**Investigation of a Commercial Product (BiOWiSH^{TM}) for
Nitrogen Management**

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for Nitrogen Management

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Abstract

Investigation of a Commercial Product (BiOWiSH™) for Nitrogen Management

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BiOWiSH–Aqua, which has the capability of treating nitrogen from wastewater through bioaugmentation, is a commercial product consisting of a blend of microorganisms developed by BiOWiSH Technologies. A study of the treatment of nitrogen compounds (i.e. NH_3 , NO_3^- , and NO_2^-) using Biowish–Aqua was conducted using small scale experiments (flask experiments) and large scale experiments (column reactor experiments). In this work, column reactors were created to test Biowish–Aqua’s nitrogen treating capabilities by providing enough depth to simulate the dissolved oxygen gradient that can be observed in a pond. The results show that the optimal growth conditions for both ammonia assimilating and denitrifying bacteria are an anoxic environment with a carbon-to-nitrogen ratio of 2:1. Under this optimal growth environment, Biowish–Aqua was able to assimilate ammonia with a zero order k value of 3.06 ppm/day. Also, under the same conditions, Biowish–Aqua was able to eliminate nitrate (NO_3^-) and nitrite (NO_2^-) at a rate of 9.58 ppm/day and 5.64 ppm/day respectively. The experiments also suggested that with a C:N ratio of 2:1, Biowish–Aqua did not have an effect in slowing the hydrolysis of urea.

Overall, this research suggests that the application of Biowish–Aqua is a feasible nitrogen removing strategy for wastewater with initial presence of ammonia and nitrate between 10 to 20 ppm.

Keywords: Ammonia assimilation, Bioaugmentation, BiOWiSH, Denitrification

To my best friends Gene and Jorge

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CHAPTER 1

Introduction and Background

The purpose of this research was to evaluate the effects of a commercial product, BiOWiSH^{TM} – Aqua, which will be referred to as Biowish throughout this paper, on nitrogen management in wastewater. Biowish is a powder blend of multi-organism and media developed for wastewater treatment purposes. Wastewater can contain large amounts of nitrogen compounds in various forms. Treating nitrogen contaminants from wastewater is an important step in the pretreatment and/or the water reclamation process. In fact, nutrients, including nitrogen, are the fifth leading type of pollutants contributing to the impairment of rivers and streams (U.S. EPA 2004). Considering the importance of nitrogen removal in wastewater, Biowish can potentially be used as a cost effective alternative and/or addition to current wastewater treatment methods through bioaugmentation, a method commonly used to introduce microbial strains to treat contaminated soil or water.

The natural or artificial enrichment of nutrients, especially phosphates and nitrates, causes eutrophication, the responses of the ecosystem to these conditions. An excessive amount of nutrients in a water body will provoke algal blooms causing high levels of organic matter, which during its decomposition will consume the available dissolved oxygen present in the water. The depletion of oxygen leads to the death of other aquatic organisms (USGS 2011). Negative impacts of eutrophication include loss of submerged aquatic vegetation, oxygen deficiency, shifts in food webs and loss of harvestable fisheries. The ultimate consequence is a loss of habitat with negative effects to the aquatic biodiversity and changes in ecosystem structure and function. Over the last three decades, it is apparent that effects of eutrophication have large-scale implications and are spreading rapidly (Rabalais 2002). Figure 1.1 is a photograph of eutrophication in Lake Okeechobee, Florida.

Figure 1.1: Example of eutrophication in Lake Okeechobee, Florida (Conley 2009)

Ammonia is mainly introduced into wastewater from the decomposition of organic nitrogen compounds such as urea and proteins in manure (U.S. EPA 2006). When urea ($(\text{NH}_2)_2\text{CO}$) is discharged, water molecules displace the amine groups producing ammonia and carbonate anion. This process that generates ammonia is described as urea hydrolysis. Out of all the nitrogenous nutrients found in wastewater, ammonia is the most harmful to aquatic life. Oxidation of ammonia increases the biological oxygen demand (BOD) as it consumes dissolved oxygen. Also, even low concentration of ammonia can cause stress and damage in several tissues on gill-bearing aquatic animals. Chronic exposure can increase the susceptibility to bacterial infection and inhibit the growth of aquatic animals (Francis-Floyd 2005). Finally, high concentrations of ammonia will cause the death of numerous aquatic organisms present in the water body.

