EVALUATING THE FATE OF MANURE NITROGEN IN CONFINED DAIRY WASTE OPERATIONS: A FULL-SCALE WASTE ANALYSIS AND START-UP PROTOCOL FOR AN ANAMMOX-BASED TREATMENT TECHNOLOGY APPLICABLE TO DAIRY WASTE MANAGEMENT

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ABSTRACT

In an effort to develop cost-effective technologies for the removal of ammonium nitrogen from dairy waste, a novel biological wastewater treatment process, utilizing anaerobic ammonium oxidation (anammox), referred to as Oxygen-Limited Autotrophic Nitrification and Denitrification (OLAND) was examined. Due to the potential use of OLAND-based systems in dairy manure management, a detailed water quality assessment of a modern dairy farm manure treatment-system was conducted. The Johnson Highland Dairy Farm, Glade Spring, Virginia, was selected for this assessment and a comprehensive analysis of the wastewater characteristics throughout the confined animal feeding operation was completed. The results suggest that ammonia concentrations in the anaerobic storage facility was high enough to justify use of treatment technologies that reduce ammonia loads in stored dairy waste. A lightly loaded Fixed Film Bioreactor (FFBR), in which the OLAND process was desired to occur, was then constructed in the laboratory and monitored over 51 days. Of particular interest was the time taken to achieve stable performance of this OLAND system. Furthermore, a protocol was developed to determine whether OLAND based metabolism was occurring. Ammonium nitrogen removal efficiency in the FFBR throughout the 51-day monitoring period was high, averaging approximately 95 % for the length of the study. From day 32 to 51, simultaneous removal of both NH_4^+ and NO_2^- with a low level of concomitant NO_3^- production was observed, a key indicator of possible anammox activity. Stoichiometric ratios calculated for the FFBR compared favorably with those already established for OLAND systems. The developed protocol, incorporating anaerobic and aerobic batch experiments, to verify the occurrence of OLAND based metabolism did not yield expected results and described poorly what was being observed in the FFBR. Volatilization of ammonia during the experimental test was suspected and should be controlled when the protocol is performed in the future.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

Acronym	Description
AAOB	Aerobic Ammonia Oxidizing Bacteria
AnAOB	Anaerobic Ammonia Oxidizing Bacteria
Anammox	Anaerobic Ammonium Oxidation
CAFO	Confined Animal Feeding Operation
CANON	Completely Autotrophic Nitrogen Removal Over Nitrite
DO	Dissolved Oxygen
FFBR	Fixed Film Bioreactor
HAO	Hydroxylamine Oxidoreductase
HH	Hydrazine Hydrolase
HRT	Hydraulic Retention Time
MLR	Mixed Liquor Recirculation
NiR	Nitrite Reductase
NOB	Nitrite-Oxidizing Bacteria
OLAND	Oxygen Limited Anaerobic Nitrification and Denitrification
SBRAS	Sequencing Batch Reactor Activated Sludge System
SCOD	Soluble Chemical Oxygen Demand
STKN	Soluble Total-Kjeldahl Nitrogen
TCOD	Total Chemical Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
TP	Total Phosphorus
TKN	Total Kjeldahl Nitrogen
VS	Volatile Solids
VSS	Volatile Suspended Solids

Chapter 1: Introduction

In recent years, agriculture has been recognized as a major source of environmental pollution. Regulatory oversight is now focusing on specific aspects of agricultural pollution. Currently, major concerns exist regarding negative environmental effects associated with nutrient losses and gaseous emissions from concentrated animal feeding operations (CAFOs), including large dairy farm operations. A major source of these nutrient losses and emissions is animal manure and one of the most significant pollutants in animal manure is nitrogen (N). Nitrogen contamination of ground and surface water and air pollution, caused by ammonia emissions, are of environmental concern.

Dairy farming is one of the major agricultural industries in Virginia, with Virginian dairy farms holding approximately 119,000 dairy cows (USDA–NASS, 2001). The Chesapeake Bay located, in part, on the eastern shore of Virginia, has been continually impinged upon through nutrient pollution. For example, agricultural operations are responsible for approximately 38 % of N loadings entering the Chesapeake Bay (Chesapeake Bay Program, 2002). As a result of increased awareness regarding agricultural pollution, more stringent environmental regulations now exist, which will have an impact on concentrated livestock production facilities both in Virginia and throughout the U.S. However, CAFOs in environmentally sensitive areas have few options for pollution control. If agricultural practices continue as they have in the past, the environment will suffer inevitable, detrimental impacts. Alternatively, a reduction in agricultural productivity will have a negative impact on a viable farm and rural economy and the supply of domestic food.

In order to address this problem, advanced wastewater treatment options are being considered as part of the solution to nutrient pollution by CAFOs. Advanced N removal systems have been in operation for many years. Traditional aerobic/anaerobic N removal wastewater treatment technologies are large and may require several reactors for standard operation. With the cost of these systems being high, both in capital and current expenditures, it is unlikely that large dairy facilities will be able to afford such treatment options for the removal of N. Therefore a need exists for the development of more cost effective advanced-wastewater-treatment options for the removal of N from dairy manures.

Recently, novel approaches have been developed for the removal of N from liquid waste streams involving oxidation of ammonia to N_2 gas via completely autotrophic processes. These new approaches combine aerobic autotrophic ammonia oxidizers with anaerobic ammonium oxidizers that are capable of autotrophic anaerobic ammonia oxidation to N_2 using NO_2^- as the electron acceptor, in the absence of molecular oxygen. One term used to describe this system, and that used in this study, is Oxygen-Limited Autotrophic Nitrification and Denitrification (OLAND). This system has been developed as a single fixed film bioreactor.

This system has several advantages over conventional N removal systems including a reduced O_2 requirement, no chemical oxygen demand requirement and only one reactor basin is required (in contrast to 2 basins for conventional N removal or suspended growth anammox technologies (van Dongen et al., 2001)). The OLAND system requires a plausible level of management, is cost effective and easily retrofitted to existing treatment facilities. Furthermore, anaerobically stabilized animal manure on dairy farms (stabilized through the employment of anaerobic lagoons, typically found on CAFOs) is low in bioavailable organic matter and high in reduced N, making the OLAND process an ideal choice for the treatment of anaerobically stabilized dairy wastes. Much research has been conducted highlighting the effectiveness of the OLAND process (Strous et al., 1997a; Pynaert et al., 2002; Fux et al., 2002); however, the OLAND system has not yet been applied to the treatment of dairy manures.

The research presented in the following chapters sets out to achieve the following objectives:

1. Conduct a detailed water quality assessment of key wastewater parameters for the manure treatment system of a modern Virginia dairy farm, in order to describe the characteristics of the wastewater that will ultimately be used by the OLAND system and establish a template for conducting further water quality assessments of dairy farm manure treatment systems,
2. Construct, maintain and monitor an OLAND Fixed Film Bioreactor (FFBR) during start-up, utilizing a synthetic wastewater, in an effort to achieve stable performance of a lightly loaded OLAND FFBR system and,
3. Develop and demonstrate a protocol that determines if OLAND based metabolism is occurring in an FFBR.

Chapter 2: Literature Review

2.1 Introduction

The following literature review is divided into three distinct sections: Dairy Manure, Traditional Nitrogen Removal and The Anammox Process. The first section, Dairy Manure, details the characteristics of dairy manure, current on farm manure treatment technologies and environmental impacts associated with N in dairy manure. In the design of any treatment system it is very important to understand the characteristics of the wastewater being treated. Furthermore, it is helpful to be aware of the current management of the waste and the reasons behind wishing to treat it. Section 2 provides an overview of N removal systems that are in common use and also details potential problems associated with these systems. In order to appreciate the value of novel N removal processes, one of which is examined in this research, it is necessary to fully understand some of the established mechanisms and technologies for N removal. Finally, section 3 provides a review of the novel anaerobic ammonia oxidation (Anammox) process, its applications in advanced wastewater treatment and advantages such applications possess over traditional N removal technologies.

2.2 Section 1: Dairy Manure

2.2.1 Dairy Manure Characteristics

The US Dairy Practices Council defines manure as the feces and urine from farm live stock (Barber, 1979). The Council points out that the characteristics of manure will depend heavily on the health, diet and development of the farm animal. A young dairy calf, for example, will produce manure with different characteristics than that from a lactating cow, due to dietary composition (Weeks, 1998).

The characteristics of manure must be known to maintain an environmentally and economically sound dairy farm operation (Morse et al., 1994). Based on a wide collection of published and unpublished data, the American Society of Agricultural Engineers (ASAE) have compiled a comprehensive standard, D384, detailing the production and characteristics of manure for many species of farm animal (ASAE, 2002). This ASAE standard is widely accepted and referenced within the US (Safley et al., 1984; Van Horn et al., 1994). It is noted in the standard that the presented values are subject to variation

on a site specific basis due to differences in animal diet, age, usage, productivity and management (ASAE, 2002).

Several studies have been completed regarding the variation of manure characteristics. Safley et al. (1984) conducted a study analyzing the characteristics of dairy manure from seven North Carolina dairy farms over a 12 month period. While many of the findings agreed with the data from D384, concentrations of nitrogen were found to be 30% greater than those reported in the Standard. The findings also highlight possible shortcomings in D384. These shortcomings, also alluded to in the standard itself (ASAE, 2002), include the general nature of the figures and the need for frequent updating due to changing management, feeding, and handling practices.

Another study of note (Morse et al., 1994) examined the production and characteristics of manure from lactating dairy cows in Florida. Among other findings, this project served as a check for the ASAE D384 standard. The results generated from the Florida farms compared well with the Standard (Morse et al., 1994).

2.2.2 Current Dairy Manure Management Technologies

Current practice for agricultural waste management, particularly dairy manures, involves a system of collection, separation and storage for a period of time prior to land spreading (L'Herrnite et al., 1992). Many large dairies have insufficient acreage for recycling nutrients (Van Horn et al., 1994). In an effort to reduce nutrient loads in dairy manures, several technologies are employed. One of the more prominent technologies is that of solids separation. Solids separation is typically found in dairies where flushing water is used to clean animal pens. Flushing, while simple and clean, generates larger volumes of slurry to be managed. Separation of coarse solids from flushed manure removes large particles, reduces the organic loading on anaerobic and aerobic lagoons fed with flush water and captures fibrous by-products that can be used as agricultural resources for other farming operations (Van Horn et al., 1994).

Stationary screening (the most common type of screening) of manure solids removes approximately 21 % of organic solids and 16 % of total solids (Auvermann and Sweeten, 1992). Prior to screening, it is now general practice to use sedimentation basins of circular or rectangular cross section (Vidak and Roberts, 1991). Several studies have been conducted examining the efficiency of such basins (Moore et al., 1975; Voermans et

al., 1990; Montoya, 1992). With a hydraulic retention time (HRT) of 3 hours, it has been found that over 60 % of total solids from dairy flush waters can be removed within the first 10 minutes of settling (Moore et al., 1975). The use of flocculating agents, such as agricultural lime ($CaCO_3$), has been found to remove approximately 90 % of total solids during settling (Montoya, 1992).

Following sedimentation and screening, processed flush waters commonly undergo some form of anaerobic treatment. Popular treatment systems include anaerobic storage systems and anaerobic lagoons. Anaerobic lagoons (characterized by a hard crust on the surface of the wastewater in the lagoon) represent the most widely employed method of anaerobically processing dairy manure (Van Horn et al., 1994). Such systems can achieve chemical oxygen demand, total solids, volatile solids and total N reductions of 75, 48, 46 and 69 %, respectively (Hill et al., 1990). However, these removal efficiencies are not always achieved. Many dairy lagoon systems are mismanaged (Hill et al., 1990). Anaerobic storage facilities differ from anaerobic lagoons in that the liquid surface of an anaerobic storage facility is not covered with a crust and can be directly exposed to the atmosphere if not covered. Aerobic treatment can also be employed, but is used to a lesser degree due to the high cost of supplying aeration.

2.2.3 Nitrogen in Dairy Waste and Associated Environmental Impacts

Four forms of nitrogen can be present in dairy manures: organic, ammonia, nitrite and nitrate nitrogen (Figure 2.1, The Nitrogen Cycle). All nitrogen that is present in organic material is defined as organic nitrogen. Decomposition of organic matter results in the generation of ammonia nitrogen, through a process called ammonification. Fresh manure contains a relatively high mass of organic nitrogen and a relatively low mass of ammonia nitrogen. Bacterial oxidation of ammonia nitrogen generates nitrite nitrogen. Fresh manure possesses very little nitrite nitrogen.

Intensive agricultural practices are a significant source of nutrient (including nitrogen) pollution. Furthermore, with regard to point and non-point sources for total N export from watersheds within the United States, animal agriculture is responsible for 8.1 to 21 % of the total N exported (Smith and Alexander, 2000). It has been established that the major inputs of N in agriculture are legume fixation and, imported feeds and fertilizers (Kohn et al., 1997). N losses and emissions can occur through production of

agricultural commodities, leaching, runoff, volatilization and biological conversion of nitrogen to N_2 (Kohn et al., 1997). These nitrogen outputs impinge upon air and water quality (NRC, 2003).

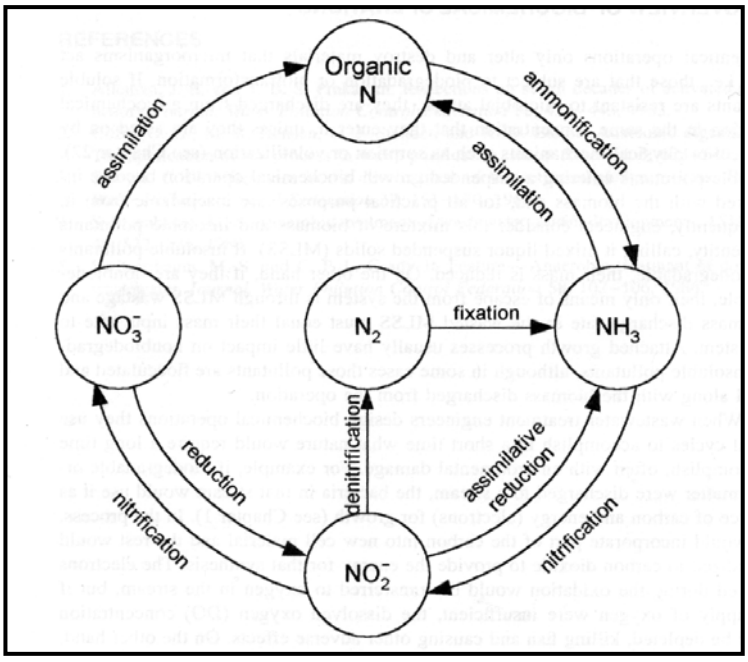

Figure 2.1: The nitrogen cycle (Grady et al., 1999)

Many rural communities depend on untreated ground water as their primary source of drinking water. Elevated levels of nitrogen, specifically nitrate nitrogen, in this untreated water can result in serious illness or death, particularly among infants (blue baby syndrome). Secondly, in U.S. waters, eutrophication, caused in part by nitrogen enrichment, continues to be a very serious problem (Bricker at al., 1999; Balls et al., 1995). Eutrophication can result in fish kills, loss of submerged aquatic life and occurrences of nuisance and toxic algal blooms (green scum on the surface of waters, due to large microorganism populations). Significant regional bodies of water, each representing vast economic, ecological, and recreational resources, are known to be impaired by eutrophication. In the northeastern U.S., these water bodies include Narragansett Bay, Long Island Sound, and the Chesapeake Bay (Chesapeake Bay Program, 2002). Agricultural operations are responsible for approximately 38 % of N loadings entering the Chesapeake Bay (Chesapeake Bay Program, 2002).

Of further concern, and interest to this research, is the volatilization of ammonia from dairy manures. In aqueous solutions NH_3, a gas, reacts with acid to form NH_4^+,

which is not gaseous. The pK_a of the NH_4^+/NH_3 couple is 9.3. In an acidic environment rapid and balanced conversion of NH_3 to NH_4^+ occurs and little NH_3 is lost to the atmosphere. However, most animal manures, lagoons and feedlot surfaces have a pH greater than 7, making H^+ scarce and thus promoting rapid losses of NH_3 to the atmosphere (Van Horn et al., 1994). Moreover, through microbial stabilization of excreted manures, gases, including ammonia, are generated. In the U.S., it is estimated that 71 % of ammonia emissions are generated by animal agriculture (EPA, 1998) with 50 % of global ammonia emissions originating from animal agriculture (Bouwman and Vanderhoek, 1997). Agricultural atmospheric ammonia is a major issue in Western Europe (Zebarth et al, 1999). European atmospheric ammonia concentrations have contributed to acid rain and the destruction of forests (Apsimon and Kruse-Plass, 1991). Moreover, ammonia volatilization is also an occupational hazard for farm workers (OSHA, 1998) and can have a deleterious effect on livestock health and impair livestock productivity (Donham, 1991). Atmospheric ammonia has been known to cause blindness in chicks and turkey poults and can be toxic to cells (Elliot et al., 1990). Ammonia volatilization also increases atmospheric N fallout, contributing to eutrophication. Accumulating ammonia gases in the atmosphere can react with acid gases to form ammonium salts, returning to soils through precipitation and subsequently releasing acid when oxidized (Likens et al., 1996).

All of the above releases are now under international scrutiny through the development of national and international reduction-protocols. Increasingly, policy makers are seeking information on the causes, mechanisms and solutions to these agricultural emissions and discharges (Jarvis and Ledgard, 2002). Such attention will necessitate large livestock operations to give serious consideration to the employment of advanced wastewater treatment operations, not just sedimentation, separation and anaerobic/aerobic lagoons. In order to maintain productivity, treatment options must be economically and financially sound.

Long-established treatment processes exist for the removal of nitrogen from liquid waste streams. However, significant advances are being made in the development of novel biological and potentially cost-effective treatment approaches for agricultural wastes, particularly in the field of ammonia-nitrogen removal (Dong and Tollner, 2003). These developments, although at their beginnings, warrant considerable attention.

Research projects dealing with such developments must examine both the current state of relevant manure-management strategies within the farming industry of interest, assess the engineering characteristics of these novel technologies and the feasibility of their industrial integration.

2.3 Section 2: Traditional Nitrogen Removal

2.3.1 Nitrogen

The element nitrogen comprises several oxidation states ranging from -3 to +5. At least one unique inorganic molecule exists at each oxidation state, brought about via the combination of nitrogen with atoms of oxygen, hydrogen and nitrogen. It is possible for each oxidation state to be present in aqueous environments, due to the fact that the oxidation of N is controlled primarily by kinetic rates of conversion and not thermodynamic equilibrium (Williams and Frausto da Silva, 1996).

