35.2
01/30	9	Low	477.3	8.6	0.0	26.8
03/24	9	Low	251.3	45.0	0.9	30.4
04/19	9	Low	251.5	36.0	0.2	29.6
05/17	9	Low	285.7	72.1	0.5	35.9
06/17	9	Low	262.6	82.7	2.6	34.9
07/24	9	Low	315.4	114.7	5.0	45.7
08/12	9	Low	282.0	104.9	3.8	39.3

APPENDIX B
SUMMARY OF CATION DATA

Appendix B. Cations

Date	Population	RAS	Sodium	Calcium	Magnesium	Potassium	Copper	Iron	Lead
1991	Density	Number	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L	(ppb)
01/16	High	2	406.0	128.3	9.0	14.0			
01/30	High	2	1880.0	117.2	8.1	10.0			
02/04	High	2	708.0	66.0	19.4	12.9	0.02	0.08	nd
02/15	High	2	512.0	79.0	15.0	17.2			
03/02	High	2	240.0	65.0	9.6	22.4	0.01	0.05	1
03/24	High	2	225.0	58.0	7.0	22.3	0.01	0.46	1
04/19	High	2	185.0	102.0	12.2	16.8	0.01	0.07	1
05/09	High	2	252.0	92.0	8.1	26.0	0.01	0.07	nd
07/23	High	2	256.0	132.0	11.2	60.0	0.01	0.16	nd
08/06	High	2	282.0	134.0	11.3	80.0	0.02	0.09	nd
01/16	Medium	3	298.0	116.2	1.2	8.8			
01/30	Medium	3	420.0	102.1	15.5	14.0			
02/04	Medium	3	266.0	48.0	10.7	15.9	0.02	0.08	1
02/15	Medium	3	250.0	92.1	9.0	17.4			
03/02	Medium	3	210.0	68.0	9.6	20.8	0.01	0.20	1
03/24	Medium	3	240.0	53.0	7.1	25.5	0.01	0.08	2
04/19	Medium	3	197.0	78.0	7.4	26.9	0.01	0.08	nd
05/09	Medium	3	240.0	101.0	8.1	39.8	0.01	0.08	nd
07/23	Medium	3	218.0	108.0	10.6	51.9	0.00	0.18	1
08/06	Medium	3	260.0	130.0	10.2	90.2	0.00	0.04	nd
01/16	Low	4	310.0	124.2	9.0	11.2			
01/30	Low	4	500.0	189.9	16.2	12.0			
02/04	Low	4	362.0	53.0	15.2	11.3	0.01	0.06	1
02/15	Low	4	330.0	75.1	11.6	17.4			
03/02	Low	4	250.0	114.0	10.7	28.0	0.01	0.11	1
03/24	Low	4	231.0	71.0	10.1	23.3	0.01	0.38	2
04/19	Low	4	175.0	91.0	11.3	34.4	0.01	0.12	1
05/09	Low	4	228.0	95.0	10.0	32.8	0.01	0.07	nd
07/23	Low	4	246.0	112.0	9.1	45.5	0.00	0.04	1
08/06	Low	4	266.0	121.0	10.2	33.4	0.00	0.04	nd
01/16	High	5	306.0	111.0	12.0	14.2			
01/30	High	5	480.0	183.8	13.1	16.0			
02/04	High	5	322.0	61.0	11.3	16.7	0.01	0.14	nd
02/15	High	5	314.0	149.0	8.8	18.8			
03/02	High	5	251.0	79.0	8.4	24.4	0.01	0.04	2
03/24	High	5	250.0	65.0	6.9	21.3	0.00	0.15	1
04/19	High	5	205.0	87.0	7.7	27.0	0.01	0.13	nd
05/09	High	5	240.0	94.0	7.8	24.6	0.01	0.08	nd
07/23	High	5	278.0	138.0	10.3	74.9	0.01	0.21	1
08/06	High	5	304.0	167.0	11.8	55.8	0.01	0.07	nd