Ingestion of nitrates can lead to acute health effects on humans. At low exposure levels infants aging from 0 to 3 months have a high risk of developing methemoglobinemia or blue baby syndrome. Nitrate can bind with hemoglobin to produce methemoglobin, impairing its effectiveness at delivering oxygen to tissues resulting in the blue tint of the skin. With higher concentrations of nitrate, older children and adults can experience the same syndrome. Higher exposure levels could increase the risk of cancer including non-Hodgkin's lymphoma and stomach and gastric cancer in adults; and brain tumors, leukemia and nasopharyngeal cancer in children (U.S. EPA 2006). As for nitrites, prenatal exposure can potentially cause malformations in fetuses. Nitrite can also react in the gastrointestinal tract to form ethylnitrosourea, which is a potent carcinogen.

The United States Environmental Protection Agency (U.S. EPA) closely monitors the maximum contaminant level (MCL) allowed to be in the water. EPA sets the current MCL for ammonia, nitrate and nitrite at 0.025 ppm, 10 ppm, and 1 ppm respectively. Untreated wastewater in the United States typically contains 20 to 40 ppm of ammonia (FAO 2012), 25 to 35 ppm of nitrate (Kuphal 2005), which is 250% to 350% of the permitted nitrate concentrations. Therefore, the nitrogen pollutants in wastewater exceed the MCL to be released to surface waters. Since the effluent water needs to comply with the EPA MCL standards, the nitrogen levels in the influent wastewater need to be reduced. Nitrification and denitrification are currently the main methods used to manage nitrogen levels in wastewater.

1.1 Nitrogen Removal Processes

The nitrogen cycle is an important cycle in which nitrogen transforms between various chemical forms and can be carried out using biological and non-biological processes. Fixation, mineralization, nitrification, and denitrification are several of the important processes in the nitrogen cycle.

1.1.1 Nitrogen Assimilation

Nitrogen assimilation is the transformation of ammonium ion into essential nitrogenous compounds with the help of enzymes. There are two known pathways for the assimilation of ammonia. One is the glutamate dehydrogenase (GDH) pathway:

where α -Ketoglutarate (α -kg) is the metabolic nitrogen transporter in bacterial cells and L-Glutamine (L-Gln) is one of the essential cellular energy source. The other is the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway:

where L-Glutamate (L-Gln) provides for cellular metabolism. As seen in the above reactions, ammonia is converted by enzymatic activities into amino acids (L-glutamine).

According to a study conducted by Hipkin, it was proven certain types of yeast, such as *Candida nitratophila*, are able to assimilate ammonium in a glucose-rich media. Ammonia cannot be assimilated by cultures deprived of carbon source (Hipkin 1989).

1.1.2 Nitrification

Nitrification is the biological transformation of ammonia to nitrate. The two step process of nitrification starts with *Nitrosomonas* converting ammonia/ammonium into nitrite

and finishes with *Nitrobacters* turning nitrite into nitrate

Nitrification is usually an aerobic process that requires dissolved oxygen and sufficient inorganic carbon source to be present in the water. Growth condition such as pH and temperature are also important factors which should be maintained at 6.5 to 7.5 and 30 to 35°C respectively.

1.1.3 Denitrification

Denitrification is the conversion of nitrate, which serves as an electron acceptor, into nitrogen gas. This process is usually facilitated by heterotrophic bacteria such as *Paracoccus denitrificans* and sometimes some autotrophic organisms such as *Thiobacillus denitrificans* (Knowles 1982). Heterotrophic processes are usually preferred due to its higher removal rates. There are possible intermediate steps illustrated as follows

in which NO, N₂O and N₂ are in the gaseous phase. Transient accumulation of denitrification intermediates are commonly observed in the reduction of nitrogen ionic oxides (Knowles 1982). Less positive aspects of heterotrophic denitrification include the requirement of exogenous supply of organic carbon which may have to be removed later in the treatment process. The organic carbon source required by denitrifiers to provide electrons can be supplied in the form of methanol or glucose. The amount of organic carbon may exert different effects on rate of denitrification. The following chemical equations explain denitrification with carbon source of methanol (CH₃OH)

For denitrification to take place, zero to minimal dissolved oxygen (0.5 ppm) concentration providing an anoxic environment (USGS 2010) is required since excessive oxygen can cause bacteria to uptake oxygen instead of nitrate for respiration.

pH is also an important factor relating to the denitrification rates. In both pure cultures and natural systems, the optimum pH is between 7.0 and 8.0. Outside that range denitrification activity is gradually inhibited. Lastly, in aquatic systems, denitrification rates show minor temperature dependence.