Nitrogen gas represents 79% of the earth's atmosphere and is of vital importance to life. Nitrogen is constantly used and replenished in a cyclical relationship known as the nitrogen cycle. From a microbiological perspective, the nitrogen cycle consists of five catabolic reactions (nitritification, nitratification, denitrification, dissimilatory nitrate reduction and anaerobic ammonium oxidation), three anabolic reactions (ammonium uptake, assimilatory nitrate reduction and nitrogen fixation) and ammonification (Brock et al., 1997). The anaerobic ammonium oxidation reaction is a recent addition to the cycle.

2.3.2 Biological Nitrogen Removal

For the elimination of nitrogen in domestic wastewater streams, the biological method of choice is the use of a nitrification/denitrification process (Fux, 2004). Due to cost limitations, many plants only employ nitrification, which is a nitrogen conversion but not a nitrogen removal technology. Nitrification, a two step process, involves the aerobic oxidation of ammonia by nitrifying bacteria where ammonium is oxidized by oxygen, via the intermediate nitrite, to nitrate (Equations 2.1 and 2.2). Examining the stoichiometry of Equation 2.1, it can be seen that acid is generated (alkalinity is consumed). The reaction detailed by Equation 2.1 yields 1.3 to 2.6 grams of biomass per

mole of oxidized ammonium, with the reaction detailed by Equation 2.2 yielding 0.9 to 1.8 grams of biomass per mole of oxidized nitrite.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad \textbf{(2.1)}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad \textbf{(2.2)}$$

Denitrification is the process whereby nitrate is reduced, under anaerobic conditions, to elemental nitrogen (N_2) via the oxidation of an organic electron donor, such as acetate (Equation 2.3). From examining the stoichiometry of Equation 2.3, it can be seen that alkalinity is generated. The reaction described by Equation 2.3 will yield 27 grams of biomass per mole of acetate consumed.

$$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \quad \textbf{(2.3)}$$

Conventional N removal typically results in a demand of 3.7 g of alkalinity per gram of N removed.

2.3.3 Microbiology of Nitrification and Denitrification

2.3.3.1 Denitrification

Nitrogen oxides, such as nitrate or nitrite, are used as terminal electron acceptors under anaerobic, microaerobic and, occasionally, aerobic conditions, with a wide variety of bacteria capable of mediating denitrification (Zumft, 1997; Metcalf and Eddy, 2003). Biological denitrification is a vital component of the global nitrogen cycle. It was initially believed that only certain bacteria mediated denitrification. Pseudomonads represent the largest group of denitrifying bacteria within a single genus (Metcalf and Eddy, 2003). However, it has been shown that halophilic and hyperthermophilic archaea and the mitochondria of fungi can also be responsible for the process (Zumft, 1997). Environmental factors affecting the rate and activity of denitrification include pH, aeration, temperature, nitrate and nitrite concentrations, and carbon availability (Bergsma et al., 2002).

2.3.3.2 Nitrification

Aerobic ammonia oxidation

As seen above, nitrification consists of two distinct steps, the first being aerobic ammonia oxidation which is mediated by autotrophic aerobic ammonia oxidizing bacteria

(AAOB) (Metcalf and Eddy, 2003). Ammonium monooxygenase and hyroxylamine oxidoreductase are the biological catalysts employed in the oxidation of ammonia to nitrite (Egli, 2000). Presently 25 species of ammonia-oxidizers have been discovered, although many more may exist (Koops and Pommerening-Roser, 2001). It has been observed that *Nitrosomonas* strains are the most important populations found in wastewater treatment systems with *N. europaea, N. oligotropha/urea* and *N. communis* frequently found (Gieseke et al., 2001).

Nitrite Oxidation

Nitrite oxidation is the second, and last, nitrification step where again, autotrophic bacteria mediate the process (Nitrite-Oxidizing Bacteria (NOB)). These are, however, distinctly different from the autotrophic bacteria involved in step 1 (Metcalf and Eddy, 2003). The pertinent enzyme, catalyzing the nitrite oxidation reaction, is the nitrite oxidoreductase (Egli, 2000). For most nitrite oxidizers, the only source of useful energy is generated from nitrite, although some *Nitrobacter* can utilize organic compounds for energy metabolism (Bock and Koops, 1992). According to Metcalf and Eddy (2003), *Nitrobacter* are the bacteria responsible for nitrite oxidation in wastewater. However, Daims et al. (2001) point out that it is *Nitrospira*-like bacteria and not *Nitrobacter* that are commonly found in most full-scale wastewater treatment plants and laboratory-scale reactors. The *Nitrospira*-like bacteria appear to prefer low nitrite and oxygen concentrations, wheras *Nitrobacter* thrive in the presence of high nitrite and oxygen concentrations (Schramm et al., 1996). Since treatment plants often suffer breakdowns in nitrification performance, it would be important to conduct further study as to the role of *Nitrospira*-like bacteria in modern biological sewage treatment (Daims et al., 2001).

2.3.4 Traditional Treatment Technologies for the Removal of Nitrogen

In order to place the novel nitrogen removal system for dairy manure studied during the course of this research in context, it is of benefit to examine the operation of current and popular biological nitrogen removal technologies. Examples of such popular technologies include the modified Ludzack-Ettinger (MLE) process and the four-stage Bardenpho process. Each process incorporates a range of different configurations but both comprise aerobic zones for nitrification, anoxic zones for denitrification and mixed

liquor recirculation (MLR) to transfer nitrate-N generated in the aerobic zone back to the initial anoxic zone (Grady et al., 1999). Sequencing batch reactor activated sludge systems (SBRAS) are also commonly used to achieve nitrification and denitrification. While many other traditional treatment technologies for the removal of nitrogen exist, further examination of MLE, four-stage Bardenpho and SBRAS processes, will serve the purpose of placing the novel nitrogen removal technologies discussed later in this chapter in perspective.

2.3.4.1 MLE Process

In order to use the readily-biodegradable substrate in wastewater as an electron donor and achieve partial denitrification, Ludzack and Ettinger (1962) separated a bioreactor into two compartments, one compartment anoxic and the other aerobic. Essentially, reduction of the nitrogen discharge could be achieved via secondary treatment (Ludzack and Ettinger, 1962). This process was later modified by Barnard whereby a stream of mixed liquor from the actively nitrifying aerobic zone is pumped back to the anoxic zone, supplying this latter zone with nitrate (Grady et al., 1999, Metcalf and Eddy, 2003). The process is represented schematically by Figure 2.2. The MLE process can easily meet a common effluent standard of less than 10 mg/L total nitrogen (Metcalf and Eddy, 2003).

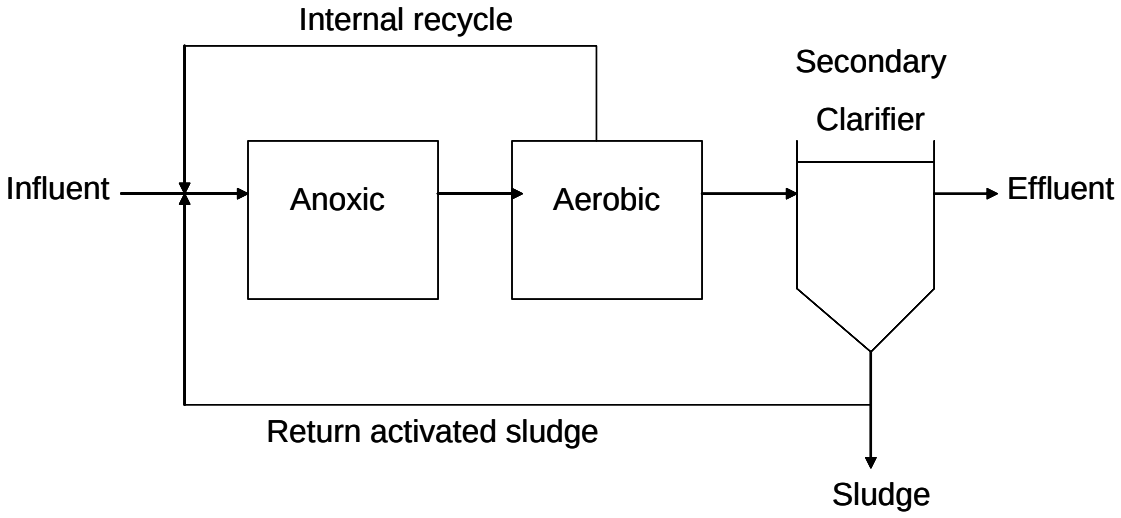

Figure 2.2: The modified Ludzach-Ettinger process

2.3.4.2 Bardenpho Process

A disadvantage of the MLE setup is that the effluent always contains appreciable quantities of nitrate-N due to nitrification occurring in the last bioreactor (Grady et al., 1999). To circumvent this problem a second anoxic and aerobic stage can be added after the final bioreactor. This is known as the Bardenpho process and is detailed in Figure 2.3. The system consists of four completely mixed basins in series, with the nitrates in the mixed liquor of the second basin serving as the electron acceptor for the first and third basin, which are both anoxic (Barnard, 1975). The purpose of the second anoxic and aerobic bioreactors is to allow further denitrification through biomass decay and the utilization of a slowly biodegradable substrate (Grady et al., 1999). At its beginnings, it was reported that the process was capable of removing greater than 90% of the influent N, without any chemical additions (Barnard et al., 1978).

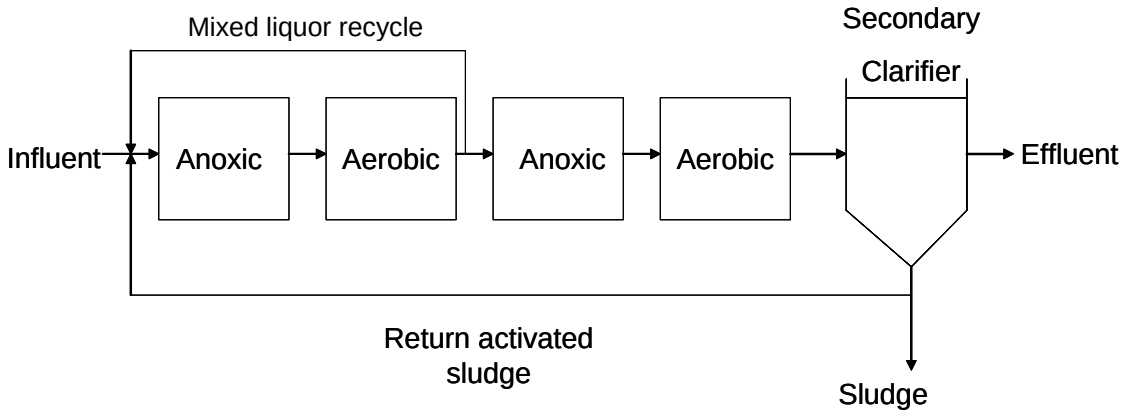

Figure 2.3: The Bardenpho process

2.3.4.3 SBRAS Configuration

SBRASs act in a similar manner to that of activated sludge processes and can be designed using similar procedures. There are, however, important differences between SBRASs and other activated sludge systems (Grady et al., 1999). Nitrate removal is accomplished by a number of steps, as shown in Figure 2.4.

Effluent NO_3^--N concentrations have been reported at less than 5 mg/L (Metcalf and Eddy, 2003). Furthermore, bench–scale studies using synthetic, high-strength waste streams have achieved a consistent total-nitrogen removal of 92% and greater, without

the addition of a supplemental carbon source (Alleman and Irvine, 1980). The use of sequencing batch reactors for the treatment of piggery effluent has been examined by Edgerton et al. (2000).

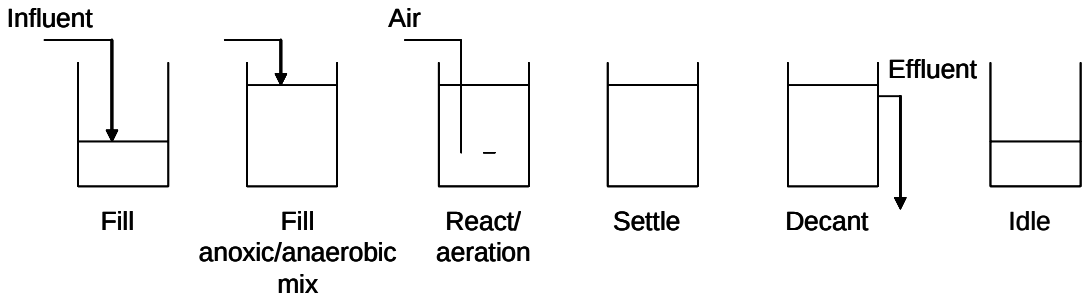

Figure 2.4: The SBRAS process

2.3.5 Problems Associated with Traditional Biological Nitrogen Removal Systems and their use for Dairy Manure Treatment

Nitrification can be affected by a number of environmental factors including pH, toxicity, metals (nickel, chromium and copper) and unionized ammonia (EPA, 1993; Metcalf and Eddy, 2003; Kelly et al., 2004). The following issues associated with traditional biological nitrogen removal are pertinent to this research.

Nitrification and denitrification, as alluded to earlier, are expensive processes. Recently, it has been estimated that a nitrifying activated-sludge plant serving a population of 100,000 would cost $13.3 million. A denitrifying activated-sludge plant, serving the same population, would cost $12.8 million (Copper et al., 1995). With the cost of these systems being high, both in capital and current expenditures, it is unlikely that dairy facilities will be able to afford such treatment options for the removal of nitrogen.

Traditional aerobic/anaerobic N removal wastewater treatment technologies are also large and may require several reactors for standard operation. However, any treatment system employed in a dairy farm setting should be easily retrofitted to existing treatment facilities, have a small footprint and require a plausible level of management. As such, traditional biological nutrient treatment facilities may not be suited to agricultural settings.

Furthermore, the long established approaches for nitrogen removal as described above require readily biodegradable organic matter and produce substantial biomass

solids, which can have additional cost implications. Traditional approaches become expensive when dealing with highly nitrogenous waste streams that have chemical oxygen demand (COD) to reduced nitrogen (total kjeldahl nitrogen, TKN) ratios less than 5 (Grady et al., 1999). The COD in anaerobically stabilized manure from dairy farm stabilization ponds is significantly decreased (van Horn et al., 1994), leading to lowered COD:TKN ratios that are problematic for nitrogen removal by conventional means. Clearly, a need exists for the development of more applicable and cost effective advanced-wastewater-treatment options for the removal of nitrogen from dairy manures. The use of anammox based systems for the treatment of dairy manures has a strong potential to address this need.

2.4 Section 3: Anaerobic Ammonium Oxidation (Anammox)

2.4.1 Overview of Anammox

Previously, it was believed that ammonia oxidation proceeded only under aerobic conditions. However, during the early 1990s, anaerobic ammonium oxidation was observed in a denitrifying fluidized bed reactor (Mulder et al., 1995). This discovery uncovered a new process in which ammonium is oxidized with nitrite serving as an electron acceptor under anaerobic conditions, producing dinitrogen gas (Mulder et al., 1995). The process was given the name Anammox, an acronym for anaerobic ammonium oxidation (Mulder et al., 1995). Confirmation of the biological nature of the anammox process was provided by a series of anaerobic batch culture experiments (van de Graff et al., 1995). From that study, it was concluded that the anaerobic oxidation of ammonium is a biological process, terminated by the production of N_2.

Employing the use of sequencing batch reactors (SBR), a comprehensive stoichiometric equation (Equation 2.4) detailing the anammox process was generated (Strous et al., 1998).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \textbf{(2.4)}$$

The microorganism mediating the anammox process has been identified as a new plactomycete and the archetype was named Candidatus *Brocadia anammoxidans* (Strous et al., 1999a). Several interesting physiological parameters and characteristics are associated with this archetype. Purified and unpurified cells were only active at cell

concentrations of 10^{10} to 10^{11} cells/ml or higher (Strous et al., 1999a). Furthermore, Candidatus *Brocadia anammoxidans* is the first autotrophic member of the order planctomycetalas, which has, to this point, only been represented by organotrophs. The maximum specific growth rate of the organism is 0.0027 hrs^{-1} (compared to a typical value of 0.034 hrs^{-1} for nitrifiers), with a doubling time of 11 days (compared to a matter of hours for nitrifiers and denitrifiers) (Strous et al., 1998). The maximum specific rate of ammonium consumption was found to be 45 ± 5 nmol $mg^{-1}_{protein}$ min^{-1} and biomass yield was calculated as 0.066 ± 0.01 mol C mol^{-1} NH_4-N (Strous et al., 1998). Clearly, anammox is a low yield process.

Another genus of anammox bacteria, Candidatus *Kuenenia stuttgatienis*, appears to be more prevalent than the previously discussed archetype (Schmid et al., 2000). During an investigation of the microbial community structure of a trickling filter biofilm with a high anaerobic ammonium oxidation activity, Candidatus *Kuenenia stuttgatienis* was found to dominate the fraction of biofilm bacteria (Schmid et al., 2000). This genus was also prominent in an enriched anammox culture from a rotating disk contactor close to Kölliken, Switzerland which was used to treat ammonium-rich leachate with low organic carbon content (Egli et al., 2001). Several full-scale industrial biofilm plants in Germany that were able to eliminate nitrogen without a carbon source, were also examined for the presence of anammox bacteria (Helmer-Madhok et al., 2002). Once again, the genus Candidatus *Kuenenia stuttgatienis* was found to dominate (Helmer-Madhok et al., 2002).

It has become apparent that anammox bacteria are more widespread than previously thought. Such bacteria contribute extensively to the nitrogen cycle within marine sediments via the production of N_2 (Thamdrup and Dalsgaard, 2002). Moreover, consortia of the anammox bacteria have been successfully selected and enriched from municipal treatment plant sludges in Sydney, Australia (Toh et al., 2002). However, to this point, isolation of anammox mediating bacteria has eluded the scientific community, although Candidatus *Brocadia anammoxidans* has been enriched up to 99.6% using density centrifugation (Strous et al., 1999a).

Several investigations studying the metabolic pathway governing the anammox process have been conducted (van de Graaf et al., 1997; Jetten et al., 2002). The main focus has been on the role played by the enzymes ammonia monooxygenase and

hydroxylamine oxidoreductase (HAO) (Jetten et al., 2002). In batch experiments with excess hydroxylamine and ammonium, an accumulation of hydrazine was observed, indicating its possible importance in the anammox process (Jetten et al., 2002). In *Nitrosomonas europaea*, the oxidation of hydrazine to dinitrogen gas is mediated by the HAO enzyme. High HAO activity has been observed in cell extracts of Candidatus *Brocadia anammoxidans* suggesting a similar use of the enzyme in the anammox process to that in *N. europaea* (Jetten et al,. 2002).