Appendix B. Continued

Date	Population	RAS	Sodium	Calcium	Magnesium	Potassium	Copper	Iron	Lead
1991	Density	Number	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(ppb)
01/16	Medium	6	326.0	124.2	18.0	10.6			
01/30	Medium	6	640.0	168.7	14.1	16.0			
02/04	Medium	6	334.0	57.0	13.5	18.3	0.01	0.11	1
02/15	Medium	6	302.0	131.0	11.7	19.0			
03/02	Medium	6	330.0	52.0	8.2	40.7	0.01	0.06	nd
03/24	Medium	6	320.0	50.0	7.3	35.3	0.01	0.11	2
04/19	Medium	6	204.0	87.0	7.9	28.0	0.01	0.14	1
05/09	Medium	6	228.0	96.0	8.2	25.0	0.02	0.13	nd
07/23	Medium	6	232.0	111.0	8.8	44.8	0.00	0.18	1
08/06	Medium	6	242.0	112.0	9.4	46.0	0.01	0.05	nd
01/16	Medium	7	328.0	123.2	11.0	12.4			
01/30	Medium	7	580.0	153.5	18.1	18.0			
02/04	Medium	7	314.0	61.0	13.2	21.3	0.01	0.13	1
02/15	Medium	7	244.0	126.0	9.3	21.6			
03/02	Medium	7	325.0	63.0	8.2	26.3	0.01	0.05	nd
03/24	Medium	7	350.0	65.0	7.3	51.2	0.02	0.24	nd
04/19	Medium	7	216.0	79.0	7.9	30.8	0.01	0.27	2
05/09	Medium	7	230.0	94.0	8.7	28.9	0.01	0.18	nd
07/23	Medium	7	226.0	121.0	9.0	40.0	0.00	0.16	1
08/06	Medium	7	201.0	131.0	9.7	39.1	0.00	0.09	nd
01/16	Low	8	266.0	103.0	9.0	2.6			
01/30	Low	8	460.0	207.5	13.1	6.0			
02/04	Low	8	280.0	40.0	12.0	11.2	0.01	0.07	1
02/15	Low	8	334.0	146.0	9.3	12.8			
03/02	Low	8	300.0	51.0	9.2	30.7	0.01	0.07	nd
03/24	Low	8	250.0	55.0	7.3	50.1	0.01	0.50	nd
04/19	Low	8	198.0	92.0	7.7	25.9	0.01	0.26	1
05/09	Low	8	220.0	81.0	7.1	26.3	0.01	0.06	2
07/23	Low	8	228.0	100.0	8.7	30.2	0.01	0.17	1
08/06	Low	8	230.0	110.0	9.6	33.6	0.00	0.06	nd
01/16	Low	9	141.0	114.0	18.0	2.6			
01/30	Low	9	540.0	167.0	18.1	6.0			
02/04	Low	9	320.0	48.0	15.9	13.1	0.02	0.04	1
02/15	Low	9	316.0	85.0	14.8	11.4			
03/02	Low	9	280.0	59.0	11.6	30.5	0.02	0.04	nd
03/24	Low	9	260.0	55.0	8.8	26.0	0.01	0.06	1
04/19	Low	9	209.0	73.0	7.8	26.3	0.01	0.08	1
05/09	Low	9	216.0	83.0	8.7	24.0	0.00	0.07	nd
07/23	Low	9	232.0	103.0	8.9	39.2	0.01	0.17	nd
08/06	Low	9	232.0	123.0	10.5	42.1	0.00	0.06	nd

APPENDIX C
CARBONACEOUS OXYGEN DEMAND DATA

Appendix C1. High density CBOD5.

RAS	Date	CBOD5
No.		mg/L
2	02/08/91	7.0
2	02/15/91	12.4
2	03/06/91	13.4
2	03/23/91	15.0
2	04/18/91	13.7
2	05/19/91	8.9
2	06/10/91	10.0
2	06/16/91	15.7
2	07/06/91	20.0
2	07/11/91	22.1
2	07/23/91	14.1
2	08/09/91	22.9
2	08/16/91	14.4
2	08/22/91	43.0
5	02/02/91	12.7
5	02/08/91	6.8
5	02/15/91	7.6
5	03/06/91	10.6
5	03/23/91	18.1
5	04/18/91	18.2
5	05/19/91	12.2
5	06/10/91	10.6
5	06/16/91	11.5
5	07/06/91	18.4
5	07/11/91	21.8
5	07/23/91	9.1
5	08/09/91	31.2
5	08/16/91	20.4
5	08/22/91	

Appendix C2. Medium density CBOD5

RAS No.	Date	CBOD5 mg/L	RAS No.	Date	CBOD5 mg/L	RAS No.	Date	CBOD5 mg/L
3	02/08/91	2.42	6	02/08/91	5.67	7	02/02/91	8.75
3	02/15/91	8.4	6	02/15/91	12.12	7	02/08/91	7.51
3	03/06/91	10.5	6	03/06/91	7.8	7	02/15/91	8.82
3	03/23/91	9.3	6	03/23/91	7.48	7	03/06/91	8.03
3	04/18/91	11.1	6	04/18/91	7.2	7	03/23/91	9.1
3	04/19/91	5.4	6	04/19/91	6.7	7	04/18/91	10.1
3	06/10/91	7.04	6	06/10/91	11.99	7	04/19/91	4.5
3	06/16/91	8.37	6	06/16/91	6.68	7	06/10/91	8.02
3	07/06/91	6.23	6	07/06/91	11.71	7	06/16/91	6.61
3	07/23/91	5.28	6	07/23/91	7.42	7	07/06/91	13.54
3	08/09/91	8.27	6	08/09/91	16.94	7	07/23/91	9.8
3	08/16/91	10.36	6	08/16/91	19.75	7	08/09/91	16.13
			6	08/22/91	21.58	7	08/16/91	10.24

Appendix C3. Low density CBOD5

RAS No.	Date	CBOD5 mg/L	RAS No.	Date	CBOD5 mg/L	RAS No.	Date	CBOD5 mg/L
4	02/08/91	3.3	8	02/08/91	3.3	9	02/02/91	2.6
4	02/15/91	2.7	8	02/15/91	5.3	9	02/08/91	0.3
4	03/06/91	4.3	8	03/06/91	7.0	9	02/15/91	3.3
4	03/23/91	5.2	8	03/23/91	5.6	9	03/06/91	7.2
4	04/18/91	6.1	8	04/18/91	5.4	9	03/23/91	6.7
4	05/19/91	4.5	8	05/19/91	3.7	9	04/18/91	6.8
4	06/10/91	10.4	8	06/10/91	5.0	9	05/19/91	3.1
4	06/16/91	10.3	8	06/16/91	5.1	9	06/10/91	6.2
4	07/06/91	7.9	8	07/06/91	5.3	9	06/16/91	6.2
4	07/23/91	4.6	8	07/23/91	5.3	9	07/06/91	6.9
4	08/09/91	13.9	8	08/09/91	11.6	9	07/23/91	6.9
4	08/16/91	8.2	8	08/16/91	5.6	9	08/09/91	10.0
						9	08/16/91	6.7
						9	08/22/91	15.1