1.2 Current Nitrogen Management Methods

The above nitrogen removal processes including nitrification and denitrification are utilized in onsite treatment systems, conventional wastewater treatment plants, and natural treatment systems.

Current practical nitrogen-removal systems for onsite applications include recirculating sand filters (RSFs) with enhanced anoxic modifications, sequencing batch reactors (SBR), and a range of aerobic nitrification processes combined with an anoxic/anaerobic denitrification process. (U.S. EPA 2012)

Aerobic/anaerobic trickling filter package plant uses synthetic media trickling filters that receive wastewater for aerobic treatment and nitrification. Filtrate moves to

the anaerobic zone to undergo denitrification by mixing with either septic tank contents or incoming septic tank effluent. The SBR system can remove significant amounts of nitrogen under anoxic conditions and that nitrification occurs during the “react” phase of the SBR cycle given sufficient hydraulic retention time (HRT). Intermittent sand filters (ISF) with anaerobic filters provide nitrification in the ISF and denitrification in a separate anaerobic filter. Recirculating sand filters (RSF) systems normally remove 40% to 50% of influent nitrogen. This can be enhanced by combining a greater supply of carbon, time, and mixing than normally offered in conventional recirculation tanks. Anaerobic/anoxic filters can be added to the RSF to enhance denitrification process (U.S. EPA 2012).

Nitrogen removal is becoming increasingly required on or near coastal waters or over aquifers used for drinking water. Typically, nitrogen removal systems are one of the last steps prior to Subsurface Wastewater Infiltration System (SWIS) disposal and the minimum total nitrogen standard that can be regularly met is about 10 mg/L (U.S. EPA 2012). Figure 1.2 is an illustration of the onsite treatment system.

Figure 1.2: Diagram of an onsite treatment system

In a WWTP, aeration/anoxic tanks serve as the instrument for removal of nitrogen compounds (ammonia, nitrate and nitrite). The ammonia in wastewater is minimized by nitrification in the aeration tanks with high content of dissolved oxygen. Following nitrification the anoxic tanks provide the appropriate low DO level to facilitate denitrification. Consisting of a series of parallel concrete chambers, aeration tanks are where sufficient oxygen is mechanically introduced into the wastewater. Anoxic tanks are designed in a similar fashion without the aeration device. Figure 1.3 is an example of an aeration tank at a WWTP.

Figure 1.3: Example of aeration tank at a WWTP (Waste'n WaterTech)

Many WWTPs use attached culture systems to grow sewage-digesting bacteria. One of the most common types of on-site treatment with attached growth design is trickle filters. In trickle filter design, wastewater passes over microbial films attached to the surfaces of the treatment unit in intermittent doses. A complex community of microorganisms develops in the treatment unit and these systems are capable of efficient removal of BOD. Also being aerobic, this design will help support the growth of nitrifying bacteria and be used to nitrify wastewater.

A different type of on-site treatment used in WWTPs is the activated sludge process. In this suspended culture system, the resident population of bacteria in solids and sludge of the treatment unit are utilized; usually by mixing the sewage to keep the bacteria in suspension. During the periods where mixing ceases, the solids are allowed to

settle and it is assumed that the sludge will become anaerobic. The anaerobic bacteria in the sludge will then denitrify the waste.

Wetlands are defined as land where the water table is at (or above) the ground surface long enough each year to maintain saturated soil conditions. They are usually shallow with a depth no greater than 0.6 m (2 ft). Natural wetlands are typically marshes or swamps used for treating wastewater. Natural wetlands possess a vast number of microbial organisms, which play an important role in the elimination of nutrients from the water. The major disadvantage of natural wetlands is that since all the regulations for surface waters apply to these bodies, wastewater needs to be pre-treated before being discharged to the wetland (Reed 1995). Figure 1.4 is an example of a natural wetland in Kenosha County, WI.