Through an investigation detailing the cellular organization of Candidatus *Brocadia anammoxidans,* it was found that the cells display a peripheral-ribosome free region, surrounding the perimeter of the cell, and an interior region bound by a single intracytoplasmic membrane (Lindsay et al., 2001). It is within this membrane bound compartment, also referred to as the anammoxosome that the highest concentrations of HAO are found (Jetten et al., 2002). It is suggested that HAO oxidizes hydrazine to dinitrogen gas and 4 electrons, which are used by nitrite reductase (NiR) to reduce nitrite to the level of hydroxylamine (Jetten et al. 2002). It is assumed that hydroxylamine and ammonium are converted to hydrazine by a putative hydrazine hydrolase (HH) (Jetten et al., 2002).

Strous et al. (1999b) have reported that anammox activity decreases with increasing nitrite concentration. Complete inhibition of anammox mediating bacteria was observed in the presence of more than 0.1g of nitrite nitrogen per liter (Strous et al., 1999b). Moreover, aerobic and microaerobic conditions inhibit the anammox process (Strous et al., 1997b). Further support of these inhibitions is provided by Egli et al. (2001). However, despite these sensitivities and rather slow growth rates of the responsible bacteria, the anammox process is of economic interest. Compared to conventional nitrification/denitrification, substantial reductions of operational costs can be expected. The aeration and carbon-source demand can be reduced by over 50 % and 100 %, respectively (Fux et al., 2002).

2.4.2 Benefits of Systems Utilizing the Anammox Process

As mentioned above, the anammox process has distinct advantages over traditional nitrification/denitrification processes, specifically with regard to oxygen and COD consumption. The partial nitritation/anammox process allows over 50% of the

oxygen to be saved and no organic source is needed (Fux et al., 2002). Via the classical nitrification/denitrification process, for every mole of NH_4^+ consumed, 40.4 g of $COD_{biomass}$ are produced, with 1.9 moles of O_2 consumed. In contrast, the partial nitritation /anammox process produces 6.1 g of $COD_{biomass}$ per mole of NH_4^+ consumed (with only 0.8 moles of O_2 being consumed) (Fux et al., 2002). As such, the biomass yield of Anammox is low; therefore, little sludge is generated (Fux et al., 2002). On this basis, distinct economic advantages are associated with the partial nitritation/anammox process. Moreover, anammox based systems are ideally suited to wastes that are low in bioavailable organic matter and high in reduced nitrogen, such as anaerobically-stabilized dairy manures. The anammox process has great potential to be employed effectively in the treatment of waste streams with low COD:TKN ratios.

Work by Fux (2003) examines the cost associated with a newly constructed partial nitritation/anammox process in comparison to a traditional nitrification/denitrification process serving the same population. It is noted that generally-valid cost analysis does not exist and site-specific factors must always be taken into account. Figures generated were based on a number of assumptions relating to reactor volume, energy and investment costs, maintenance requirements, chemical usage and, sludge disposal. With this said, the figures do provide a strong case for the significant economic benefits associated with the anammox process. It was determined that partial nitritation/anammox is always more economical than nitrification/denitrification even if nitrogen is the intermediate product and all the electron donors are obtained free of charge (Fux, 2003). The partial nitritation/anammox cost was estimated at 3.25 $\$/kgN_{eliminated}$ whereas the *cheapest* nitrification/denitrification process also serving the same population (100,000 population equivalents) was 3.97 $\$/kgN_{eliminated}$. The separate biological treatment of sludge liquors compared even more favorably to the cost of extending the activated sludge system of the main stream (10.4 $\$/kgN_{eliminated}$) (Fux, 2003).

2.4.3 Applications of Anammox

2.4.3.1 Partial nitritation and anaerobic ammonium oxidation in two separate reactors

Stable nitritation in a first reactor can be combined with anaerobic ammonium oxidation in a second reactor. Aerobic and anaerobic ammonium oxidation are not

operated in a single stage but can be controlled separately via two reactors in series. The best-known application is the Sharon/anammox process (van Dongen et al., 2001), shown in Figure 2.5. The Sharon process is a nitritation/denitritation process without sludge retention. Sharon is an acronym for Single Reactor System for High-activity Ammonia Removal over Nitrite and, was developed in the 1990s at Delft University of Technology.

The Sharon process employs a conventional CSTR with suspended biomass, in which nitrogen is removed by nitrification/denitrification via nitrite (Hellinga et al., 1998). In the Sharon/anammox configuration, it is commonly understood that, in this context, the term Sharon refers only to partial nitritation without the addition of any external carbon source ("Half-Sharon") (Fux et al., 2002).

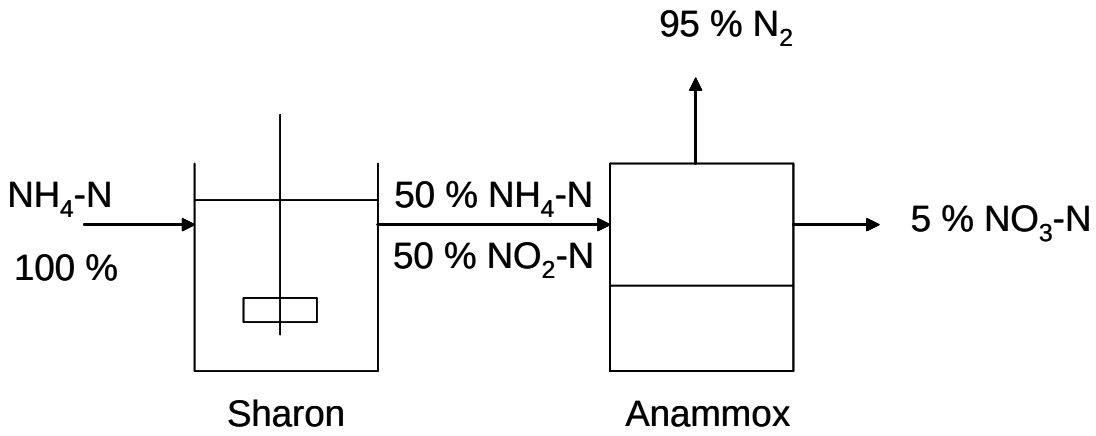

Figure 2.5: The combined Sharon/Anammox process for the removal of ammonium from sludge digestion effluent (adapted from Jetten et al. 2002)

Figure 2.6 represents a basic flow scheme of the Sharon-anammox concept, which has been partially implemented at a wastewater treatment plant in Rotterdam, the Netherlands (van Dongen et al., 2001). The aerobically digested sludge recycle water typically contains 15% of the total plant ammonia load with only 1% of the hydraulic load. The ammonia in the sludge liquor is removed by applying a partial oxidation of ammonium to nitrite via conventional AAOB where-after the nitrite is reduced to N_2 with ammonium as the electron donor via Anammox (van Dongen et al. 2001). Van Dongen et al. (2001) state that in using this process, the oxygen requirement for nitrogen removal is reduced by 60%, no COD is needed, the sludge production is significantly reduced and the net CO_2 emissions are greatly lowered, when compared to traditional biological N

removal systems. Jetten et al. (1997) have also identified the use of the Sharon/Anammox process as a key component in improving the sustainability of current and future wastewater treatment systems, due to the reduced cost of treatment.

In the context of applying such a system to the treatment of dairy manure, the Sharon/Anammox process, incorporating two separate reactors, certainly allows for nitrogen removal from wastes with a low COD:TKN ratio. However, the potentially large footprint associated with a two reactor set-up may not be advantageous in a CAFO.

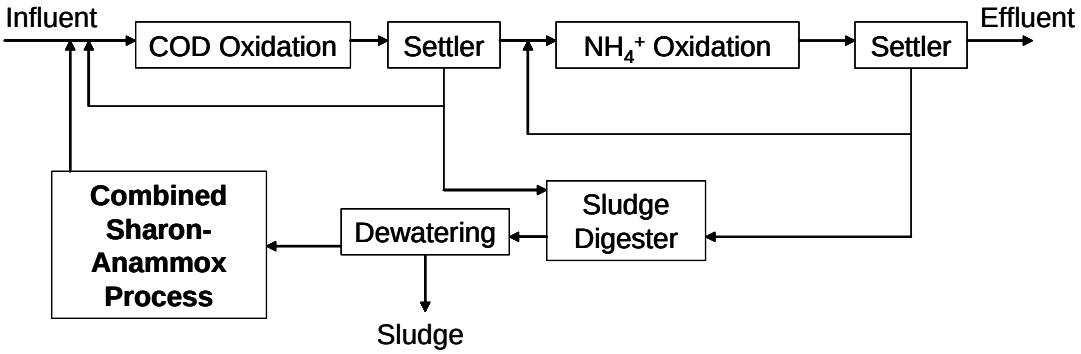

Figure 2.6: Schematic representation of the implemented combined Sharon/Anammox process in Rotterdam, Netherlands (adapted from Jetten et al. 2002)

2.4.3.2 The Canon System

Recently developed at Delft University of Technology, Netherlands, the completely autotrophic nitrogen removal over nitrite (Canon) process has been designed and developed as a single-reactor biological nitrogen removal process (Sliekers et al., 2002). The compact-biofilm Canon reactor is ideally suited for wastewater with small nitrogen loads (Hao et al., 2002) and therefore may not be ideally suited to treatment of dairy manures. The Canon system proceeds under oxygen limiting conditions with the aerobic ammonia oxidizers present being required to remove virtually all of the oxygen from the liquid (Sliekers et al., 2002). Results from investigations conducted by Sliekers et al. (2002) found ammonia was mainly converted to N_2 (85%) and the remainder (15%) was recovered as NO_3^-, with *nitrosomonas*-like bacteria partially converting ammonia to nitrite and anammox bacteria converting the mixture of ammonia and nitrite to dinitrogen gas and nitrate (Equation 2.4).

A model detailing biofilm reactions, kinetics and, general stoichiometry, developed by Hao et al. (2002), has been utilized in analyzing the sensitivities of the Canon process. It was determined that reduced oxygen levels (Hao et al., 2002) and increased ammonium loads (Third et al., 2001) can stress the nitrite oxidizers and lead to a reduction in nitrate production.

2.4.3.3 The OLAND System

The OLAND process is an oxygen-limited autotrophic nitrification/denitrification system for the removal of nitrogen from nitrogen-rich wastewater in one step (i.e. a single reactor) (Kuai et al., 1998). Originally, it was believed that normal nitrifiers, dominated by ammonia oxidizers, mediated the OLAND process; however, it was later discovered that plactomycete-like bacteria were also responsible for the process (Wyffels et al., 2003). The OLAND process couples known AAOB that convert NH_3 to NO_2^- with Anammox bacteria. Equations 2.5 and 2.6 summarize the stoichiometry of the two metabolic steps in the OLAND process, and Equation 2.7 shows the overall stoichiometry of the coupled OLAND process (Pynaert et al., 2004). AAOB are of particular importance in this process because it is postulated that these bacteria play an active role in the anoxic part of the oxygen-limited biofilm, next to the known anaerobic ammonia oxidizing bacteria (AnAOB), which differentiates OLAND from the Canon process (Pynaert et al., 2003).

AAOB: $1.3\ NH_4^+ + 1.95\ O_2 \rightarrow 1.3\ NO_2^- + 2.6\ H^+ + 1.3\ H_2O$ **(2.5)**

Anammox: $NH_4^+ + 1.30\ NO_2^- + 0.117\ H^+ \rightarrow 1.058\ N_2 + 0.183\ NO_3^- + 2.05\ H_2O$ **(2.6)**

N-Removal: $2.3NH_4^+ + 1.95\ O_2 \rightarrow 0.183\ NO_3^- + 1.058N_2 + 2.483\ H^+ + 3.35H_2O$ **(2.7)**

The OLAND process produces 0.17 grams of biomass per gram of N removed (compared to 1.9 grams of biomass per gram of N removed in conventional N removal systems). Further laboratory scale studies, examining the OLAND system in an RBC biofilm and Fixed Film Bioreactor (FFBR), were conducted and described in detail by Pynaert et al. (2004). Like the Sharon/Anammox process described above, the OLAND system has the ability to remove nitrogen from wastes with a low COD:TKN ratio. In addition, OLAND based systems have the advantage of being one step systems, leading to a small footprint.

As such, the OLAND process appears to be the most suitable system for use on dairy farms and, therefore, was chosen for this study.

Chapter 3: Manure Treatment System Water Quality Assessment
Johnson Highland Dairy Farm, Glade Spring, Virginia

3.1 Introduction

During the months of May and June 2004, the Johnson Highland Dairy Farm, Glade Spring, VA, was visited on two occasions. Five consecutive days (5/20/2004 to 5/24/2004; sample event 1) and one further day (6/29/2004; sample event 2) were spent on the farm. Information and data that pertain to the manure treatment system employed were gathered during these times. Samples were collected at specific points within the system during each visit. In this report, the manure treatment system is described and the results for both physical and chemical water quality characteristics are reported and analyzed for a range of water quality parameters.

3.2 Manure Treatment System Overview

The Johnson Highland Dairy encompasses 1,100 acres. The farm contains two barns: a main barn and a loafing barn. Approximately 370 Holstein cows are milked twice daily with an average milk yield of 32.5 kg/head/day. Together, these 370 cows consume over 15,540 kg of feed/day, as shown in Table 3.1. Liquid manure production is 21,955 m^3/year. Recycled, anaerobically treated water is used to flush each barn. A schematic highlighting the manure treatment system is shown in Figure 3.1.

The main barn is split into four groups, as shown in Figure 3.2. Four alleys run through the barn where the manure is deposited by the cows. The outermost alleys are approximately 3 m wide and the inner alleys are approximately 4 m wide (Figure 3.2). The total flushing alley width is 14 m and the barn length is 132 m (Figure 3.2). Each group can be flushed separately. Flushes are carried out twice a day simultaneously with a mechanical scraper after the cows from a particular group have vacated the barn for milking. Flush-water from the top half of the barn is collected in a trench in the middle of the barn which is then piped by gravity to the mixing basin (Figure 3.1). Flush-water from the bottom half of the barn runs directly into a trench adjacent to the mixing basin (Figure 3.1). After a transition period of 3 seconds, the steady state flow rate of flush water in the outer most alleys is 0.25 m^3/sec and 0.29 m^3/sec in the inner alleys. A typical volume of flush water used for one group in the main barn is 25 m^3. The total volume of flush water used to flush the entire main barn (all four groups) is 100 m^3/flush.

Fresh water from the parlor (7.6 m^3/flush, twice a day) also collects in the mixing basin, but reportedly contains little manure. A smaller loafing barn is located adjacent to the parlor where a small number of cows are held. This barn is also flushed twice a day and the flush-water from the barn passes by gravity through a pipe into the mixing basin. However, manure-volumes produced here are reported to be small in comparison to the main barn.

In the mixing basin, water stagnates until mixing and mechanical solids separation commences. The mechanical separator runs for 45 minutes twice a day. Running along the base of the main barn and adjacent to the barn-side of the mixing basin is a collection trench that is used to collect flush water from the main barn before entering the mixing basin (Figure 3.2). The surface area of the trench and mixing basin are 26.8 m^2 and 98.3 m^2, respectively. The average residence time of flush water in the mixing basin is 4 hours.

The liquid fraction leaving the mechanical solids separator is transferred to the 9,085 m^3 anaerobic storage facility. The residence time within the anaerobic storage facility is 35 days. Water from the anaerobic storage facility is drawn into two flush water storage-tanks, mixed with fresh water and subsequently used for flushing. One tank is associated with each barn. The volume of the tank adjacent to the main barn (used for main barn flush) is 100 m^3. The volume of the tank adjacent to the loafing barn (used for loafing barn flush) is 53 m^3. The tanks are filled twice a day. Freshwater pumps convey 0.35 m^3/min of fresh water from the local creek and run for 1 hour during each filling cycle. Approximately 15% of each flush is fresh water.

Table 3.1: Number of cows in each group with associated daily feed intake.

Group #	# Cows in each group	Average feed intake (kg/head/day)	Dry matter in feed[#] (kg/head/day)
1	83	42	22
2	83	45	23
3	84	44	23
4	62	45	23
Transition*	38	32	17
Slow**	11	45	23
Fresh***	9	24	12
Total	**370**	42	22

* Cows in various stages of lactation
** Cows which take a longer period of time to milk
***Cows 14 days after calving
[#] Based on a dry matter content of 51.6% in a feed sample taken on 5/20/2004

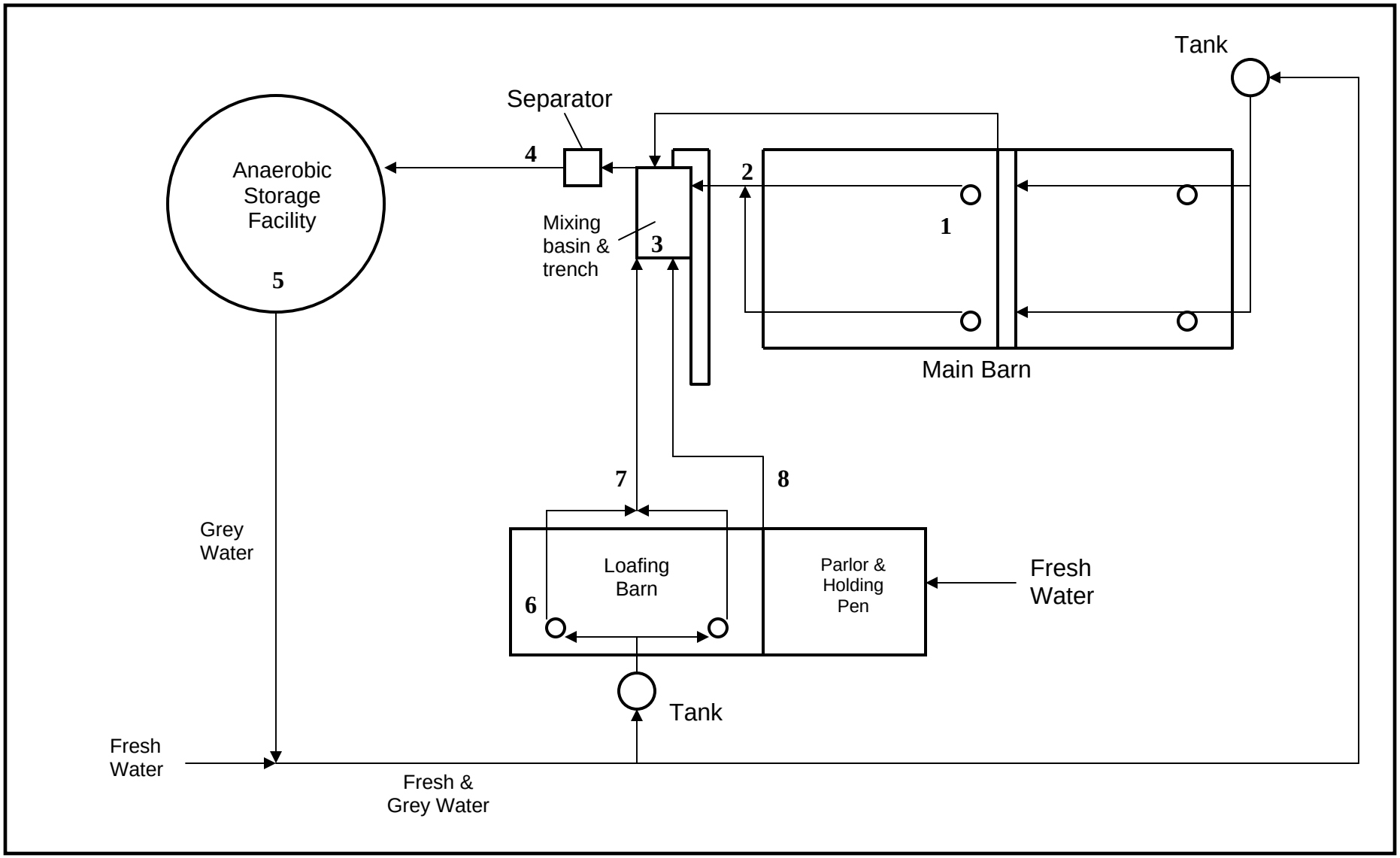

Figure 3.1: Schematic of manure treatment system (Points 1 to 5 sampled during sample event 1 (5/24/2004), points 1 to 8 sampled during sample event 2 (6/29/2004)). Not to Scale.