APPENDIX D
CHEMICAL OXYGEN DEMAND DATA

Appendix D1. Chemical Oxygen Demand

Date	RAS 2	RAS 3	RAS 4	RAS 5	RAS 6	RAS 7	RAS 8	RAS 9
01/23/91	48.0	24.5	20.4	16.3	24.5	36.7	20.4	28.6
01/30/91	40.8	24.8	16.3	28.6	40.8	43.0	32.6	30.6
02/15/91	32.6	49.0	44.9	49.0	40.8	44.9	24.5	32.7
03/06/91	32.0	32.0	32.0	34.0	60.0	32.0	30.4	80.0
03/24/91	32.8	38.4	42.4	50.4	29.6	32.0	40.0	24.0
04/19/91	16.1	40.2	36.2	48.2	38.2	20.1	32.1	55.0
05/17/91	63.5	69.5	47.6	103.1	83.3	79.3	67.4	63.5
05/25/91	29.9	46.9	38.4	55.5	85.3	46.9	51.2	55.5
06/03/91	56.0	48.8	40.0	52.0	60.0	40.0	34.0	48.0
06/12/91	50.0	57.6	49.9	78.7	34.6	49.9	38.4	42.2
06/17/91	57.4	49.2	53.3	65.6	49.2	36.9	45.1	57.4
07/10/91	57.9	45.5	49.7	70.3	43.5	49.7	47.6	41.4
07/24/91	84.2	63.2	71.6	88.4	67.4	75.8	54.7	75.8
08/11/91	105.3	50.5	54.7	96.8	71.6	71.6	54.7	50.5
08/21/91	74.8	47.2	51.2	47.2	78.7	47.2	39.3	39.3

APPENDIX E
DISSOLVED ORGANIC CARBON DATA

Appendix E. Dissolved organic carbon

Date	RAS 2	RAS 3	RAS 4	RAS 5	RAS 6	RAS 7	RAS 8	RAS 9
01/16/91	10.20	5.70	20.00	7.00	8.10	7.20	48.10	1.90
01/23/91	6.40	6.20	6.90	7.90	6.70	9.50	4.10	2.30
01/30/91	8.85	11.28	7.97	12.85	11.11	10.77	6.55	6.15
02/15/91	9.68	10.29	8.77	13.86	8.35	9.90	5.90	5.81
03/06/91	10.52	11.83	9.86	11.67	11.03	8.84	9.10	7.99
03/24/91	10.91	9.57	10.25	15.40	7.65	9.87	15.99	8.98
04/19/91	8.82	17.97	12.49	21.33	13.79	15.00	16.23	14.28
05/17/91	17.86	20.48	17.46	28.40	20.93	22.61	16.69	17.10
06/04/91	20.73	21.29	18.84	25.90	17.80	20.72	21.30	21.46
06/12/91	35.57	33.85	27.64	47.27	27.96	25.96	23.14	26.20
07/10/91	18.70	16.05	17.46	25.70	18.17	17.87	15.17	12.17
07/24/91	22.86	16.64	17.01	23.82	18.46	23.32	15.47	16.29
08/11/91	32.68	17.14	18.68	33.35	20.59	23.80	15.10	14.56
08/21/91	24.52	16.10	17.24	19.14	25.82	17.79	14.74	14.58

APPENDIX F
SUSPENDED SOLIDS

Appendix F. Suspended Solids

Date	RAS		RAS		RAS		RAS		RAS		RAS		RAS		RAS	
	2		3		4		5		6		7		8		9	
	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS
01/13/91	6.9	5.8	6.2	5.1	4.7	3.3	7.2	5.6	21.9	19.1	7.7	6.4	2.8	2.4	0.9	0.9
01/30/91	22.1	15.6	8.1	3.5	4.5	3.6	35.5	23.7	9.0	8.0	14.4	8.3	3.6	3.6	2.5	2.5
02/15/91	15.8	12.0	12.6	9.4	1.8	1.0	6.8	6.6	17.2	8.2	8.4	6.2	5.4	3.5	2.0	0.8
03/06/91	22.7	16.4	15.6	10.3	5.4	3.2	15.6	10.2	8.3	6.1	10.4	7.2	8.8	6.6	10.7	10.7
03/24/91	20.8	16.8	9.2	6.4	6.8	5.6	27.6	22.8	18.2	16.4	13.2	9.6	10.0	6.4	5.6	2.0
04/19/91	19.2	15.6	7.6	7.2	4.2	3.8	14.6	12.4	8.1	6.6	12.0	8.8	5.8	5.0	5.8	4.0
05/17/91	13.6	10.9	12.2	10.6	7.4	5.8	25.1	20.9	8.4	5.4	8.6	6.6	6.1	3.5	3.0	2.0
06/04/91	18.5	16.7	5.1	5.1	11.1	10.0	10.1	9.0	16.9	13.5	8.0	6.8	4.9	4.4	4.3	3.9
06/12/91	16.9	14.3	4.1	12.3	16.1	13.9	8.9	8.2	11.2	10.1	10.6	8.8	10.0	6.9	9.8	8.5
06/17/91	12.8	8.1	10.5	8.7	14.6	10.6	12.6	11.1	9.8	7.8	8.2	6.0	4.2	2.4	8.2	5.2
07/10/91	12.8	10.8	7.6	6.4	7.2	6.2	21.6	18.6	11.6	9.0	16.2	13.0	28.8	18.2	10.2	9.2
07/24/91	25.0	19.0	25.4	19.1	15.0	13.4	18.4	15.2	7.6	7.6	49.2	41.4	12.7	7.9	10.9	7.0
08/11/91	16.0	12.0	11.1	9.8	14.3	12.3	100.0	56.2	11.6	10.2	31.2	17.3	13.3	12.0	11.9	9.8
08/21/91	88.1	41.7	13.8	11.7	9.3	8.4	20.8	16.4	12.4	10.2	15.2	11.8	13.3	11.0	12.2	9.6

APPENDIX G
DOC MOLECULAR WEIGHT DISTRIBUTION DATA

Appendix G. DOC Molecular weight data.