Figure 1.4: Example of natural wetland (Kenosha County, WI)

Constructed wetlands are systems constructed specifically for the purpose of treating wastewater. Constructed wetlands can be further divided into two categories: free-water-surface (FWS), and subsurface-flow (SF). FWS wetland is similar to a natural marsh, where the water has atmospheric exposure. SF wetland contains a permeable media, usually gravel or soil, through which the water level is kept below the top of the bed. In addition to the advantages that natural wetlands offer, constructed wetlands are not bound by normal surface water regulations, which signify that, unlike natural wetlands, pre-treatment of wastewater is not required. Furthermore, constructed wetlands have a great aesthetic appeal, create habitats for different kinds of birds and mammals, and lower the overall maintenance cost of the wastewater treatment facility (Reed 1995). Figure 1.5 shows an example of a constructed wetland in City of Columbia, MO.

Figure 1.5: Example of constructed wetland (City of Columbia, MO)

Finally, aquatic systems are shallow bodies of water with several species of aquatic plants. The plants will obtain the oxygen and carbon dioxide they need from either the atmosphere or from the wastewater itself. The aquatic plants will also serve wastewater treatment purposes by absorbing the minerals and nutrients they need from the water and consume the nitrogen contents present. Figure 1.6 shows an example of aquatic system treatment facility in the US.

Figure 1.6: Example of aquatic treatment system (NSI 2012)

1.3 Using Biowish for Nitrogen Management

Biowish is a possible cost effective alternative and/or addition to current wastewater treatment technologies. Among the various blends of Biowish^{TM} products, Biowish^{TM} – Aqua (Biowish) is developed for wastewater treatment purposes to facilitate the biological removal of nutrients, suspended solids, pathogens and other contaminants.

Biowish is available as a powder with the majority being non-living components to support growth and less than 1% active ingredients. It is mainly composed of organic ingredients such as rice brans, soy bean with full fat content and pre-cultured organisms. Activation is required prior to treatment dosage to create a live microbial culture with optimal cell count. Activation can be as basic as rehydration of the Biowish contents. The simple activation process and dosing procedure makes Biowish a suitable product in all existing biological plants, including those with minimal batch environment. Figure 1.7 is an illustration of the packaging of Biowish.

Applications of Biowish include the treatment of municipal/private sewage, and industrial wastewater. One of the case studies performed in India with duration of 110 days showed a 30% reduction in aeration energy consumption, 100% elimination of chemicals needed for solid settling and 100% sludge consumption in a spice oleoresin factory. Another case study conducted at the Wickwar Brewing Company in England in July 2009, indicate rapid and complete sludge reduction along with odor elimination (BiOWiSH Technologies 2012).

Figure 1.7: Illustration of Biowish

1.4 Thesis Outline

Chapter 2 explains the different experiments conducted to explore the application of Biowish in the management of nitrogen. It covers the basic principles of operation of the instruments used.

Chapter 3 presents the experimental results obtained, and **Chapter 4** discusses the results. Experiment 1 explores Biowish's ammonia removing capabilities. Experiment 2 tested the denitrification effects and conditions of Biowish. Experiment 3 studied Biowish's ability to remove nitrite. Experiment 4 utilizes Biowish organisms to treat

water containing both ammonia and nitrate. Lastly, Experiment 5 assesses the urea hydrolysis decelerating properties of Biowish.

Chapter 5 concludes the paper and proposes future work. Future work includes: experimenting Biowish in saline conditions, adding a soil layer to column reactors prior to upscaling the experiment into an actual pond, using actual wastewater to compare Biowish's nitrogen managing capabilities, and studying the effects of temperature fluctuations on Biowish growth.

CHAPTER 2

Materials and Methods

2.0 Experiment Setup

2.0.1 Activation Procedure for Biowish

Activating Biowish organisms provides a proper growth environment to obtain maximum cell count levels before the start of each experiment. Figure 2.1 shows a growth curve for Biowish in a reactor which illustrates the exponential growth after the lag phase. Biowish was pre-activated prior to all experiments by measuring 10.0 g of Biowish powder and adding it into 2.0 L of tap water to make a solution with final concentration of 5000 ppm. The mixture was then stirred well, covered with parafilm, and allowed to incubate at 30°C for 48 hours before using it for the experiments.

Figure 2.1: A BiOWiSH^{TM} – Aqua growth curve (Ng 2012)

2.0.2 Flask Experiments

2.0.2.1 Design Objective

To gain an understanding of conversion and/or degradation of nitrogen compounds, initial experiments were performed in Erlenmeyer flasks. The objective was to obtain information from these flask experiments to better design and conduct the larger scale column experiments.