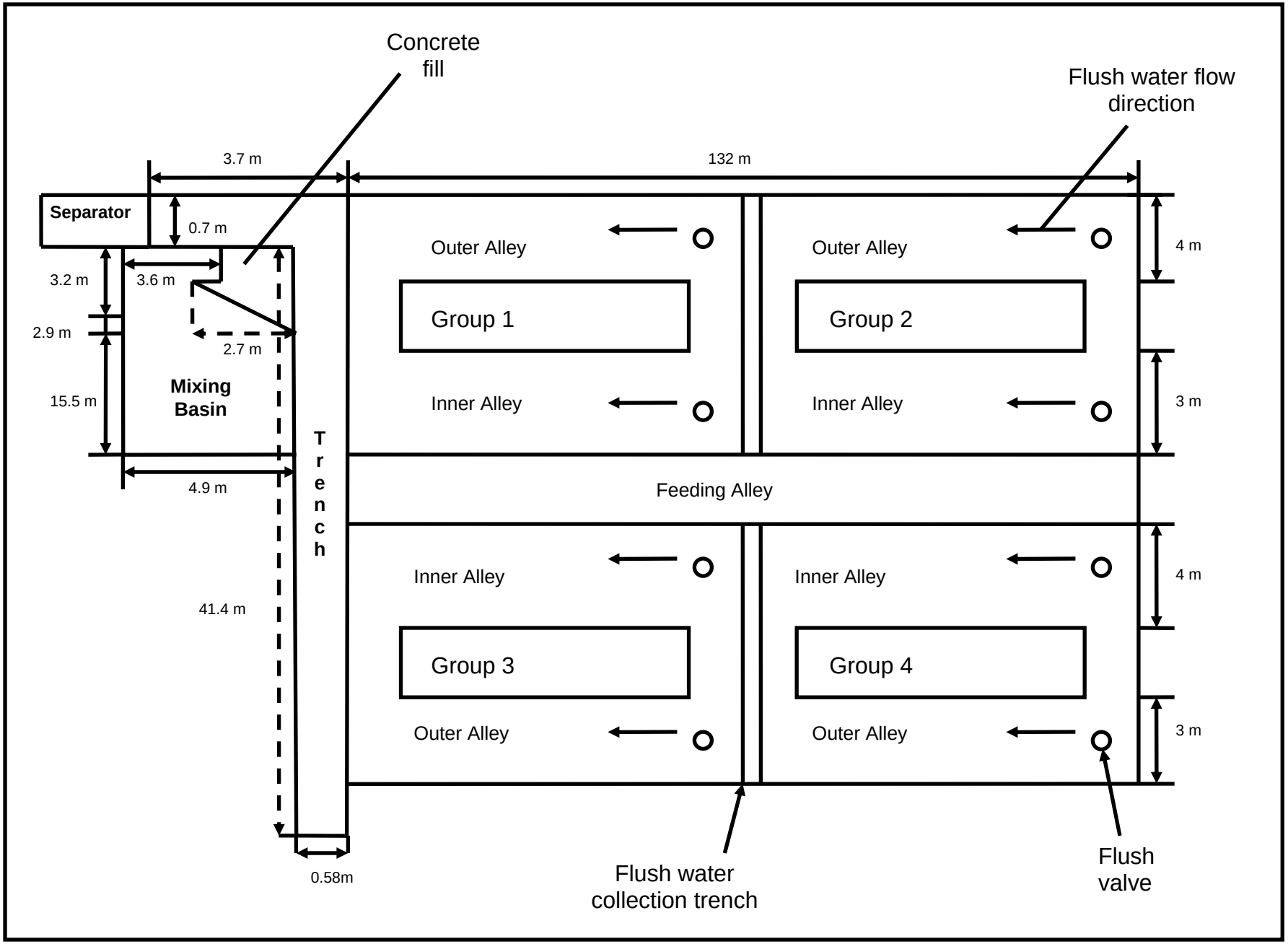

Figure 3.2: Schematic of main barn, mixing basin, trench & separator. Not to scale.

3.3 Water Quality Parameter Analysis

Table 3.2 lists the parameters determined for each sample and the storage methods used for preservation of the samples, for both sampling events. The pH at each location was also determined during sample event 2.

In order to obtain a preliminary description of the chemical characteristics of manure as it travels through the manure treatment system, five sample points (Figure 3.1) were chosen on the first sampling event (5/24/2004). It is important to note that on this day, due to fresh water pumps running for an extended period of time, flush water contained an unusually high fraction of creek water and was cleaner than normal. The five sample points chosen were:

1) Water from the flush valve of the main barn,
2) Effluent from the exit of the main barn,
3) Mixing basin, during mixing,
4) Liquid fraction leaving separator, and
5) Exit of the anaerobic storage facility.

A grab sample was obtained for each sample analysis. Three aliquots were analyzed for each sample with the exception of the nitrate and nitrite analysis when only two aliquots were analyzed.

Some measurements determined from sample event 1 are likely to be in error due to inadequate mixing of the sample bucket when sample aliquots were removed, and the location of sample extraction. The results of this analysis are summarized in Appendix 2. However, some points of interest were raised by the results gathered from sample event 1, particularly with regard to sample storage procedures for NH_3-N. It appears that freezing the NH_3-N samples, rather than acidifying them below pH 2, yielded more accurate results (Appendix 2).

During the second sampling event (6/29/2004), three more locations were sampled in addition to the five locations assessed during sample event 1 (Figure 3.1). The results of the analysis from the second sampling event are found in Table 3.3. The eight sample points chosen for sample event 2 were:

1) Water from the flush valve of the main barn,
2) Effluent from the exit of the main barn,
3) Mixing basin, during mixing,
4) Liquid fraction leaving separator,
5) Exit of the anaerobic storage facility,
6) Water from the flush valve of the loafing barn,
7) Effluent from the exit of the loafing barn, and
8) Effluent from the exit of the parlor.

Again, a grab sample was obtained for each sample analysis. Three aliquots were analyzed for each sample with the exception of the nitrate and nitrite analysis when only two aliquots were analyzed.

A new protocol was implemented with all samples containing solids during the second sampling event. During the second sampling event all sample buckets were well mixed, using a yard stick, when removing sample aliquots. Consequently, the water quality parameter analysis in this report will focus entirely on data gathered from the second sampling event.

Table 3.2: Parameters determined for each sample and storage procedures used (Standard Method, 1998).

Parameter	Storage Procedure	Max. storage recommended
Total Solids (TS)	Refrigerate	7 days
Volatile Solids (VS)	Refrigerate	7 days
Total Suspended Solids (TSS)	Refrigerate	7 days
Volatile Suspended Solids (VSS)	Refrigerate	7 days
Alkalinity	Refrigerate	24 hours
Total Chemical Oxygen Demand (TCOD)	Reduce pH below 2 & refrigerate	28 days
Soluble Chemical Oxygen Demand (SCOD)	Filter, reduce pH below 2 and refrigerate	28 days
Total Phosphorus (TP)	Refrigerate	28 days
Soluble Phosphorus (SP)	Filter and refrigerate	28 days
Total Total-Kjeldahl Nitrogen (TTKN)	Reduce pH below 2 and refrigerate	28 days
Soluble Total-Kjeldahl Nitrogen (STKN)	Filter, reduce pH below 2 and refrigerate	28 days
Ammonia Nitrogen	Acidify below pH 2 or freeze	28 days
Nitrate (NO_3^-)	Filter and freeze	28 days
Nitrite (NO_2^-)	Filter and freeze	28 days

Table 3.3: Results from chemical analysis, carried out on each sample for specified locations on the Johnson Dairy Farm (based on sample event 2).

Location (sample point number)	Flush Valve Main Barn (1)	Exit of Main Barn (2)	Mixing Basin (3)	Separator Effluent (4)	Anaerobic Storage Facility Effluent (5)	Flush Valve Loafing Barn (6)	Exit of Loafing Barn (7)	Exit of Parlor (8)
TS (g/L)	7.25 ±0.04	43.31 ±0.05	15.03 ±0.02	14.66 ±0.02	7.20 ±0.03	7.15 ±0.05	10.13 ±0.03	0.05 ±0.01
VS (g/L)	3.37 ±0.08	37.19 ±0.05	9.67 ±0.03	9.80 ±0.02	3.40 ±0.03	3.31 ±0.06	5.93 ±0.06	0.02 ±0.00
TSS (g/L)	0.97 ±0.05	17.20 ±0.17	5.50 ±0.30	4.77 ±0.45	1.50 ±0.10	1.40 ±0.00	1.93 ±0.05	0.16 ±0.01
VSS (g/L)	0.83 ±0.06	15.23 ±0.15	5.30 ±0.30	4.27 ±0.95	0.93 ±0.06	1.30 ±0.00	1.70 ±0.10	0.11 ±0.00
Alkalinity (mg $CaCO_3$/L)	3,160 ±302	3,510 ±8	3,540 ±8	3,050 ±16	3,630 ±0	3,180 ±0	3,290 ±8	152 ±0
Total COD (mg O_2/L)	2,650 ±0	23,400 ±2736	12,600 ±170	10,000 ±0	4,420 ±0	4,420 ±0	7,070 ±0	7 ±0
Sol. COD (mg O_2/L)	294 ±0	3,290 ±85	3,240 ±0	2,800 ±0	294 ±0	736 ±0	809 ±104	17 ±0
Total TKN (mg N/L)	829 ±0	1,450 ±7	1,100 ±0	1,130 ±35	811 ±1	799 ±1	904 ±1	4.98 ±1
Sol. TKN (mg N/L)	598 ±0	661 ±60	661 ±34	661 ±14	543 ±0	535 ±9	576 ±15	14.2 ±13
NH_3-N - Frozen (mg N/L)	560 ±0	638 ±0	668 ±2	587 ±16	631 ±15	620 ±2	566 ±0	6 ±0
Total P (mg/L)	96 ±9	174 ±20	178 ±23	156 ±2	90 ±0	89 ±13	121 ±18	0.45 ±0.00
Sol P (mg/L)	6.09 ±0.08	5.32 ±1.57	5.71 ±0.85	3.18 ±0.34	4.80 ±2.43	6.85 ±0.40	6.77 ±0.08	0.76 ±0.64
NO_2^- (mg/L as N)	4.08 ±0.56	3.89 ± 2.31	3.45 ± 3.12	4.42 ± 0.54	3.21 ± 0.54	1.54 ± 2.17	2.60 ± 2.36	0.00 ± 0.00
NO_3^- (mg/L as N)	1.94 ±0.04	3.24 ±0.04	3.50 ±0.11	3.62 ±0.01	2.01 ±0.08	1.93 ±0.17	2.35 ±0.22	9.56 ±1.11
pH	7.83	7.13	7.50	7.57	7.91	7.82	7.72	7.55
Flow (m^3/day)	197	198	303	259	259	106	106	15.1

3.4 Separator Efficiency

Table 3.4 highlights the efficiency with which solids were removed by the mechanical solids separator and mixing basin. Approximately 2,390 kg TSS/day are removed by the mixing basin and mechanical solids separator. An estimate of dry mass recovered by the solids separator is needed to determine what fraction of this TSS removal is achieved by the mixing basin. Although solids removal is not a goal of the mixing basin, analysis of ammonia loads into the mixing basin from all sources (186,000 g NH_3-N/day) is 8 % lower than that leaving the mixing basin (203,000 g NH_3-N/day). This suggests possible anaerobic stabilization of retained suspended solids in the mixing basin. It is suggested that further assessment of this element of the Johnson Highland Dairy Farm waste management system be performed to clarify whether suspended solids are in fact accumulating in the mixing basin.

Table 3.4: Removal efficiency of the mechanical solids separator with regard to solids.

	Exit of Main Barn	Exit of Loafing Barn	Exit of Parlor	Total Separator Influent	Separator Effluent	g/day Removed	% Removal
TS (g/day)	8,580,000	1,070,000	707	9,660,000	3,790,000	5,870,000	61
VS (g/day)	7,370,000	628,000	311	8,000,000	2,530,000	5,470,000	68
TSS* (g/day)	3,410,000	202,000	2,380	3,640,000	1,230,000	2,390,000	66
VSS* (g/day)	3,020,000	180,000	1640	3,200,000	1,100,000	2,100,000	66

**A 10:1 dilution was used for TSS and VSS samples from each location (except the exit of the parlor, where no dilution was used).*

COD (Chemical Oxygen Demand) is a measure of the electrons available in the bonds of an organic compound, expressed in terms of the amount of oxygen required to accept them when the compound is completely oxidized to carbon dioxide and water. A

certain amount of organic matter is required if biological nitrogen removal is to be achieved in the anaerobic storage facility. The COD/TKN ratio typically indicates how efficient biological nitrogen removal will be, with higher values indicating greater removal efficiency. The COD/TKN ratio for the separator effluent is 10:1, suggesting an excellent potential for biological nitrogen removal. Similarly, a minimum organic matter requirement exists for biological removal of phosphorus, which can be expressed using the COD/TP ratio. The COD/TP ratio for the separator effluent is 65:1, suggesting that if phosphorus removal was desired, an abundance of energy is available to achieve phosphorus removal by biological means.

3.5 Anaerobic Storage Facility Efficiency

Table 3.5 highlights the current removal efficiency of the anaerobic storage facility with regard to soluble and total COD. As with the mixing basin, NH_3-N concentrations in the anaerobic storage facility water increase relative to wastewater leaving the separator (also shown in Table 3.5). This indicates that anaerobic stabilization is occurring, and is a goal of the anaerobic storage facility.

Table 3.5: Removal efficiency in the anaerobic storage facility with regard to soluble and total COD and ammonia.

	Exiting Separator	Exiting Anaerobic Storage Facility	% Removal
SCOD (g O_2/day)	724,000	76,000	89
TCOD (g O_2/day)	2,590,000	1,140,000	56
NH_3 (g N/day)	152,000	163,095	-7

3.6 Nitrification, Anaerobic Storage Facility Aeration and the Effects of pH and Alkalinity

The term nitrification is used to describe a two-step biological process in which ammonia (NH_4-N) is oxidized to nitrite (NO_2-N) and nitrite is then oxidized to nitrate (NO_3-N). There are a number of reasons for wishing to stimulate nitrification in a

wastewater treatment setup. Waters receiving excessive amounts of ammonia can prove toxic to fish. Moreover, excessive concentrations of nitrogen entering water bodies can lead to eutrophication. In addition, excessive levels of ammonia in discharged wastewater can hinder the use of that water for water-reuse applications including groundwater recharge. Furthermore, in dairy operations, volatilization of ammonia in barns can impair animal health (Robertson et al., 1990).

In typical wastewater treatment operations, aerobic autotrophic bacteria are responsible for nitrification. As nitrification is a two-step process, two groups of bacteria mediate the process. In the first stage, ammonia is oxidized to nitrite by one group of autotrophic bacteria. In the second stage a distinctly different group of autotrophic bacteria oxidize nitrite to nitrate. This biological process can be described using the stoichiometric chemical equations detailed below.

The first group of autotrophic bacteria carries out the following conversion (ammonia to nitrite):

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \qquad \textbf{(3.1)}$$

Nitrite is then oxidized by the second group of autotrophic bacteria as follows:

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \textbf{(3.2)}$$

The total oxidation reaction (i.e. the sum of reactions 3.1 & 3.2) is summarized by reaction 3.3.

$$2NH_4^+ + 4O_2 \rightarrow 2NO_3^- + 4H^+ + 2H_2O \qquad \textbf{(3.3)}$$

Based on reaction 3.3, it can be observed that a significant amount of oxygen is required per gram of nitrogen removed. The oxygen required for complete oxidation of ammonia is 4.57 g O_2/g N oxidized. In order to achieve this level of oxidation within an anaerobic storage facility, a powerful mechanical aerator is required.

Nitrification is sensitive to pH and the rate of nitrification declines significantly when pH drops below 6.8. Linked to the maintenance of the appropriate pH range is the level of alkalinity in the wastewater within the anaerobic storage facility. The alkalinity of a water is the measure of its capacity to neutralize acids. Alkalinity acts as a buffer that prevents dramatic drops in pH. Nitrification consumes alkalinity and, therefore, reduces the buffer capacity of the wastewater. Where low alkalinity is expected, alkalinity can be

added in the form of lime, soda ash (sodium bicarbonate) or caustic soda (sodium hydroxide) in order to maintain the desired pH for optimal nitrification.