	RAS 2	RAS 6	RAS 9
	High Density	Medium Density	Low Desnity
	DOC mg/L	DOC mg/L	DOC mg/L
Raw Water	20.12	14.74	13.89
Passed thru YM30	14.36	10.83	10.08
Passed thru YM10	12.38	9.66	7.77
Passed thru YM3	9.9	6.59	6.63
Passed thru YM1	4.15	4.16	4.14

APPENDIX H
DIURNAL CYCLE ANALYSIS DATA

Appendix H1.High density system 24 hour data.

RAS 2						TAN (mg/L)			
TIME	TEMP	pH	NO2	ALK	DO				
of day	celcius		mg/L	as CaCO3 mg/L	mg/L	TANK	RBC Stage 1	RBC Stage 2	RBC Stage 3
9am	24.3	6.9	0.350	75	7.9	1.99	1.7	1.62	1.63
11am	24.4	6.9			5.1	2.28	1.84	1.72	1.68
1pm	24.5	6.8	0.540	76	4.9	2.63	2.13	1.94	1.86
3pm	24.8	6.8	0.550		5.2	2.51	0.46	1.28	1.44
5pm	24.5	6.9	0.500	118	5.4	2.21	1.67	1.54	1.52
7pm	24.7	6.9	0.434	110	4.6	2.12	1.60	1.45	1.41
9pm		6.9	0.514	103	5.1	2.26	1.72	1.61	1.54
11pm	25.0	6.9	0.512	96	6.0	2.24	1.72	1.53	1.54
1am	25.1	6.9	0.496	89	6.2	2.05	1.68	1.60	1.61
3am	25.2	6.9	0.436	81	7.3	2.08	1.62	1.54	1.51
5am	25.4	6.9	0.414	76	8.0	2.06	1.68	1.57	1.57
7am	25.5	6.9	0.356	71	8.5	1.90	1.63	1.46	1.56
9am	25.4	6.8	0.414	70	6.9	1.91	1.63	1.59	1.53

Appendix H2.Medium density system 24 hour data.

TANK 6						TAN (mg/L)				
TIME	TEMP	pH	NO2	ALK	DO					
OF DAY	CELSIUS		mg/L	as CaCO3 mg/L	mg/L	TANK	SUMP	Stage 1	Stage 2	Stage 3
9	24.1	7.1	0.330	107	9.4	1.16	1.12	1.03	0.98	0.95
11	24.2	7.1			7.4	1.27	1.15	1.04	0.93	0.86
1	24.3	7.0	0.500	99	7.8	1.45	1.36	1.19	1.01	1.01
3	24.1	7.2	0.430		8.1	1.35	1.24	1.13	1.04	0.96
5	24.2	7.1	0.440	143	7.6	1.26	1.16	0.93	0.96	0.88
7	24.4	7.0	0.380	134	7.1	1.26	1.13	0.99	0.86	0.77
9		7.1	0.450	131	8.2	1.33	1.22	1.07	0.98	0.89
11	24.7	7.2	0.444	125	9.0	1.28	1.15	0.94	0.89	0.81
1	24.9	7.2	0.430	120	9.4	1.13	1.07	0.92	0.89	0.80
3	25.0	7.2	0.350	113	9.7	1.10	1.02	0.90	0.82	0.78
5	25.1	7.2	0.318	110	10.0	1.09	0.99	0.91	0.83	0.79
7	25.2	7.2	0.284	107	10.1	1.04	1.04	0.88	0.79	0.74
9	25.2	7.1	0.356	110	8.8	1.01	0.99	0.89	0.86	0.81

Appendix H3.Low density system 24 hour data.

RAS 9						TAN (mg/L)			
TIME	TEMP	pH	NO2	ALK	DO				
of day	celcius		mg/L	as CaCO3 mg/L	mg/L	TANK	RBC Stage 1	RBC Stage 2	RBC Stage 3
9am	23.6	7.2	0.140	61	10.7	0.76	0.73	0.63	0.64
11am	23.7	7.3			9.4	0.80	0.72	0.65	0.62
1pm	23.8	7.1	0.210	58	9.7	1.08	0.91	0.76	0.73
3pm	23.4	7.3	0.200		9.3	0.99	0.90	0.71	0.64
5pm	23.7	7.4	0.200	113	9.0	0.86	0.76	0.67	0.62
7pm	23.8	7.3	0.136	110	9.3	0.80	0.66	0.58	0.52
9pm		7.4	0.134	105	9.5	0.76	0.69	0.62	0.60
11pm	24.1	7.3	0.120	101	10.0	0.75	0.66	0.61	0.56
1am	24.3	7.4	0.111	100	9.9	0.73	0.65	0.59	0.55
3am	24.4	7.4	0.100	97	10.0	0.73	0.65	0.61	0.55
5am	24.6	7.4	0.095	95	10.3	0.67	0.61	0.50	0.53
7am	24.6	7.4	0.091	93	10.6	0.71	0.65	0.56	0.57
9am	24.6	7.4	0.158	91	10.3	0.55	0.52	0.47	0.44