2.0.2.2 Experimental Procedures

Necessary calculations were performed to obtain the mass and/or volume of chemicals needed as shown in Appendix A. A 300 mL mixture containing the desired amount of nitrogen compounds, organic carbon source and Biowish supernatant was prepared in clean, 500 mL Erlenmeyer flasks. The Biowish supernatant was prepared by stirring the pre-activated Biowish mixture for 15 seconds, letting it precipitate for 30 seconds, and extracting the desired volume from the liquid portion. Table 2.1 lists the chemicals used to create the solution. In order to maintain a pH between 7 and 8, a solution of 0.2 M HCl was added. Control flasks that do not contain either Biowish or glucose were also prepared for comparison. Initial samples were taken before placing the flasks in 30°C shaking incubator (New Brunswick Scientific Co. Inc. Series 25 Incubator Shaker). Later samples were taken every 8 to 12 hours to be analyzed. The methods for analysis included: Ion Chromatography (Dionex DX-120), ammonia probe (Van London pHoenix NH31501C), urea assay kit (BioAssay Systems' QuantiChrom DIUR-500), pH monitoring (Mettler Toledo S20 SevenEasy^{TM} pH). Figure 2.2 illustrates an example of a flask experiment setup.

Table 2.1: Chemicals used as nitrogen source in the experiment

Nutrient	Chemical	State
NH_3	NH_4OH (2660 ppm)	Aqueous
NO_3^-	KNO_3	Solid
NO_2^-	NaNO_2	Solid
Urea	Urea	Solid

Figure 2.2: Example of a flask experiment setup

2.0.3 Column Reactors

2.0.3.1 Design Objective and Fabrication

Column-shaped reactors were originally chosen to provide stratified dissolved oxygen conditions which mimic a natural water body. The depths selected for the column reactors were 4 ft, 6 ft, and 7 ft because they are representative of the conventional nitrogen treatment ponds.

The column reactor and the sampling device were constructed with schedule 40 Poly Vinyl Chloride (PVC) piping products. PVC was selected for its inert characteristics (withstands chemical attack by most acids, alkalis, salts, fungi and bacterial action), water and temperature resistance, and durability. It is non-toxic, odorless and machinable, which makes it easy for both alteration and installation of various attachments.

The reactor materials were purchased at The Home Depot. Each column reactor required: one 6 in. diameter pipe cut to size as the main chamber body; one cap to seal the bottom pipe opening; one 6 in. male adapter to hold the reactor in place; one 2 in. riser and ½ in. threaded ball valve for each foot of the reactor depth as sampling ports. A bottle of PVC cement was also purchased for bonding each part and sealing any leakage.

To construct each column reactor, the main chamber body was fitted with the cap and the connections sealed with PVC cement. The male adapter was glued to the bottom of the cap to create a stable flat surface that allows the reactor to be positioned upright. Sampling ports were installed at every foot of the body by drilling 1 in. diameter holes

prior to screwing in the PVC ball valves. The junctions were then sealed with PVC cement to prevent leakages. Figure 2.3 is a photograph showing all the column reactors and figure 2.4 illustrates the assembly of the column reactor.

Figure 2.3: Column reactor setup with ports

Figure 2.4: Sketch of a 4 ft column reactor

For time-sensitive readings such as dissolved oxygen (DO) and oxidation-reduction potential (ORP), a sampling device was built with one ½ in. PVC elbow, one ½ in. riser, and one female adapter to house the different electrodes needed to take measurements. The above mentioned components were connected together to form the sampling device shown in Figure 2.5. The sampling device screws on to the sampling port and allows different probe tips to remain suspended in the mixture.

Figure 2.5: Sampling device for column reactors

Six column reactors were built to simulate natural pond environment: one 7 ft tall column (38.9 L in volume), one 6 ft tall column (33.3 L), and four 4 ft tall columns (22.2 L). Sampling ports were positioned at every foot for sampling and monitoring water conditions at different depths.

2.0.3.2 Experimental Procedure

Necessary calculations were performed to obtain the mass and/or volume of chemicals needed as shown in Appendix A. Clean column reactors were filled half way (around 10 L) with tap water. Then, the calculated amount of nitrogen compounds, organic carbon, and Biowish supernatant was added (refer to 2.0.2.2 flask procedures). A 0.2 M HCl solution was used to adjust the pH close to neutral. The solution was thoroughly mixed with 1in. diameter PVC pipes and the remaining water needed to fill the column reactor was added (22.2 L for 4 ft columns and 33.3 L for 6 ft columns 38.9 L for 7 ft columns). After the column reactors were completely filled, the solution was stirred for another minute to ensure the mixture was homogeneously distributed. Column reactors were kept at 30°C with aquarium heaters (Aqueon 06107) purchased at Petco. The aquarium heaters are equipped with a thermostat which allowed the temperature to remain constant and prevented overheating.