Based on an NH_4^+ concentration of 631 mg N/L in the anaerobic storage facility, the amount of alkalinity needed by the anaerobic storage facility in order for nitrification to occur (upon aeration), is estimated to be 23.2 g/L (Appendix 1, Treatability in the Anaerobic Storage Facility). This estimate is extremely conservative, as it assumes that no ammonia is volatilized and no ammonia is redirected to cell growth for bacteria growing in the anaerobic storage facility. Presently, the alkalinity of the anaerobic storage facility is 3.63 g/L as $CaCO_3$. Although the original estimate is likely to be conservative, the much lower alkalinity found in the anaerobic storage facility is of concern. The consequence of this depends upon the level of dissolved oxygen (DO) that is achieved in the anaerobic storage facility. If a measurable DO is achieved, then it is possible that the pH will drop below the preferred physiological range for growth of both COD reducing and nitrifying bacteria because nitrification may occur and consume all of the alkalinity. The degree to which the anaerobic storage facility achieves stabilization may be adversely affected. The current pH (7.91) within the anaerobic storage facility is ideally suited to promoting maximal activity of nitrifying bacteria.

Furthermore, if low DO concentrations are maintained (more likely, due to the high COD load to the anaerobic storage facility), nitrification can still occur but only partially. Under low DO conditions the second step in nitrification (reaction 3.2) is easily inhibited. The result would be nitrite formation and possible accumulation (nitrite is toxic to infant humans and can result in methemoglobinemia in cows). In summary, the influence of adding aeration to the anaerobic storage facility at the Highland Dairy Farm should be carefully evaluated to avoid adverse consequences due to oxidation of nitrogenous compounds. While the stimulation of nitrification in the anaerobic set-up is desirable (in order to curb atmospheric ammonia pollution and nitrogen losses to local and regional waters), adding aeration to the anaerobic storage facility should be approached with caution.

3.7 Conclusion

In the development of new or existing treatment facilities, the strengths and characteristics of the wastewater treated must be known. The final goal of the OLAND

based system examined in this thesis is to treat anaerobically stabilized dairy manure (not presented in this research). Fundamental to this objective is the ability to accurately assess the changing wastewater characteristics on a dairy farm. Presented in this chapter is a comprehensive template for conducting a water quality parameter sampling event for the manure treatment setup of such a facility. Following this template the wastewater characteristics at each stage of operation of a dairy farm manure treatment system can be assessed. This analyses involves an in-depth understanding of the manure treatment system employed (achieved through site visits), the development of treatment schematics, the selection of sample points and chemical parameters to be determined and, the consequent analyses and examination of results obtained. In order to acquire a comprehensive assessment, that takes seasonal variations into account, sampling events must be conducted on several strategically chosen occasions for a period of one year (i.e. more than two sampling events are required). Also presented in this chapter are the key issues and analyses to be conducted for the development of an aerobic storage facility from an anaerobic set-up.

Chapter 4: Start-up of an Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND) Fixed Film Bioreactor (FFBR) for the Treatment of Ammonium Rich Wastewater

4.1 Introduction

In an effort to introduce a novel and cost effective biological N removal waste treatment strategy for dairy manures, an alternative process to the conventional aerobic/anaerobic N removal wastewater treatment technologies was examined. The novel biological process studied in this project is called Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND). Ideally suited to the treatment of anaerobically-stabilized dairy manures, OLAND couples low oxygen partial nitrification with anaerobic, autotrophic denitrification. Based on previous research (Pynaert et al., 2004), the OLAND process has the possibility to deliver overall N removal rates that are competitive when compared to conventional N removal systems.

Based on the configuration of Pynaert et al. (2004), a Fixed Film Bioreactor (FFBR) was developed. The FFBR was designed to remove NH_4^+ and NO_2^- (as the electron acceptor) from a synthetic wastewater stream. The FFBR, utilizing the OLAND process, focused on single-stage N removal in a biofilm reactor, without the need for an organic carbon source. Two concurrent reactions take place during the OLAND process, namely nitritation and Anammox, or anaerobic ammonia oxidation. Nitritation occurs due to the presence of aerobic ammonia-oxidizing bacteria (AAOB) while Anammox occurs due to the presence of anaerobic ammonia-oxidizing bacteria (AnAOB), belonging to a new deep branching group within the *Planctomycetales.* Equations 4.1 and 4.2 summarize the stoichiometry of the two metabolic steps in the OLAND process, and equation 4.3 shows the overall stoichiometry of the coupled OLAND process.

AAOB: $1.3\ NH_4^+ + 1.95\ O_2 \rightarrow 1.3\ NO_2^- + 2.6\ H^+ + 1.3\ H_2O$ **(4.1)**

Anammox: $NH_4^+ + 1.30\ NO_2^- + 0.117\ H^+ \rightarrow 1.058\ N_2 + 0.183\ NO_3^- + 2.05\ H_2O$ **(4.2)**

N-Removal: $2.3\ NH_4^+ + 1.95\ O_2 \rightarrow 0.183\ NO_3^- + 1.058\ N_2 + 2.483\ H^+ + 3.35\ H_2O$ **(4.3)**

The goal of this project was to construct, start-up and maintain an OLAND Fixed Film Bioreactor (FFBR) inoculated with OLAND Sludge from the Microbial Ecology and Technology (LabMET) Laboratory run by Dr. Willy Verstraete, University of Ghent,

Belgium. Of specific interest was to determine the required time period to achieve stable performance of a lightly loaded OLAND system and demonstrate a protocol for verifying that OLAND-based metabolism was occurring. The reactor was monitored for 51 days after inoculation.

4.2 Materials and Methods

4.2.1 Synthetic Wastewater and Inoculum

A synthetic wastewater (Pynaert et al., 2004) was used to feed the reactor. The feed consisted of 1 g/L $NaHCO_3$, 30 mg/L KH_2PO_4, 189 mg/L NH_4Cl (49.5 mg/L NH_4^+-N, or 3.5 mM NH_4^+), 238 mg/L $NaNO_2$ (48.3 mg/L NO_2^--N, or 3.5 mM NO_2^-), giving approximately 100 mg of N/L, and 2 ml/L of Trace Element Solution (EDTA 5g/L, $ZnSO_47H_2O$ 2.2 g/L, $CoCl_26H_2O$ 1.6g/L, $MnCl_24H_2O$ 5.1g/L, $CuSO_45H_2O$ 1.6g/L, $(NH_4)_6Mo_7O_{24}4H_2O$ 1.1g/L, $CaCl_22H_2O$ 5.5g/L and $FeSO_47H_2O$ 5.0g/L). Cold tap water was used for feed preparation and the pH of the feed was adjusted to 7.0 using 0.5 M HCL before addition to the reactor. The FFBR was inoculated with 50 ml of OLAND sludge obtained from LabMET, University of Ghent, Belgium.

4.2.2 Reactor Design

A closed cylinder, down-flow FFBR was constructed (internal diameter 10 cm, height approximately 92 cm, total volume calculated to be 7.22 L). Plastic carrier material (KONTAKT 565 from NSW Environmental Systems, Roanoke, Virginia, USA) was used for biofilm growth (Figure 4.1). The specific characteristics of this media are a diameter of 2.2 cm with a length of 0.64 cm, a dry weight of 122 kg/m^3, a surface area of 564 m^2/m^3 and a void space of 91 %.

Figure 4.1 KONTAKT 565 Media, NSW Environmental Systems. Plan shown on left and comparison to US quarter shown on right.

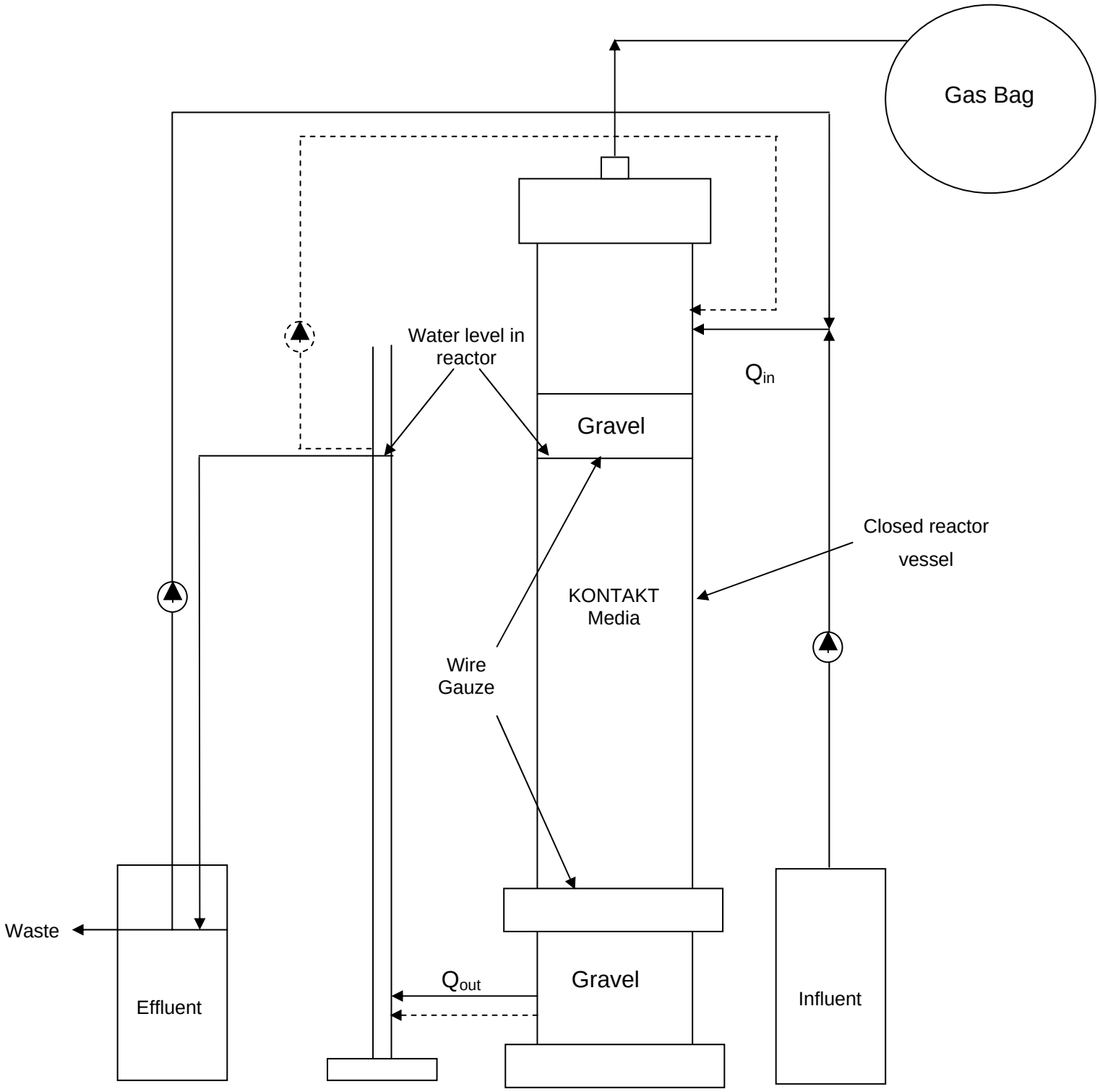

Figure 4.2: FFBR utilizing the OLAND process
(Dashed flow lines correspond to phase 1, Batch set-up (day 0 to 14),
solid flow lines correspond to phases 2, Continuous Flow set-up (day 15 to 51))

Five L of synthetic wastewater were added to the reactor, with the media and synthetic wastewater taking up 6 L of reactor volume. Figure 4.2 provides a graphical overview of the reactor design. No exogenous aeration was provided so the only oxygen loaded to the system was that dissolved in the feed media. Gravel layers, each held in place by wire gauze, were placed at the bottom (to prevent media collapse) and top (to keep media below the liquid surface) of the media surface. The continuous influent flow-rate was 1.33 L/d, giving a hydraulic retention time (HRT) of 3.76 days. Ammonia loading to the column was 83.8 mg NH_4^+/day (4.6 mmoles NH_4^+/day). Room temperature was maintained throughout the experiment. Gases produced were collected in a 10-L Tedlar gasbag, connected to the cap that sealed the reactor at the top.

Two feed phases were used over the length of the experiment. Initially, the reactor was set up as a closed-loop batch system with all effluent being returned to the reactor as influent (day 0 to 14). Here, the internal flow rate was 2.23 L/day. In the second phase (day 15 to 51), the reactor was set-up as a continuous flow system (Q_{in} = 1.33 L/d) with partial recycle of the effluent ($Q_{recycle}$ = 1 L/d). In both cases flow was obtained using L/S 13 tubing and pump heads with 1:100 peristaltic pump drivers (Cole Parmer, Illinois).

4.2.3 Start-up of Reactor

4.2.3.1 Phase 1: Batch set-up, Day 0 to 14 (Oct 7th to Oct 21st 2004)

Water was pumped through the FFBR set-up for 7 days to ensure the system was watertight. Next, the reactor was inoculated with OLAND sludge and fed with 5 L of synthetic wastewater at 11am on the 7th October 2004 (day 0). The synthetic wastewater and inoculum were recirculated internally at an empty bed hydraulic loading of 0.45 L/day for 14 days (until the 21st of October, day 14), as shown by Figure 4.2 (dashed flow lines). This initial batch protocol was used to encourage biomass adhesion to the carrier material (Characklis and Marshall, 1990).

4.2.3.2 Phase 2: Continuous flow set-up, Day 15 to 51 (October 22nd to November 27th)

On October 22nd (day 15) the FFBR set-up was changed to that of a continuous-flow reactor (Figure 4.2, solid flow lines). Partial recirculation of effluent wastewater was also incorporated into the set-up. The flow rate from the influent bucket was 0.33 L/d and the flow rate of recycled effluent was 1 L/d. This brought the Q_{in} of the reactor to a total

of 1.33 L/d. On day 15, 3 L of feed was added to the influent bucket and 1 L was added to the effluent bucket. Sampling of this new set-up began on day 19.

Periodic refilling of the influent bucket took place every three to four days from day 15 onwards. Initially, the effluent jar had a capacity of 3 L, with an estimated HRT of 7 days. Wastage of accumulating effluent was carried out manually. On the 18th of November (day 42) the effluent bucket was changed to a self-decanting jar with a constant volume of 750 ml, and a HRT of approximately 1.75 days. This change took place in order to minimize potential influences that the 7-day HRT may have exerted on the reactor's performance.

On day 58, media was removed from the column and a series of batch experiments were run with the biomass in an effort to more directly confirm the stoichiometry of the process

4.2.4 Chemical Analysis (Day 0 to 51)

Table 4.1 details the water quality parameters analyzed and the frequency of sampling from day 0 to 51.

Table 4.1: Water quality parameters analyzed and the frequency of sampling from day 0 to 51.

Reactor set-up	**Water Quality Parameters Analyzed (Standard Methods Protocol number (APHA, 1998))**	**Frequency of sampling**
Batch Set-up (Day 1 to 5)	pH (4500-H^+B), DO (4500-OG), Nitrate & Nitrite (4110 C), Ammonia (4500 – NH_3 B)	Once a day
Batch Set-up (Day 8 to 11)	pH (4500-H^+B), DO (4500-OG), Nitrate & Nitrite (4110 C), Ammonia (4500 – NH_3 B)	Twice a day
Continuous Flow set-up (Day 15 to 51)	pH (4500-H^+B), DO (4500-OG), Nitrate & Nitrite (4110 C), Ammonia (4500 – NH_3 B), VSS (2540 E), TSS (2540 B)	Three times a week
	Alkalinity (2320 B)	Once a week

Dissolved oxygen (DO) and pH were measured using electronic meters (YSI Model 58 DO Meter, Sunnyvale, CA and Accumet pH Meter 910, New York, respectively), nitrate and nitrite were measured using an ion chromatograph (Dionex DX-120 Sunnyvale, CA) and ammonia was measured using the distillation procedure, as detailed in Standard Methods (APHA, 1998). Volatile and total suspended solids and alkalinity were also measured using procedures described in Standard Methods (APHA, 1998).

For samples withdrawn during the batch set-up (day 0 to14), analysis was carried out in triplicate for each sample with the exception of the nitrate and nitrite analyses when two aliquots were analyzed. During the continuous flow set-up, due to sample volume constraints, 2 aliquots of each sample withdrawn were analyzed for VSS/TSS, nitrate, nitrite and ammonia. Three aliquots were analyzed for alkalinity.

4.2.5 Batch experiments

Two batch experiments (aerobic with NH_4^+ spike and anaerobic with NH_4^+ and NO_2^- spikes) were conducted at the end of the five-week monitoring period of the continuous-flow FFBR. Both batch experiments were carried out in duplicate; two aerobic vessels (Aerobic 1 and Aerobic 2) and 2 anaerobic vessels (Anaerobic 3 and Anaerobic 4) were used.

The aerobic batch experiments were carried out in two 500 ml Erlenmeyer Flasks. DO conditions were maintained, through aeration with a diffuser stone and an aquarium pump, at greater than 8 mg/L (saturation) for the period of the experiment. The purpose of the aerobic batch experiment, spiked with a known concentration of NH_4Cl, was to measure the nitrification capacity of the AAOB and the nitrite-oxidizing bacteria (NOB) community possibly present in the FFBR. AnAOB were not expected to influence the aerobic batch experiment results due to the requirement of AnAOB for oxygen-limited conditions. Each flask contained feed solution (1 g/L $NaHCO_3$, 30 mg/L KH_2PO_4, 2ml Trace Element Solution per L) and 107 mg/L of NH_4Cl (2 mmole NH_4^+, 28 mg/L NH_4^+-N). Biomass washed from 500 ml of media from the FFBR was added to each vessel. The final volume of the vessels was brought to 500 ml using cold tap water. The initial pH was brought to 7.0 using 0.5 M HCl and the experiment was carried out at room temperature. To obtain samples, mixing and aeration to the flasks was stopped for

approximately 5 minutes, allowing the biomass to settle (in order to conserve biomass). The mixed liquor sample withdrawn (20 ml) was filtered through a 0.45 μm filter (Whatman, 934-AH, glass microfibre filter) and analyzed for NH_4^+, nitrate, nitrite and pH (using methods described above). The TSS, VSS and DO of each vessel were measured at the end of the aerobic batch experiment using methods described above. Due to sample volume limitations, 2 aliquots for each sample were analyzed.