APPENDIX I
MICROTOX DATA

MICROTOX DATA SUMMARY

RAS #	Day into Trial	EC10	EC10	EC20	EC20	Max. Light Loss
		15 min.	30 min.	15 min.	30 min.	Observed %
2	7					4
2	34		43.0			14
2	46		48.0			12
2	81	26.0	21.0	40.0	30.0	29
2	100	33.0	22.5	22.5	36.0	41
2	211					0
3	7					0
3	34					1
3	211					5
4	7					2
4	34					1
4	101					0
4	180					4
4	211					9
5	7		47			29
5	35	21.0	15.0	50.0	39.0	39
5	101					0
5	211					0
6	5					0
6	45	38.0	28.0		45	23
6	212					0
7	5					0
7	45	32.0	23.0		37.0	29
7	101	28.0	15.0		28.0	28
7	180					4
8	5		31.0			38
8	46					5
8	46					0
8	110		42.0			12

RAS #	Day into Trial	EC10	EC10	EC20	EC20	Max. Light Loss
		15 min.	30 min.	15 min.	30 min.	Observed %
8	180		30.0			13
8	212					0
9	5		15.0			23
9	46	24.0	24.0	41.0	41.0	25
9	110					8
9	212					6

APPENDIX J
SUSPENDED SOLID PARTICLE DISTRIBUTION
AND
CLARIFIER PERFORMANCE DATA

Appendix J.Solids Clarifier performance data.

Date	RAS	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
	No.	Retained	Retained	Retained	Retained	Retained	Retained	Retained	Retained	TSS	TSS
		By 105um	By 105um	By 70um	By 70um	By 30um	By 30um	By 1.5um	By 1.5um		
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
08/18	5	5.54	0.24	1.21	0.71	1.69	0.71	33.25	19.90	41.69	21.56
08/18	7	2.20	0.23	0.49	0.00	0.46	0.24	9.02	8.24	12.17	8.71
08/18	9	1.05	1.05	0.21	0.21	0.85	0.42	8.63	7.40	10.74	9.08
09/18	5	6.85	2.75	1.98	1.18	3.92	2.34	23.71	18.82	35.60	26.60
10/16	5	15.24	1.16	4.29	0.29	2.86	0.29	8.57	2.61	30.96	4.35
08/21	2									88.90	30.67
08/21	4									12.75	6.09
08/21	9									15.25	10.35
09/11	2									9.76	2.52
09/18	7									23.60	6.24
10/02	5									15.00	8.68
10/09	5									15.33	4.17
09/25	5									27.31	8.13

APPENDIX K
SYSTEM EFFLUENT DATA

Appendix K. System effluent data.

Date	RAS	TSS	VSS	FSS	COD	Date	RAS	CBOD5	Date	RAS	TKN
1991	No.	mg/L	mg/L	mg/L	mg/L	1991	No.	mg/l	1991	No.	mg/L
03/24	2	200.0	132.0	68.0	272.0	03/15	7	75.1	03/25	3	43.4
03/29	2	152.2	118.9	33.3	224.0	03/23	3	78.6	03/25	5	42.0
03/30	2	166.0	133.0	33.0	216.0	03/23	5	105.1	03/25	6	11.8
03/24	3	280.0	200.0	80.0	304.0	03/23	6	51.7	03/29	2	13.3
05/29	4	660.0	456.0	204.0	448.0	03/23	2	78.4	03/29	5	18.9
03/24	5	380.0	285.0	95.0	414.0	03/29	5	184.7	03/29	7	20.3
03/29	5	345.0	272.5	72.5	256.0	03/29	6	117.8	03/29	9	14.7
03/30	5	360.0	300.0	60.0	432.0	03/29	7	135.6	03/30	2	14.4
05/21	5	245.7	181.4	64.3	279.5	03/29	9	119.9	03/30	5	23.8
05/29	5	472.0	366.0	106.0	464.0	03/29	2	115.3	03/30	6	20.1
05/30	5	510.0	436.0	74.0	528.0	03/30	2	154.0	03/30	8	12.6
08/12	5	909.0	628.0	281.0	502.3	03/30	3	143.7	05/29	5	31.9
03/24	6	165.0	85.0	80.0	180.0	03/30	5	187.2	05/30	5	29.7
03/30	6	276.0	201.3	74.7	312.0	03/30	8	127.0	05/30	7	17.9
05/29	6	414.0	312.0	102.0	360.0	05/21	5	85.0	05/30	9	23.5
03/29	7	353.3	255.0	98.3	320.0	05/21	7	89.0	08/12	5	41.3
05/21	7	371.0	314.3	56.7	320.0	05/29	7	62.3	08/12	7	36.8
05/29	7	317.3	241.3	76.0	224.0	05/29	6	102.8			
05/30	7	254.7	206.7	48.0	256.0	05/29	5	119.8			
08/12	7	716.7	527.8	188.9	379.0	05/29	4	122.2			
03/30	8	235.0	186.3	48.7	164.0	05/30	5	147.9			
03/29	9	251.3	172.5	78.8	200.0	05/30	7	88.6			
05/30	9	393.3	289.3	104.0	368.0	05/30	9	98.2			
08/12	9	476.7	357.5	119.2	226.0	08/12	5	184.2			
						08/12	7	165.0			
						08/12	9	126.6			
						08/18	4	202.5			
						08/18	5	264.3			
						08/18	7	165.9			

Appendix K. Continued

Date	RAS	Total	Dissolved
	No.	Phosphate	Phosphate
1991		mg/L	mg/L
08/19	4	78.3	22.6
08/19	5	105.0	25.0
08/19	7	69.0	21.9
08/13	5	96.8	36.8
08/13	7	94.5	25.0
08/13	9	64.5	20.2

APPENDIX L
LITHIUM TRACER STUDY RAW DATA

Appendix L. Lithium tracer data.