The purpose of the anaerobic batch experiments was to measure the ammonia-oxidizing activity of the AnAOB in the FFBR. Anaerobic conditions were generated in two 1-L glass jars closed tightly with rubber stoppers, in which 2 glass tubes were inserted for sample collection. One tube penetrated the liquid surface with the other remaining above the liquid surface. Samples were retrieved by pushing N_2 gas through the tube above the liquid surface. Liquid that exited through the tube penetrating the liquid surface was collected. Each vessel was filled with feed solution (1 g/L $NaHCO_3$, 30 mg/L KH_2PO_4, 2ml Trace element Solution per L) a spike of 107 mg/L NH_4Cl (2 mmole NH_4^+, 28 mg/L NH_4^+-N) and 138 mg/L $NaNO_2$ (2 mmole NO_2^-, 28 mg/L NO_2^--N), and biomass washed from 500 ml of carrier material from the FFBR. Each vessel was brought to a total volume of 500 ml using cold tap water. After all solutions and biomass were added, the vessels were purged with N_2 gas for 30 minutes and sealed. In order to obtain samples, mixing of the flasks was stopped for approximately 5 minutes, allowing the biomass to settle. The mixed liquor sample withdrawn (20 ml) was filtered through a 0.45 μm filter (Whatman, 934-AH, glass microfibre filter) and analyzed for NH_4^+, nitrate, nitrite and pH (using methods described above). The TSS, VSS and DO of each vessel were measured at the end of the anaerobic batch experiment (using methods described above). Again, due to sample-volume limitations, 2 aliquots for each sample were analyzed.

To determine the required time period over which to run the experiments, a measurement of VSS in the FFBR was required. To measure VSS, 300 ml of media was extracted from the reactor and the attached biomass was removed. The distribution of biomass throughout the reactor was not consistent. Furthermore, the biomass was not attached well to the media. Therefore, media was strategically removed from the reactor to ensure a representative sample of biomass was obtained. Using the product of the VSS measurement and relevant specific kinetic rates reported in Pynaert et al. (2003), an

expression detailing the consumption of N (in mg/L) over time was determined. Knowing the concentrations of each spike added to the batch vessels, the length of time required for complete removal of N in each vessel was determined.

Based on an aerobic ammonia oxidation potential of 147.8 mg N g VSS^{-1} day^{-1} (Pynaert et al., 2003), a VSS of 205 mg/L in the reactor (Appendix 3, Table A3.4) and an initial concentration of approximately 30 mg N L^{-1} day^{-1}, it was determined that the aerobic batch test should be conducted for 24 hours for full N removal. A sample frequency of once every 3 hours was chosen. Twenty ml of mixed liquor was removed from the vessel with 10 ml for nitrate/nitrite analyses (filtered and frozen for storage) and 10 ml for ammonia analysis (with the pH dropped below 2 and refrigerated for storage).

The reported specific anaerobic ammonia oxidation rate of 75 mg N g VSS^{-1} day^{-1} (Pynaert et al., 2003) was used in a similar fashion to determine the appropriate running time for the anaerobic batch experiments. It was calculated that 48 hours would be an appropriate period to allow for complete N removal. Based on this period, a sample frequency of once every 6 hours was chosen and 20 ml of the mixed liquor was removed from the vessels, stored and analyzed as with the aerobic samples.

4.3 Results and Discussion

4.3.1 Start up (Day 0 to 51)

4.3.1.1 Batch Phase (Day 0 to 14)

The results of the chemical analyses on samples drawn from days 1 through 5 are represented graphically in Figure 4.3. The average pH over days 1 to 5 was 7.3 and DO was measured at less than 1 mg O_2/L.

Removal efficiency of NH_4^+ was high (100 % removal by day 5), whereas nitrite removal efficiency was lower (45.5 % removal by day 5). The sludge inoculum, being starved for over 3 weeks during shipment and arrival to the laboratory at Virginia Tech, consumed available NH_4^+ rapidly. During days 1 to 5, 1.59 mmoles of NO_2^-/L were consumed. Following the anammox process (equation 4.2), it would be expected that 1.22 mmoles of NH_4^+/L would have been consumed. However, 3.5 mmoles of NH_4^+/L were consumed. Given saturated DO in the feed and a DO of 0.9 mg O_2/L in the effluent, 7.57 mg O_2/L (0.24 mmoles of O_2/L) were consumed, assuming reactor feed was the only

source of DO (other sources may exist including diffusion through tubing and reactor seals). This does not correspond with stoichiometry detailed in equation 4.1, based on residual NH_4^+ left after Anammox is considered (2.28 mmoles NH_4^+/L) and assuming no NOB activity. Furthermore, in contrast to anammox stoichiometry, negligible amounts of NO_3^-, a product of the anammox process, were produced. For each mole of ammonia consumed, 0.079 moles of nitrate would have been expected if stable OLAND were occurring. This was clearly not the case, referring to Figure 4.3.

Based on the overall NH_4^+ consumption of 3.5 mmoles, approximately 40 ml of N_2 gas should have been generated. Given the use of a 10-L Tedlar gas bag for the collection of gases from the reactor, the generation of such a small volume of gas would have been difficult to observe. As such, little gas accumulation was observed during days 1 to 5.

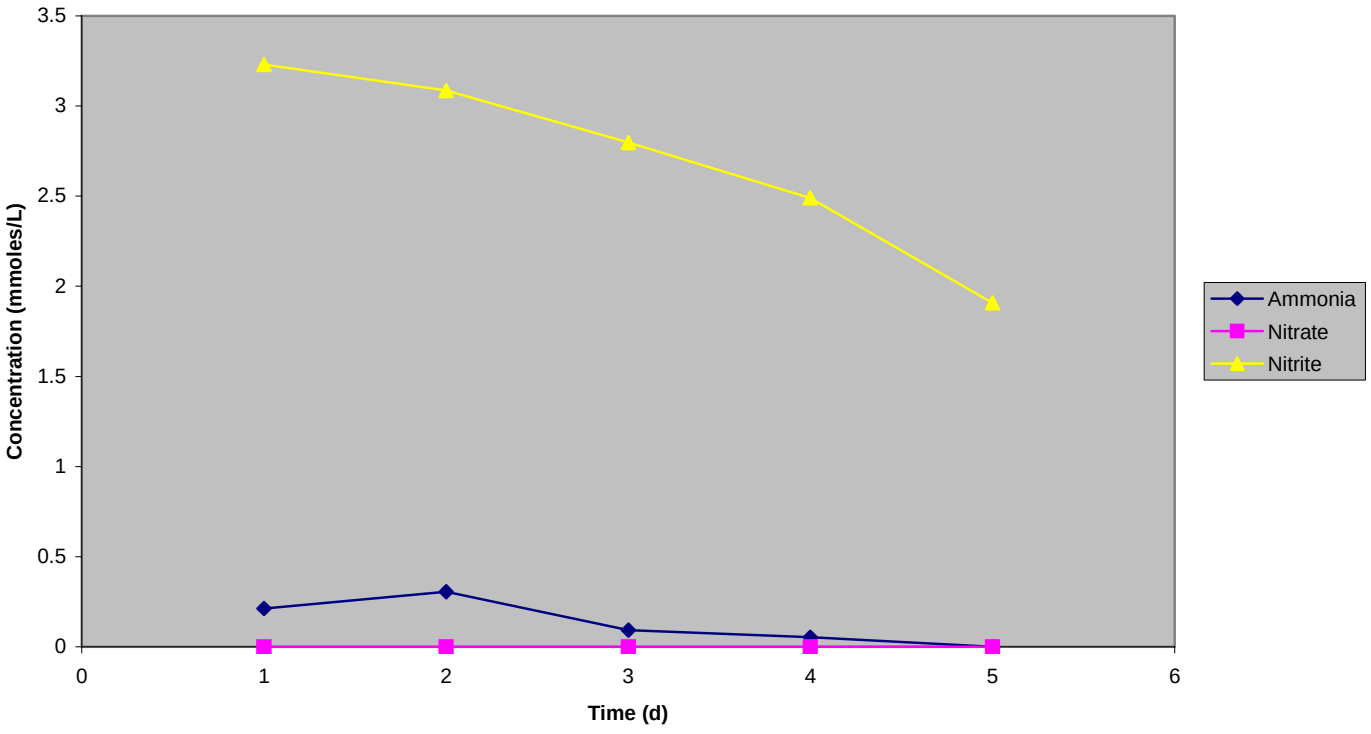

Figure 4.3: Results from chemical analysis carried out on samples withdrawn on days 1 to 5 (batch set-up).

On October 15th (day 8), the equivalent of 6 L fresh feed (in a concentrated form) was added to the reactor at 11a.m. As 26.7 mg NO_2^--N/L were remaining in the reactor on day 5 it was calculated that after feed addition on day 8, the initial concentration of

NO_2^--N rose to approximately 85 mg NO_2^--N/L (6 mmoles of NO_2^-/L). The results of the chemical analyses on these samples are graphically represented in Figure 4.4. DO was consistently below 0.9 mg O_2/L and the pH was 7.4 during days 8 through 12.

As can be seen from Figure 4.4, NH_4^+ is rapidly consumed in the reactor. It takes a period of approximately 24 hours for the influent NH_4^+ concentration of 3.5 mmoles NH_4^+/L to be fully removed. For each mole of ammonia consumed, the production of 0.079 moles of nitrate would have been expected if stable OLAND were occurring. This was clearly not seen (Figure 4.4).

During days 8 to 12, 2.73 mmoles of NO_2^-/L were consumed. Following the anammox process (equation 4.2), it would be expected that 2.1 mmoles of NH_4^+/L would have been consumed. However, 3.5 mmoles of NH_4^+/L were consumed. Given saturated DO in the feed and a DO of 0.86 mg O_2/L in the effluent, 7.61 mg O_2/L (0.24 mmoles of O_2/L) were consumed. This does not correspond with stoichiometry detailed in equation 4.1, based on residual NH_4^+ left after Anammox is considered (1.4 mmoles NH_4^+/L) and assuming no NOB activity. However, this is also based on the assumption that the only source of DO is through the feed solution, which may not be the case.

Based on the overall NH_4^+ consumption of 3.5 mmoles, approximately 40 ml of N_2 gas should have been generated. As stated above, given the use of a 10-L Tedlar gas bag for the collection of gases from the reactor, the generation of such a small volume of gas would have been difficult to observe. Therefore, little gas accumulation was observed during days 8 to 12.

It should be noted that the primary purpose of this phase of reactor initiation was to acclimate the inoculum to the new environment and promote attachment to the carrier material. As such, it was expected that stable OLAND performance might not be achieved.

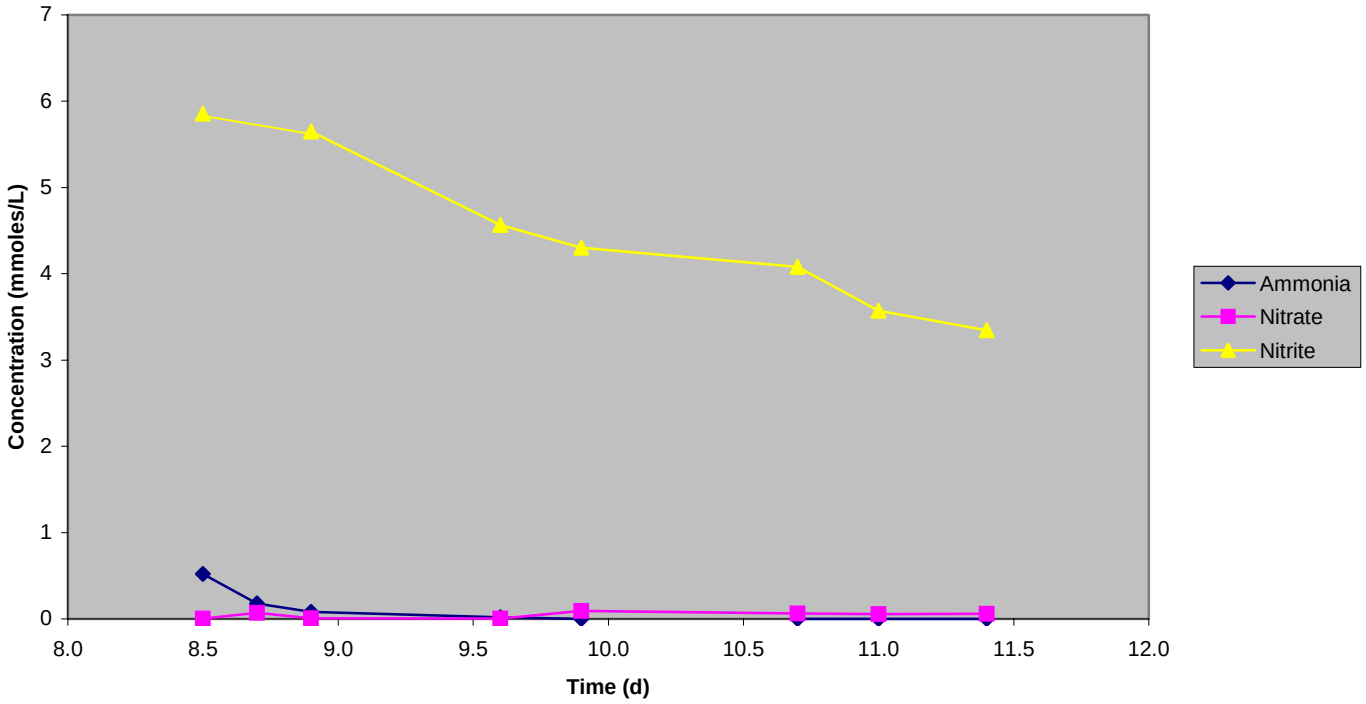

Figure 4.4: Results from chemical analysis carried out on samples withdrawn between days 8 to 12 (batch set-up).

4.3.1.2 Continuous Phase (day 15 to 51)

Figure 4.5 details the results from chemical analysis carried out on samples withdrawn during the continuous phase set-up of the FFBR (days 15 to 51) and Table 4.2 list selected stoichiometric values (actual and expected) associated with these results. By day 23, the pH in the FFBR reached 8.0, and remained at that value throughout the period of monitoring. NH_4^+ removal efficiency was continually high, averaging 95.7% over the 36 days of the continuous-flow set-up. NO_2^- was decreasingly abundant in the effluent stream from day 21 onward. As can be seen from Figure 4.5, total N removal is apparent. DO in the FFBR remained low, typically less than 0.25 mg/L, and alkalinity remained high (greater than 812 mg $CaCO_3$/L) throughout the monitoring period.

By day 33, nitrate production was observed and continued to increase up to day 51. Simultaneous removal of both NH_4^+ and NO_2^- with concomitant NO_3^- production can clearly be seen from day 32 to day 51 (Figure 4.5). This would suggest anaerobic ammonium oxidation is possibly taking place in the FFBR (Pynaert et al., 2003).

Examining Table 4.2, based on residual NH_4^+ after Anammox is taken into account and assuming no NOB activity, the concentration of DO consumed does not correspond to that which would be expected when following equation 1 (assuming feed

solution is the only source of DO, ignoring other possible sources such as diffusion through tubing etc.). This is the case for days 19 to 51. Furthermore, a greater concentration of NO_3^- was being generated from day 33 to 51, suggesting possible NOB activity (equation 4.4). The high level of NH_4^+ removal may suggest that the anammox process was occurring at a greater extent than the NH_4^+ removal process mediated by the AAOB.

$$NO_2^- + 1/2O_2 \rightarrow NO_3^- \qquad \textbf{(4.4)}$$

Other metabolisms, such as those from heterotrophic denitrifiers, may have contributed further to the stoichiometry observed (however, this is unlikely due to the low biomass yield in the reactor).

It is also possible that some of the NH_4^+ in the feed was being stripped as it sat in the influent jar, while being continually stirred. Such stripping could have a significant impact on the actual stoichiometry matching the expected stoichiometry as detailed in Table 4.2. For example, on day 51, were 0.5 mmoles/L of ammonium stripped from the feed jar, expected DO consumption would match closely the actual DO consumption. On this basis and with an adjusted NH_4^+ removal of 2.77 moles/L, the DO consumption expected would have been 0.27 mmoles/L. This compares well with an actual DO consumption of 0.24 mmoles/L.

The stoichiometric values listed in Table 4.2 indicate that the OLAND process is, most likely, one of the metabolisms occurring in the reactor. Stabilization of the OLAND system is possibly occurring, when causes of discrepancies are taken into account. However, elucidating metabolism occurring in a reactor can be difficult when examining combined stoichiometry. To determine what metabolisms are occurring in the reactor, batch experiments were needed.

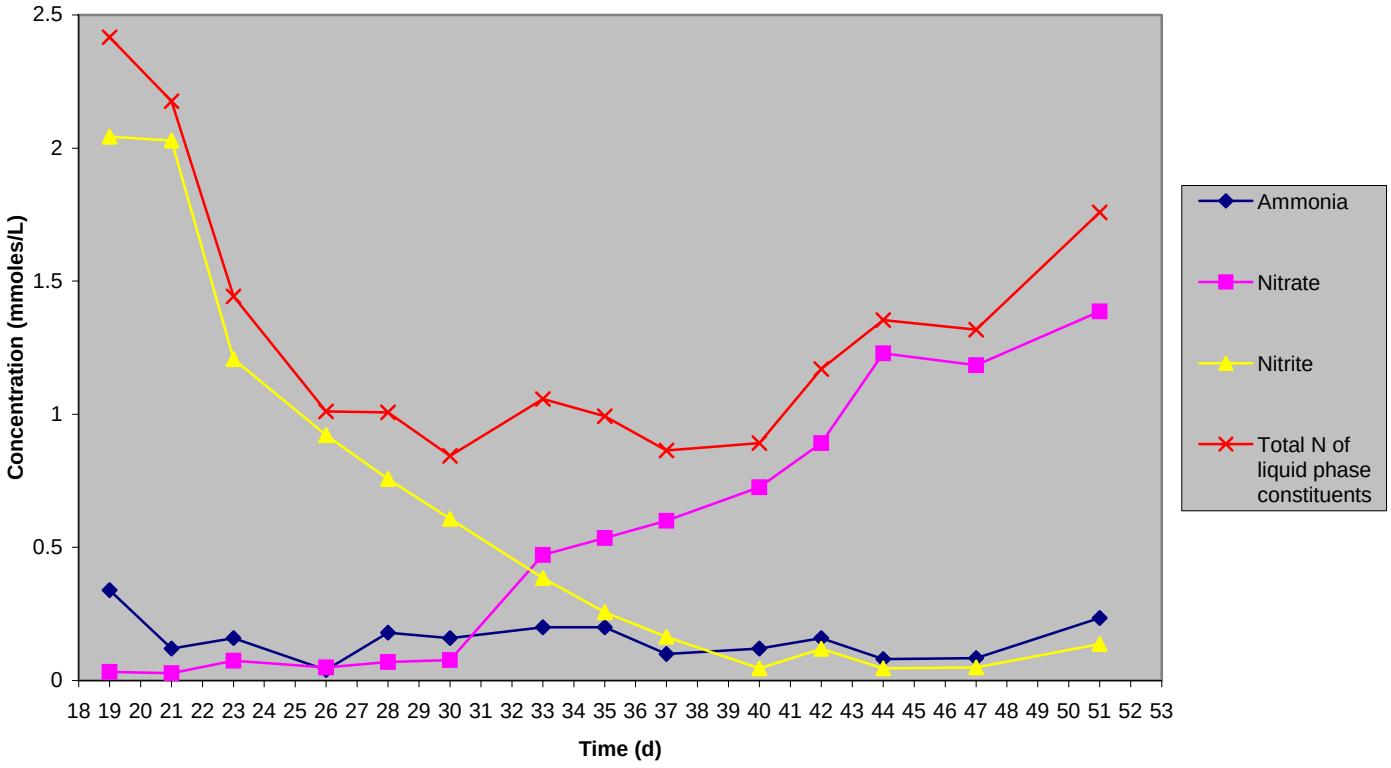

Figure 4.5: Results from chemical analysis carried out on samples withdrawn periodically from days 19 to 51 (continuous flow set-up).

Table 4.2: Selected stoichiometric values (expected and actual) for sample withdrawn on days 19 to 51 (continuous flow set-up). It is assumed that there is no NOB activity.

Sample Day	DO (mg O_2/L)	NO_2^- Consumed (mmole/L)	Expected NO_3^- production based on Anammox (mmole/L)	Actual NO_3^- produced (mmole/L)	Expected NH_4^+ Consumption via Anammox (mmole/L)	Actual NH_4^+ Consumed (mmole/L)	Residual left for AAOB after Anammox (mmole/L)	Expected DO consumption based on residual NH_4^+ (mmole/L)	Actual DO Consumption (mmole/L)	Expected N_2 gas production (L)
Week 1										
19	0.85	1.46	0.21	0.03	1.12	3.16	2.04	3.06	0.24	0.03
21	0.22	1.47	0.21	0.03	1.13	3.38	2.25	3.37	0.26	0.04
23	0.20	2.29	0.32	0.08	1.76	3.34	1.58	2.36	0.26	0.04
Week 2										
26	0.18	2.58	0.36	0.05	1.98	3.46	1.48	2.21	0.26	0.04
28	0.24	2.74	0.39	0.07	2.11	3.32	1.21	1.82	0.26	0.04
30	0.28	2.89	0.41	0.08	2.23	3.34	1.11	1.67	0.25	0.04
Week 3										
33	0.20	3.11	0.44	0.47	2.40	3.30	0.90	1.36	0.26	0.04
35	0.20	3.24	0.46	0.54	2.49	3.30	0.81	1.21	0.26	0.04
37	0.20	3.34	0.47	0.60	2.57	3.40	0.83	1.25	0.26	0.04
Week 4										
40	0.24	3.45	0.49	0.73	2.66	3.38	0.72	1.08	0.26	0.04
42	0.28	3.38	0.48	0.89	2.60	3.34	0.74	1.11	0.25	0.04
44	0.28	3.45	0.49	1.23	2.66	3.42	0.76	1.14	0.25	0.04
Week 5										
47	0.28	3.45	0.49	1.19	2.65	3.42	0.76	1.14	0.25	0.04
51	0.74	3.36	0.47	1.39	2.59	3.27	0.68	1.02	0.24	0.04

4.3.2 Batch Experiments

Figures 4.6 and 4.7 graphically represent the results obtained for batch experiments Aerobic 1 and Aerobic 2 (replicate experiments), respectively. Figures 4.8 and 4.9 represent the results obtained form anaerobic batch experiments Anaerobic 3 and Anaerobic 4 (replicate experiment), respectively. With the half saturation coefficient for nitrifiers being as low as 1 mg/ L as N and an initial concentration of 28 mg NH_4^+-N/L, the system was treated as zero order.

The primary purpose of the batch experiments was to test the hypothesis that the biofilm from the FFBR was indeed removing N through a combination of aerobic and anaerobic ammonia oxidation. In the aerobic experiments two possible reactions can occur, that due to AAOB (Equation 4.1) and that due to NOB (Equation 4.4).

From the two aerobic experiments (DO maintained at greater than 8 mg O_2/L), the aerobic ammonia consumption rate of the biofilm, based on ammonia consumed over the first 12 hours, was quantified as 300 mg N g VSS^{-1} day^{-1} (treating the system as zero order). Examining Figures 4.6 and 4.7 it can be seen that sustained nitrite formation did not occur. Furthermore, the formation of NO_3^- was found to be negligible (< 1 mg N g VSS^{-1} day^{-1}). With little NO_3^- being produced, any significant presence of NOB in the FFBR can be ruled out (and also the activity of AnAOB during the aerobic batch experiment). Based on initial and final concentrations of both NH_4^+ and NO_2^-, molar ratios of NH_4^+ removed to NO_2^- generated were found to be 0.01 and 0.2 for batch experiments Aerobic 1 and Aerobic 2, respectively. However, assuming AAOB alone are active during the aerobic batch experiments, for every mole of NH_4^+ consumed per L, 1 mole of NO_2^- per L should be formed. Clearly, a significant amount of NO_2^- was not produced, contrary to what would be expected.

The pH in each aerobic vessel rose to 8.9 within the first 3 hours of operation, and stayed at this value. Considering this pH, it can be suggested that NH_4^+ was not consumed by the biomass but rather NH_4^+ lost was due to the volatilization of NH_3 from the vessels. Employing equation 4.5 (Anthonisen et al., 1976), the amount of free ammonia as NH_3 generated, based on both the pH of the batch vessel and the initial concentration of NH_4^+-N added, can be calculated.

$$\text{Free Ammonia as } NH_3 \text{ (mg/L)} = 1.21 \times \frac{\text{total ammonia as N (mg/L)} \times 10^{pH}}{e^{(6{,}344/273 + {}^{o}C)} + 10^{pH}} \quad \textbf{(4.5)}$$

With a pH of 8.9, *at least* 12 mg/L of NH_3 (0.7 mmoles NH_3/L) were produced in each aerobic batch vessel over the course of the experiments. Considering negligible production of NO_2^-, the result calculated from equation 4.5 explains more clearly the drop in NH_4^+ concentration from the start to finish of the aerobic batch experiments.

For the anaerobic experiments, due to the low DO environment created (< 0.6 mg O_2/L), it was expected that only the anammox process would take place. The specific anaerobic ammonia consumption rate, based on removal of ammonia over the first 12 hours, was found to be 262 mg N g VSS^{-1} day^{-1} (with the system being treated as zero order). However, the nitrite concentration did not decrease during the experiment and little nitrate was produced. In order to suggest anaerobic ammonium oxidation was taking place, simultaneous removal of both NH_4^+ and NO_2^- with concomitant NO_3^- production (0.26 moles produced for every mole of NH_4^+ consumed) should have been observed. This was clearly not the case, as reflected by the results shown in Figures 4.8 and 4.9. Referring to Equation 4.2, if the anammox process was taking place in the anaerobic batch vessels it would be expected that for every mole of NH_4^+ consumed, 1.3 moles of NO_2^- should also be consumed. The molar ratio of NO_2^- removed to NH_4^+ removed for batch experiments Anaerobic 3 and Anaerobic 4 was found to be 0.47 and 0.01, respectively. These ratios for the anaerobic experiments do not compare well with expected values.

The pH in each anaerobic vessel rose to 8.5 within the first 6 hours of operation, and stayed at this level (± 0.1). Again this suggests that rather than NH_4^+ being consumed by the biomass, it is more likely that NH_4^+ loss was due to the volatilization of NH_3 from the vessels. Based on a pH of 8.5 and employing equation 4.5, over the course of the two anaerobic batch experiments at least 6 mg/L of NH_3 (0.4 mmoles NH_3/L) were produced. In light of little NO_2^- being consumed and insignificant NO_3^- generation, the results calculated from equation 4.5 explain more clearly the drop in NH_4^+ concentration from the start to finish of the anaerobic batch experiments.

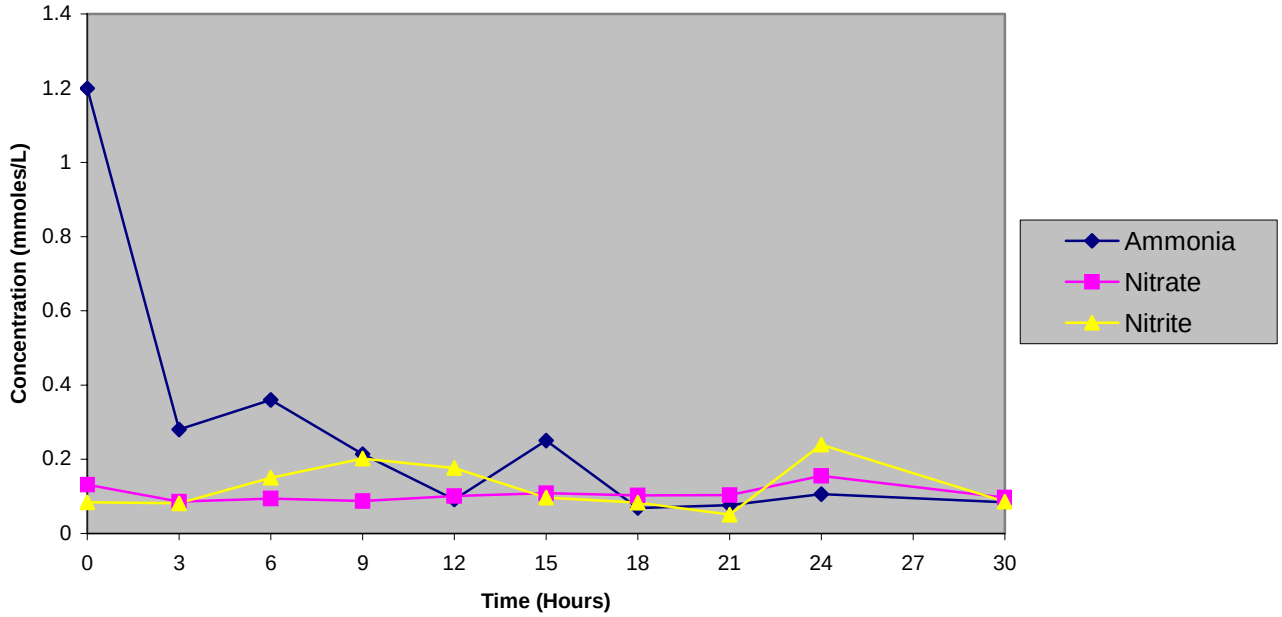

Figure 4.6: Performance of aerobic batch vessel 1

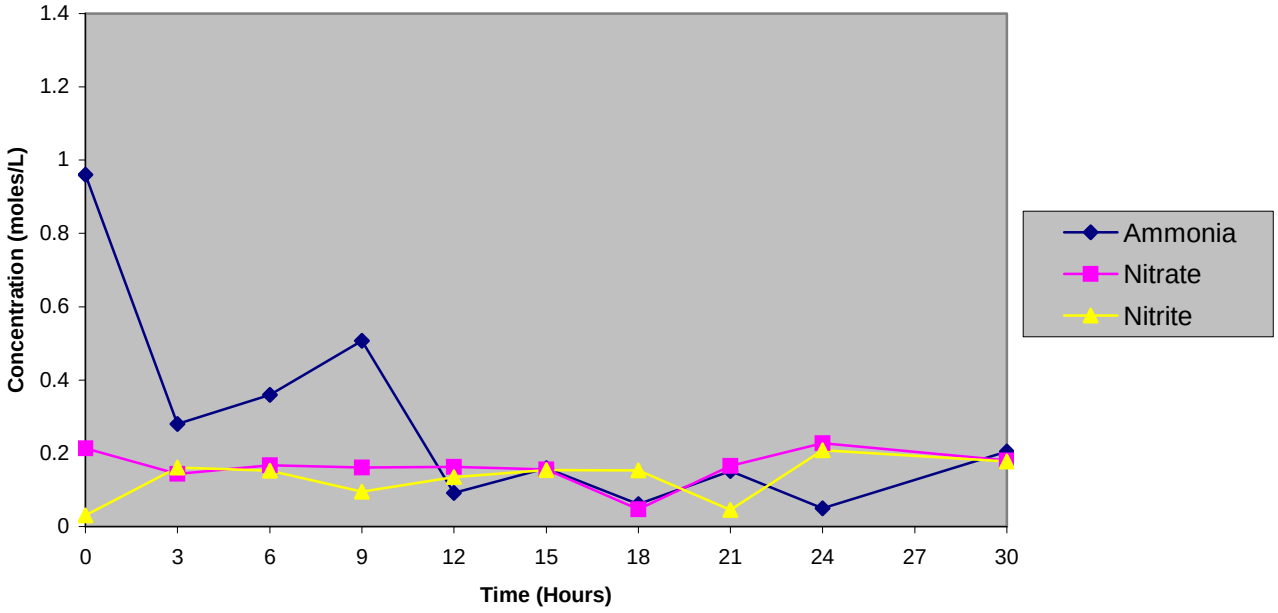

Figure 4.7: Performance of aerobic batch vessel 2.

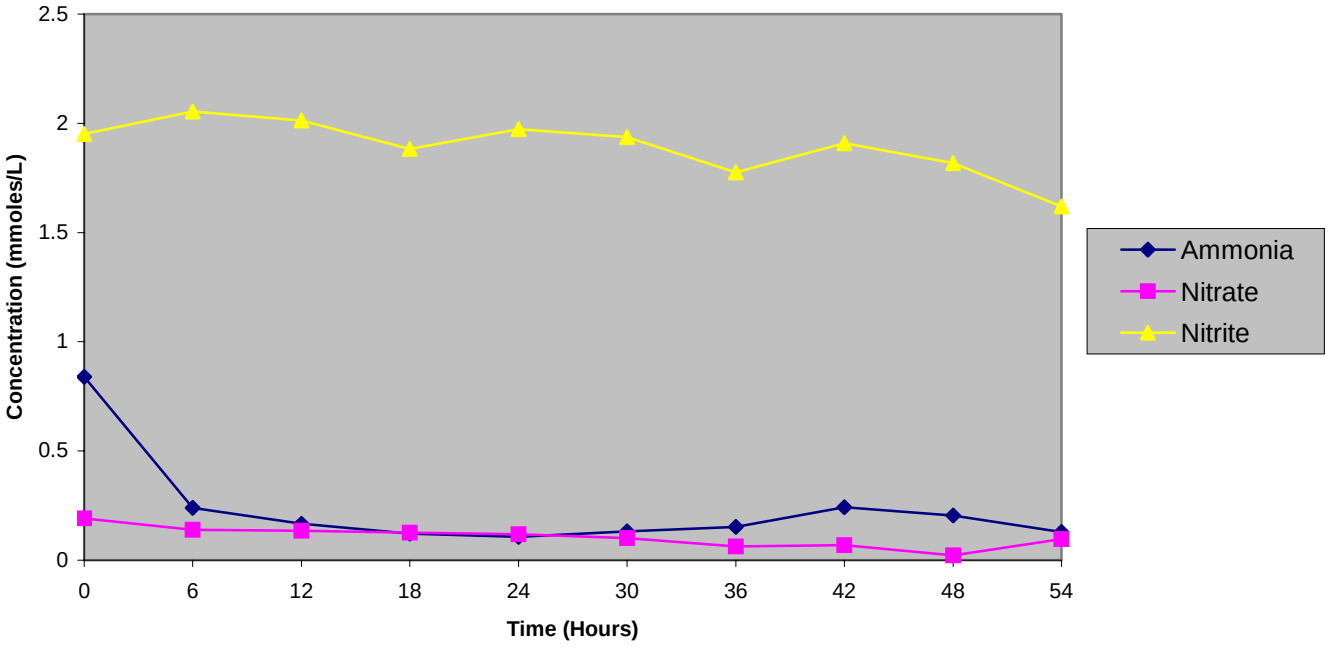

Figure 4.8: Performance of anaerobic batch vessel 3.

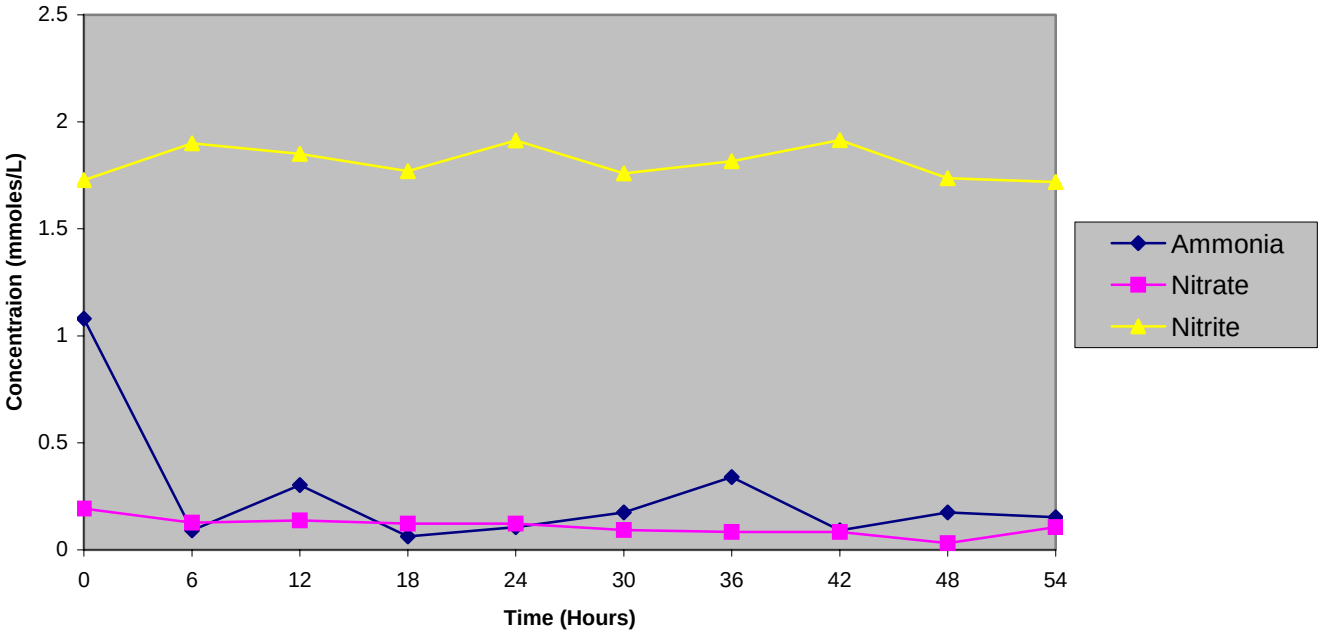

Figure 4.9: Performance of anaerobic batch vessel 4.