Time	RBC	RBC	RBC
(min	Stage 1	Stage 2	Stage 3
	Lithium (mg/L	Lithium (mg/L	Lithium (mg/L
0	11.93	0.09	0.07
1	5.95	3.53	0.35
2	4.23	4.10	1.27
3	2.58	3.51	2.00
4	1.92	2.90	2.58
5	1.31	2.37	2.65
6	0.91	2.01	2.55
7	0.65	1.54	2.37
8	0.49	1.06	2.08
9	0.40	1.07	1.80
10	0.37	0.74	1.55
11	0.34	0.65	1.35
12	0.36	0.55	1.13
13	0.38	0.46	0.95
14	0.41	0.46	0.82
15	0.45	0.45	0.69
16	0.47	0.47	0.63
17	0.50	0.48	0.60
18	0.52	0.50	0.55
19	0.54	0.51	0.53
20	0.56	0.54	0.53
22	0.59	0.58	0.53
24	0.61	0.60	0.55
26	0.61	0.61	0.57
28	0.61	0.61	0.59
30	0.61	0.62	0.61

APPENDIX M
ROTATING BIOLOGICAL CONTACTOR ORGANICS DATA

Appendix M. RBC organic data.

RAS	Date	Tank	Stage 1	Stage 2	Stage 3	Tank	Stage1	Stage2	Stage3
No.		BOD	BOD	BOD	BOD	DOC	DOC	DOC	DOC
	1991	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
2	08/02	28.24	30.14	27.61	29.10		35.50	29.50	25.57
2	08/09	22.86	22.46	21.54	21.76	27.36	24.54	23.29	23.52
2	08/16	14.44	13.80	14.22	12.80	30.89	29.99	28.94	28.79
5	05/20	12.20	12.20	12.60	11.50		23.10	22.40	21.80
5	05/27		13.40	13.00	12.40		29.56	23.06	23.48
5	06/10	10.60	14.30	13.60	11.40		34.74	33.50	30.05
5	06/16	11.46	17.16	9.53	12.54		21.65	27.64	22.85
5	07/06					23.11	21.53	39.50	17.73
5	07/23	9.14	11.04	10.04	10.60	22.88	22.44	26.27	21.20
7	05/20						19.60	19.90	18.80
7	05/27		5.40	5.00	5.00		18.70	17.50	16.20
7	06/10	8.02	8.99	7.30	6.72		18.18	17.53	18.04
7	06/16	6.61	5.89	6.05	5.51		13.56	17.18	12.95
7	07/06	13.54	13.60	12.60	13.25	10.40	11.01	11.94	12.32
7	07/23	9.80	10.70	9.27	8.98	19.92	19.48	15.91	19.00
7	08/09	16.13	16.46	15.76	15.64	16.16	18.02	15.02	18.54
9	05/20	3.10	6.00	1.60	1.80		16.00	14.80	16.30
9	05/27		6.90	6.60	6.40		18.10	18.20	18.50
9	06/10	6.16	8.80	11.19	6.15		18.42	19.11	18.99
9	06/16	8.18	5.93	5.97	5.76		13.57	13.48	13.81
9	07/06	6.89	6.91	6.63	6.63	13.39	14.08	7.84	8.62
9	07/23	6.89	7.18	9.31	7.44	16.21	14.00	13.36	12.71
9	08/09	9.95	10.30	10.12	9.49	13.66	15.29	14.58	13.02
9	08/16	6.65	7.67	7.53	2.24	15.59	15.18	15.18	15.20

APPENDIX N
ROTATING BIOLOGICAL CONTACTOR NITRIFICATION DATA

Appendix N. RBC nitrification data.