4.4 Conclusions

An FFBR OLAND system, inoculated with OLAND sludge, was constructed. This system was monitored and maintained over a 51-day period. By day 51 simultaneous removal of both NH_4^+ and NO_2^- with concomitant NO_3^- production was observed, a key indicator of possible anammox activity. While the OLAND system did not reach stabilization by day 51, the trend followed by stoichiometric parameters over the 51 days suggested that a stable OLAND system was being approached. However, this approach could have been disrupted by the possible presence of NOB, indicated by a higher than expected generation of NO_3^- during the continuous flow set-up from day 33 onwards. Furthermore, a degree of inaccuracy in stoichiometric parameters determined may have occurred due to stripping of NH_4^+ from the synthetic feed. Throughout the monitoring period, removal of ammonia was continually high, typically over 96 %. While ammonia loading conditions were low and kept constant at 83.8 mg NH_4^+/day (4.6 mmoles NH_4^+/day), results indicate that the FFBR OLAND system was highly effective at removing ammonia.

In order to verify that OLAND based metabolism was occurring within the reactor, batch experiments at the end of the 51-day monitoring period were conducted. In contrast to what was being observed in the FFBR, the batch experiments suggested that OLAND based metabolism was not occurring in the reactor. However, the reliability of the batch experiments was low based on the high pH observed in each batch vessel, leading to a significant degree of NH_3 volatilization.

It is suggested that for future FFBR OLAND systems, the influent to the reactor be frequently monitored to determine if NH_3 volatilization is occurring. Furthermore in the conduct of batch experiments, the pH of the system must be maintained at an acceptable level to prevent volatilization of NH_3. It is also suggested that the N load to the reactor be increased periodically during start-up to allow for greater biomass growth. Maintaining the load at a constant value limited the growth-potential of the biomass. Finally, further research examining the use of the OLAND based system in a dairy setting is needed prior to utilizing the FFBR in the field. Such research includes the establishment of laboratory scale systems using anaerobically stabilized dairy manure and a pilot scale set-up of the OLAND FFBR system.

Chapter 5: Engineering Significance

Today's largest farms, which produce the majority of society's needs for food and fiber, are more akin to industrial activities and can produce a significant amount of pollution. Dairy farms are no exception. With the growth of concentrated livestock facilities, serious issues of excessive N input into sensitive watersheds and gaseous N emissions into the atmosphere have arisen. On foot of these problems much research has been conducted to reduce the amount of N exiting the farm. Research has ranged from animal diet composition to the use of wastewater treatment units for the reduction of N released from the farm. However, little research has examined the use of a novel biological process called OLAND to reduce nitrogen in agricultural waste, particularly dairy manure.

When implementing a proposed wastewater treatment system or altering an existing set-up, the characteristics of the wastewater treated must be known. Each individual dairy operation will display varying wastewater characteristics, which must be determined for effective use of the treatment technology developed. This study also provides a detailed template for conducting such an analysis for dairy farming facilities. By following this template, the strengths and characteristics of dairy-farm wastewater, on a particular day and at each stage of operation, can be assessed.

Any wastewater treatment unit proposed for the removal of N in an agricultural setting must be cost effective, require a plausible level of management skill, have a small footprint and be easily retrofitted to existing treatment facilities. The OLAND system has strong potential to satisfactorily address each of the points. This study provides a preliminary exanimation for how an OLAND based treatment system should be established and maintained during the first months of start-up. Presented is a detailed procedure for the start-up and stabilization of an OLAND FFBR system and a protocol to verify that OLAND based metabolism is occurring. The system developed is simple in construction and capable of high ammonia removal efficiencies. This study is the first stage in demonstrating the application of OLAND technology to anaerobically stabilized dairy manures.

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Appendix 1: Physical Parameters and Efficiency Calculations, Manure Treatment System Water Quality Assessment, Johnson Highland Dairy Farm, Glade Spring, Virginia

A1.1 Physical Parameters of Manure Treatment System

Main Barn:

- Barn length = 132 m
- Total flushing alley width = 2 x (3 m + 4 m) = 14 m
- Flow exiting the main barn = 2 flushes/day x 100 m^3/flush = 200 m^3/day

Mixing Basin & Mixing Basin Trench:

- Area of total trench = 26.8 m^2
- Area of mixing basin = 98.3 m^2
- Flow exiting the mixing basin and trench to separator = 2 flushes/day x (100 + 52) m^3/flush = 304 m^3/day

Flush Water Storage Tank associated with Main Barn:

- Circumference = 10.44 m
- Active height = 11.43 m
- Cross Sectional Area (CSA) = 8.64 m^2
- Volume = 98.74 m^3
- Flow from flush valve in barn is based on the volume in the storage tank, i.e. flow exiting flush valve = 2 flushes/day x 98.74 m^3= 197.5 m^3/day
- Pipe diameter to barn = 1.07 m (x 2 pipes)

Flush Water Storage Tank associated with Loafing Barn:

- Circumference = 10.44 m
- Active height = 10.44 m
- CSA = 8.64 m^2
- Volume = 52 m^3 (assumed to be equal to the volume exiting the loafing barn per flush)
- Flow from flush valve in barn is based on the volume in the storage tank, i.e. flow exiting flush valve = 2 flushes/day x 52 m^3= 104 m^3/day
- Pipe diameter to barn = 1.07 m (x 1 pipe)

Steady State Flow Rate in Main Barn:

- Outer alley = 0.25 m^3/sec

- CSA of flow = 3.81 cm x 3 m
- Velocity = 21.9 m/10.58 sec = 2.07 m/sec
- Velocity was measured by determining the time taken for a half submerged object in the flush flow to travel a known distance (here distance = 21.9 m)
- Flow rate = CSA*Velocity

- Inner Alley = 0.29 m^3/sec

  - CSA of flow = 3.81 cm x 4 m
  - Velocity = 51.2 m/27 sec = 1.89 m/sec
  - Velocity measured in similar fashion to outer ally measurement (here distance = 51.2 m)
  - Flow rate = CSA*Velocity

Volume of Water used in Typical Flush of One Group:

- Increase in water-table level in mixing basin & trench due to fill from flush = 0.2 m
- Total area of mixing basin & trench = 125 m^2
- Vol. = 875 ft^3

Flush of Total Main Barn:

- 4 x 25 m^3 = 100 m^3

Residence Time in the anaerobic storage facility:

- Vol.= 9,085 m^3
- Assume: Anaerobic storage facility acts as a Continuous Stirred Tank Reactor (CSTR).
- Assume: Liquid loss in solids during separation is equal to volume of fresh water added to water withdrawn from the anaerobic storage facility (anaerobic storage facility volume remains constant), that is, 15% of the total liquid volume from the flush does not enter the anaerobic storage facility, but is lost in the solids during mechanical separation.
- Approx. fill from each flush after separation = (1-0.15) x (100 + 52) m^3 = 130 m^3
- Approx fill/day = 2 x 130 m^3 = 260 m^3/day
- Residence time = 9,085 m^3 / 260 m^3/day = 35 days
- Flow exiting anaerobic storage facility = 2 x 130 m^3 = 260 m^3/day = Flow of liquid fraction exiting separator

Residence Time in Mixing Basin & Trench:

- As milking commences, flushing of each group area begins after the respective group has left the main barn. Total milking time for all groups takes approximately 5 hours. Only one group vacates the main barn at a time, after which flushing can take place. The mixing basin and separator are turned on

approximately 1.5 to 2 hours after all milking has concluded. The residence time in the mixing basin and trench is approximately 6 hours from first flushing to 2 hours for the final flush. The average residence time in the mixing basin and trench is 4 hours.

Flow Rate for Parlor

- Volume exiting parlor per flush = 7.57 m^3
- Flow rate exiting parlor = 7.57 m^3 x 2 flushes/day = 15.14 m^3/day

A1.2 Estimated removal of g TSS/Day

- Volume passing through separator per day = 260 m^3/day (from main barn, loafing barn and parlor)
- TSS in separator effluent = 4.7667 g/L = 1,225,128 g TSS/day
- TSS entering from main barn, loafing barn and parlor = 3,613,562 g TSS/day
- g TSS removed/day = 2,388,434 g TSS/day

A1.3 Treatability in the Anaerobic Storage Facility

- Alkalinity in anaerobic storage facility = 3,630 mg $CaCO_3$/L
- Assume aerobic growth of autotrophs with ammonia as the electron donor is represented by the following stoichiometric equation:

  $NH_4^+ + 3.300O_2 + 6.708\ HCO_3^- \rightarrow 0.129\ C_5H_7O_2N + 3.373\ NO_3^- + 1.041\ H_2O + 6.463\ H_2CO_3$

- Assume: $NH_4^+ = NH_3$-N = 631 mg N/L
- Moles/L NH_4^+ = 0.057
- 0.057 * 6.708 = 0.38 moles HCO_3^-/L
- 0.38 moles HCO_3^-/L * 61g/mole = 23.2 g/L alkalinity needed
- We have 3.63 g/L as $CaCO_3$; therefore insufficient alkalinity is present for nitrification upon aeration of anaerobic storage facility. Unless pH is controlled, the pH will drop below the physiological range of specific microorganisms necessary for nitrification and the system performance will be adversely affected.

Appendix 2: Summary of Water Quality Data from Sample Event 1 (5/24/2004), Manure Treatment System Water Quality Assessment, Johnson Highland Dairy Farm, Glade Spring, Virginia

Table A2.1: Results from chemical analysis carried out on each sample for specified locations on the Johnson Dairy Farm based on sample event 1 (5/24/2004).

	Location (sample point number)				
Parameter measured	**Flush Valve Main Barn (1)**	**Exit of Main Barn (2)**	**Mixing Basin (3)**	**Separator (4)**	**Anaerobic Storage Facility (5)**
TS (g/L)	0.43 ± 0.21	13.40 ± 3.49	8.47 ± 0.14	9.73 ± 0.48	11.00 ± 0.22
VS (g/L)	0.18 ± 0.03	10.86 ± 2.87	5.19 ± 0.09	6.39 ± 0.37	6.44 ± 0.15
TSS (g/L)	0.02 ± 0.01	6.77 ± 0.40	2.20 ± 1.41	3.40 ± 0.65	3.40 ± 0.26
VSS (g/L)	0.02 ± 0.01	6.53 ± 0.35	1.85 ± 1.06	3.37 ± 0.60	3.33 ± 0.21
Alkalinity (mg $CaCO_3$/L)	171 ± 1	932 ± 0	2,510 ± 16	2,430 ± 35	3,770 ± 56
Total COD (mg O_2/L)	11 ± 2	2,630 ± 0	2,000 ± 0	3,650 ± 1420	2,630 ± 1090
Sol. COD (mg O_2/L)	3 ± 2	2,260 ± 0	1,160 ± 0	2,160 ± 364	1,470 ± 315
Total TKN (mg N/L)	6 ± 0	796 ± 2	543 ± 29	567 ± 60	770 ± 33
Sol. TKN (mg N/L)	3 ± 0	157 ± 2	336 ± 10	365 ± 15	452 ± 37
NH_3-N - Acidified (mg N/L)	0 ± 0	84 ± 0	327 ± 5	274 ± 46	479 ± 18
NH_3-N - Frozen (mg N/L)	4 ± 4	190 ± 8	420 ± 14	450 ± 74	706 ± 4
Sol P (mg/L)	0.02 ± 0.01	3.52 ± 0.07	1.71 ± 0.05	2.82 ± 0.28	4.00 ± 0.05
Total P (mg/L)	1	71	80	83	130
NO_2^- (mg/L as N)	0.3 ± 0.5	0.6 ± 0.3	1.1 ± 0.8	1.7 ± 0.0	2.0 ± 0.1
NO_3^- (mg/L as N)	2.6 ± 0.8	1.9 ± 0.1	0.7 ± 0.0	0.0 ± 0.0	0.6 ± 0.0

Appendix 3: Chemical Analysis Results for OLAND FFBR Operation During Days 0 to 51, and for Aerobic and Anaerobic Batch Experiments

Table A3.1: Results from chemical analysis carried out on samples withdrawn on days 1 to 5 (Batch set-up).

Sample Day	pH	DO (mg/L)	Nitrate (mg – N/L)	Nitrite (mg - N/L)	Ammonia (mg NH_4-N/L)
1	7.2	-	0.00	45.2	2.99
2	7.2	-	0.00	43.2	4.29
3	7.4	-	0.00	39.2	1.31
4	7.3	0.82	0.00	34.9	0.75
5	7.3	0.97	0.00	26.7	0.75

Table A3.2: Results from chemical analysis carried out on samples withdrawn days 8 to 11 (Batch set-up)

Sample Day	pH	DO (mg/L)	Nitrate (mg – N/L)	Nitrite (mg - N/L)	Ammonia (mg NH_4^+-N/L)
8.5	7.1		0.05	82.0	7.28
8.7	7.4	0.86	1.00	28.7	2.52
8.9	7.4		0.08	79.1	1.12
9.6	7.4		0.07	63.9	0.28
9.9	7.4		1.28	60.2	0.00
10.7	7.4	0.87	0.87	57.1	0.00
11.0	7.4		0.77	50.0	0.00
11.4	7.4		0.82	46.8	0.00

Table A3.3: Results from chemical analysis carried out on samples withdrawn days 15 to 51 (Continuous flow set-up).

Sample Day	pH	DO (mg/L)	Nitrate (mg -N/L)	Nitrite (mg -N/L)	Alkalinity (mg $CaCO_3$ -N/L)	TSS (g/L)	VSS (g/L)	Ammonia (mg NH_4-N/L)
Week 1								
19	7.5	0.85	0.46	28.6	960	0.0138	0.0031	4.76
21	7.8	0.22	0.39	28.4		0.0108	0.0062	1.68
23	7.9	0.20	1.05	16.9		0.0154	0.0031	2.24
Week 2								
26	8.0	0.18	0.69	12.9	905	0.0165	0.0118	0.56
28	8.0	0.24	0.98	10.6		0.0118	0.0094	2.52
30	8.0	0.28	1.07	8.50		0.0129	0.0118	2.24
Week 3								
33	8.0	0.20	6.60	5.40	868	0.0100	0.0070	2.80
35	8.0	0.20	7.50	3.60		0.0080	0.0040	2.80
37	7.9	0.20	8.40	2.30		0.0070	0.0060	1.40
Week 4								
40	8.0	0.24	10.2	0.64	812	0.0090	0.0040	1.68
42	8.0	0.28	12.5	1.66		0.0270	0.0230	2.24
44	8.0	0.28	17.2	0.64		0.0160	0.0150	1.12
Week 5								
47	8.0	0.28	16.6	0.69	824	0.0170	0.0060	1.17
51	8.0	0.74	19.4	1.92		0.0130	0.0050	3.29

Table A3.4: VSS/TSS values for batch experiments and the FFBR.

Batch Vessels TSS/VSS			*Reactor TSS/VSS*	
Vessel	*TSS (mg/L)*	*VSS (mg/L)*		
Aerobic 1	161	133	*TSS (mg/L)*	231
Aerobic 2	64	52	*VSS (mg/L)*	205
Anaerobic 3	92	73		
Anaerobic 4	68	57		

Table A3.5: Results from chemical analysis carried out on samples withdrawn from vessel Aerobic 1.

Hours after time zero	pH	DO (mg/L)	Ammonia (mg NH_4^+ -N /L)	Nitrate (mg - N/L)	Nitrite (mg –N/L)
0	7.0		16.8	1.84	1.18
3			3.92	1.20	1.14
6	8.9		5.04	1.32	2.10
9			3.00	1.22	2.83
12			1.28	1.41	2.47
15			3.50	1.53	1.35
18	8.9		0.96	1.44	1.16
21			1.07	1.45	0.71
24	8.9	9.1	1.49	2.18	3.35
30	8.9		1.17	1.35	1.20

Table A3.6: Results from chemical analysis carried out on samples withdrawn from vessel Aerobic 2.

Hours after time zero	pH	DO (mg/L)	Ammonia (mg NH_4^+ -N /L)	Nitrate (mg - N/L)	Nitrite (mg –N/L)
0	7		13.4	2.99	0.42
3			3.92	2.02	2.26
6	8.9		5.04	2.35	2.14
9			7.10	2.26	1.33
12			1.28	2.29	1.89
15			2.23	2.18	2.16
18	8.9		0.88	0.66	2.15
21			2.13	2.32	0.64
24	8.9	8.95	0.70	3.19	2.92
30	8.9		2.87	2.53	2.49

Table A3.7: Results from chemical analysis carried out on samples withdrawn from vessel Anaerobic 3.

Hours after time zero	pH	DO (mg/L)	Ammonia (mg NH_4^+ -N /L)	Nitrate (mg - N/L)	Nitrite (mg –N/L)
0	7		11.8	2.69	27.3
6	8.4		3.36	1.94	28.7
12	8.5		2.34	1.88	28.2
18			1.70	1.76	26.4
24	8.5		1.49	1.66	27.6
30	8.6		1.84	1.42	27.1
36			2.13	0.88	24.9
42	8.5		3.40	0.95	26.7
48			2.87	0.31	25.5
54		0.5	1.81	1.35	22.8

Table A3.8: Results from chemical analysis carried out on samples withdrawn from vessel Anaerobic 4.

Hours after time zero	pH	DO (mg/L)	Ammonia (mg NH_4^+ -N /L)	Nitrate (mg - N/L)	Nitrite (mg –N/L)
0	7		15.1	2.70	24.2
6	8.6		1.28	1.77	26.6
12	8.5		4.24	1.92	25.9
18			0.88	1.71	24.8
24	8.5		1.49	1.71	26.8
30	8.6		2.44	1.29	24.6
36			4.77	1.16	25.4
42	8.6		1.28	1.18	26.8
48			2.44	0.43	24.3
54		0.6	2.13	1.48	24.1