RAS			TAN	TAN	TAN	TAN	NO-2	NO-2	NO-2	NO-2
No.	Date	Flow	Influent	Stage 1	Stage 2	Stage 3	Influent	Stage 1	Stage 2	Stage 3
		lit/min	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
2	08/02/91	282.0	3.60	2.50	2.40	2.10				
2	08/09/91	282.0	2.02	1.75	1.47	1.42	2.38	2.46	2.33	2.45
2	08/16/91	282.0	1.76	1.39	1.21	1.13				
2	08/20/91	282.0	1.70	1.46	1.31	1.30				
2	08/23/91	282.0	2.53	2.13	1.94	1.86				
2	09/03/91	282.0	1.19	0.93	0.79	0.79	0.49	0.43	0.34	0.33
2	09/04/91	282.0	0.91	0.80	0.68	0.65	0.31	0.30	0.23	0.21
2	09/05/91	282.0	1.32	1.05	0.91	0.83	0.45	0.40	0.34	0.35
3	08/20/91	279.0	0.93	0.73	0.69	0.70				
4	08/20/91	280.5	0.67	0.64	0.58	0.54				
5	05/27/91	252.0	1.89	1.77	1.60	1.56	0.64	0.65	0.63	0.63
5	06/10/91	252.0	2.44	2.30	1.94	1.79	1.14	1.19	1.21	1.24
5	06/16/91	252.0	1.26	1.19	1.02	0.93	0.54	0.60	0.51	0.54
5	07/06/91	252.0	1.70	1.59	1.49	1.36	0.46	0.52	0.50	0.43
5	07/23/91	252.0	1.61	1.59	1.35	1.22	0.40	0.40	0.42	0.33
5	08/20/91	252.0	2.19	2.12	1.97	1.91				
5	09/03/91	252.0	1.42	1.29	1.17	1.01	0.73	0.75	0.69	0.68
5	09/04/91	252.0	1.42	1.30	1.15	1.02	0.57	0.56	0.55	0.47
5	09/05/91	252.0	1.56	1.40	1.22	1.10	0.61	0.55	0.54	0.47
5	09/11/91	252.0	1.84	1.58	1.32	1.08	0.54	0.53	0.44	0.44
5	09/12/91	252.0	1.89	1.67	1.39	1.11	0.57	0.54	0.48	0.46
5	09/13/91	252.0	1.77	1.49	1.26	1.03	0.56	0.49	0.44	0.44
5	09/16/91	252.0	1.71	1.50	1.35	1.05	0.54	0.54	0.45	0.45
5	09/23/91	252.0	2.09	1.85	1.56	1.36	0.63	0.62	0.50	0.48
5	09/26/91	252.0	1.83	1.57	1.36	1.06	0.66	0.66	0.54	0.53
5	10/03/91	252.0	1.56	1.34	1.18	1.02	0.45	0.45	0.34	0.31
6	08/20/91	285.4	1.04	0.96	0.88	0.80				
6	08/23/91	285.4	1.36	1.19	1.01	1.01				

Appendix N. Continued.

RAS			TAN	TAN	TAN	TAN	NO-2	NO-2	NO-2	NO-2
No.	Date	Flow	Influent	Stage 1	Stage 2	Stage 3	Influent	Stage1	Stage2	Stage 3
		lit/min	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
7	01/22/92	3.8	1.19	0.55	0.61	0.40	0.96	0.14	0.08	0.05
7	01/24/92	3.8	0.84	0.28	0.18	0.13	0.46	0.07	0.03	0.04
7	01/25/92	3.8	0.89	0.39	0.31	0.31	0.40	0.05	0.03	0.02
7	01/26/92	3.8	1.06	0.46	0.38	0.36	0.65	0.09	0.04	0.04
7	10/30/91	22.7	1.55	1.09	0.91	0.80	0.82	0.66	0.55	0.48
7	11/04/91	22.7	1.72	1.15	0.94	0.82	1.15	0.85	0.68	0.58
7	11/05/91	22.7	1.36	1.10	0.99	0.97	0.55	0.42	0.56	0.38
7	11/14/91	22.7	1.55	1.01	0.85	0.73	1.07	0.75	0.64	0.53
7	11/15/91	22.7	1.47	1.00	0.80	0.70	1.07	0.80	0.61	0.50
7	11/27/91	22.7	0.99	0.63	0.54	0.43	0.33	0.22	0.32	0.15
7	12/02/91	22.7	1.14	0.66	0.58	0.53	0.68	0.44	0.30	0.24
7	12/03/91	22.7	0.91	0.58	0.51	0.51	0.56	0.31	0.26	0.19
7	12/04/91	22.7	1.06	0.64	0.54	0.54	0.58	0.34	0.22	0.18
7	12/05/91	22.7	0.95	0.69	0.63	0.58	0.50	0.29	0.21	0.16
7	12/06/91	22.7	1.45	0.83	0.68	0.67	0.74	0.41	0.30	0.24
7	12/09/91	43.5	1.01	0.82	0.73	0.69	0.43	0.34	0.28	0.26
7	12/10/91	43.5	1.03	0.84	0.82	0.79	0.37	0.44	0.27	0.22
7	12/11/91	43.5	0.94	0.76	0.69	0.65	0.40	0.34	0.28	0.23
7	12/12/91	43.5	0.82	0.64	0.58	0.56	0.27	0.22	0.18	0.15
7	12/13/91	43.5	1.07	0.85	0.77	0.71	0.51	0.64	0.34	0.29
7	12/16/91	43.5	0.86	0.62	0.54	0.52	0.26	0.23	0.19	0.15
7	12/17/91	43.5	0.63	0.57	0.51	0.47	0.19	0.16	0.12	0.11
7	12/19/91	43.5	0.83	0.71	0.67	0.69	0.24	0.20	0.18	0.13
7	12/27/91	43.5	1.16	0.95	0.87	0.85	0.40	0.32	0.25	0.21
7	01/02/92	43.5	1.11	0.98	0.83	0.89				
7	01/06/92	64.4	0.77	0.70	0.65	0.61	0.26	0.26	0.21	0.17
7	01/07/92	64.4	0.78	0.69	0.58	0.58				
7	01/08/92	64.4	0.76	0.65	0.58	0.58				
7	01/17/92	64.4	0.87	0.71	0.60	0.57	0.23	0.20	0.17	0.15
7	01/18/92	64.4	1.03	0.86	0.73	0.71	0.34	0.33	0.24	0.21
7	01/19/92	64.4	1.11	0.94	0.81	0.72	0.38	0.35	0.29	0.23

Appendix N. Continued.

RAS			TAN	TAN	TAN	TAN	NO-2	NO-2	NO-2	NO-2
No.	Date	Flow	Influent	Stage 1	Stage 2	Stage 3	Influent	Stage1	Stage2	Stage3
		lit/min	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
7	10/30/91	162.4	1.58	1.42	1.26	1.18	0.85	0.90	0.71	0.87
7	11/04/91	162.4	1.73	1.57	1.39	1.30	1.09	0.84	1.18	1.22
7	11/05/91	162.4	1.34	1.19	1.07	1.05	0.58	0.69	0.60	0.59
7	11/14/91	162.4	1.55	1.26	1.15	1.02	1.01	1.15	1.22	1.23
7	11/15/91	162.4	1.54	1.37	1.21	1.03	1.04	1.14	1.17	1.17
7	11/27/91	162.4	1.12	1.11	0.80	0.73	0.30			0.33
7	12/02/91	162.4	1.15	0.93	0.89	0.78	0.76	0.73	0.73	0.71
7	12/03/91	162.4	0.91	0.80	0.73	0.66	0.48	0.63	0.53	0.57
7	12/04/91	162.4	1.00	0.85	0.79	0.75	0.57	0.58	0.55	0.54
7	12/05/91	162.4	0.97	0.86	0.81	0.73	0.48	0.51	0.50	0.49
7	12/06/91	162.4	1.40	1.32	1.15	1.04	0.72	0.84	0.82	0.80
7	12/09/91	162.4	0.97	0.87	0.79	0.80	0.41	0.43	0.43	0.42
7	12/10/91	162.4	1.00	0.96	0.92	0.79	0.38	0.37	0.40	0.37
7	12/11/91	162.4	0.94	0.87	0.76	0.74	0.40	0.42	0.42	0.39
7	12/12/91	162.4	0.81	0.72	0.63	0.63	0.27	0.29	0.29	0.29
7	12/13/91	162.4	1.06	0.95	0.90	0.81	0.49	0.64	0.53	0.52
7	12/16/91	162.4	0.87	0.81	0.68	0.65	0.25	0.28	0.25	0.25
7	12/17/91	162.4	0.65	0.63	0.59	0.54	0.22	0.22	0.22	0.20
7	12/19/91	162.4	0.82	0.80	0.78	0.78	0.24	0.24	0.24	0.23
7	12/27/91	162.4	1.15	1.06	0.99	0.95	0.40	0.42	0.41	0.40
7	01/02/92	162.4	1.14	1.02	0.98	0.98				
7	05/20/91	289.2	0.70	0.56	0.55	0.51	0.36	0.26	0.28	0.26
7	05/27/91	289.2	1.20	1.14	1.04	0.97	0.15	0.13	0.14	0.12
7	06/10/91	289.2	1.48	1.28	1.19	1.01	0.27	0.28	0.28	0.25
7	06/16/91	289.2	0.90	0.71	0.64	0.56	0.20	0.28	0.17	0.14
7	07/06/91	289.2	1.03	0.96	0.88	0.87	0.30	0.22	0.20	0.17
7	07/23/91	289.2	1.38	1.27	1.13	1.09	0.25	0.24	0.20	0.18
7	08/09/91	289.2	1.57	1.34	1.13	1.09	0.54	0.57	0.55	0.50
7	08/20/91	289.2	0.96	0.91	0.85	0.86				
7	01/24/92	289.2	0.99	0.91	0.83	0.71	0.67	0.69	0.68	0.67

Appendix N. Continued.

RAS			TAN	TAN	TAN	TAN	NO-2	NO-2	NO-2	NO-2
No.	Date	Flow	Influent	Stage 1	Stage 2	Stage 3	Influent	Stage1	Stage2	Stage 3
		lit/min	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
8	08/20/91	274.8	0.59	0.54	0.53	0.45				
9	05/20/91	277.8	0.48	0.39	0.36	0.41	0.22	0.19	0.20	0.19
9	05/27/91	277.8	0.93	0.93	0.82	0.81	0.08	0.10	0.11	0.09
9	06/10/91	277.8	1.16	1.15	1.09	0.90	0.30	0.32	0.32	0.32
9	06/16/91	277.8	0.59	0.52	0.47	0.45	0.18	0.17	0.16	0.16
9	07/06/91	277.8	0.59	0.55	0.58	0.52	0.18	0.18	0.21	0.14
9	07/23/91	277.8	0.95	0.85	0.84	0.78	0.28	0.31	0.27	0.24
9	08/09/91	277.8	0.67	0.55	0.50	0.47	0.15	0.17	0.14	0.15
9	08/16/91	277.8	0.72	0.63	0.58	0.54				
9	08/20/91	277.8	0.66	0.66	0.58	0.56				
9	08/23/91	277.80	0.98	0.91	0.76	0.73				

VITA

Chris Easter was born on July 15, 1953 in Richmond, Virginia. He grew up in the small town of West Point, Va. enjoying the rivers, woods, baseball, football and friends. High-school found him back in Richmond where wrestling became a passion for four years. Chris completed two undergraduate degrees, B.A. in Biology (1975 University of Richmond) and B.S. Agricultural Engineering (1989 Virginia Tech). During most of the time between these two degrees He was a Naval Flight Officer, Tactical Coordinator and Mission Commander on P-3 Orion antisubmarine warfare aircraft in the U.S. Navy.

At the end of his Agricultural Engineering degree Chris was hired by the Fisheries and Wildlife Department at Virginia Tech as a staff research engineer to outfit a new aquaculture research facility. During this employment he completed his Masters degree in Environmental Engineering specializing in water and wastewater applications. At the end of his Masters studies in June of 1992 Chris accepted employment with an environmental engineering consulting firm (CH2M HILL) in the water and wastewater division as a staff engineer in Redding, California.