After column reactors were fully prepared, initial samples were taken. Samples were collected at each port at 8 to 12 hour intervals. DO, ORP, OD, and pH were monitored intermittently as needed while samples were analyzed for ammonia (as needed), nitrate and nitrite concentrations.

To monitor the experiment conditions, the following instruments were calibrated and used: Dissolved oxygen (DO) probe (YSI 5905) and meter (YSI 58), oxidation-reduction potential (ORP) probe (Vernier ORP sensor), spectrophotometer (Hitachi U-3010), and pH probe and meter (Mettler Toledo S20 SevenEasy™ pH).

2.1 Experiment 1 – Ammonia Removal

To conduct this experiment, a mixture of NH_3 and Biowish was prepared and placed in shaker (refer to Table 2.1). The nominal concentrations of NH_3 and Biowish for the initial mixture were 15 ppm and 75 ppm respectively. This experiment was run for 96 hours, and to monitor the performance of Biowish in the elimination of NH_3 from the mixture, samples were taken and analyzed every 24 hours.

In this experiment, two control samples were prepared. Control 1 did not include any carbon in the mixture and Control 2 had a C:N ratio of 4:1 but it did not contain Biowish. The organic carbon content was then varied in the inoculated flasks to see its effect on the performance of Biowish in the ammonia removal process. Table 2.2 shows the different concentrations of chemical used in each shaker flask.

Table 2.2: Experimental conditions for Biowish, nitrogen source and carbon source in experiment 1

	C:N	Biowish	N source	C source
			NH ₄ OH (ammonia as N)	C ₆ H ₁₂ O ₆
Control 1	-	4.5 ml (75 ppm)	4 mL (15 ppm)	-
Control 2	4:1	-	4 mL (15 ppm)	45 mg
Flask 1	2:1	4.5 ml (75 ppm)	4 mL (15 ppm)	22.5 mg
Flask 2	4:1	4.5 ml (75 ppm)	4 mL (15 ppm)	45 mg
Flask 3	6:1	4.5 ml (75 ppm)	4 mL (15 ppm)	135 mg

2.2 Experiment 2 – Denitrification

To conduct this experiment, a mixture of NO₃⁻ (10 ppm) and Biowish (50 ppm) was prepared, and the mixture was placed in 4 ft, 6 ft and 7 ft column reactors. To obtain the optimal conditions for Biowish to eliminate the NO₃⁻ present in the initial solution, the organic carbon (glucose) content was varied as specified in Table 2.3.

After the experiment started, a small sample was collected from each column reactor's port to test the initial conditions of the experiment. A similar task was carried out at the 6th, 20th, 30th, 44th and 53rd hour to assess the NO_3^- and NO_2^- content in the mixture.

Table 2.3: Experimental conditions for Biowish, nitrogen source and carbon source in experiment 2

	Height	C:N	Biowish	N source	C source
				KNO_3 (NO_3^- as N)	$\text{C}_6\text{H}_{12}\text{O}_6$
Control	4 ft	-	222 ml (50 ppm)	1.60 g (10 ppm)	-
Column Reactor 1	4 ft	2:1	222 ml (50 ppm)	1.60 g (10 ppm)	1.11 g
Column Reactor 2	4 ft	4:1	222 ml (50 ppm)	1.60 g (10 ppm)	2.22 g
Column Reactor 3	4 ft	6:1	222 ml (50 ppm)	1.60 g (10 ppm)	3.33 g
Column Reactor 4	6 ft	6:1	333 ml (50 ppm)	2.40 g (10 ppm)	5.00 g
Column Reactor 5	7 ft	6:1	389 ml (50 ppm)	2.80 g (10 ppm)	5.83 g

2.3 Experiment 3 – Nitrite Removal

This experiment was started with two 4 ft control columns and two 4 ft Biowish inoculated columns using sodium nitrite as nitrogen source and glucose as a carbon

source. The amount of nutrient and Biowish addition are listed in Table 2.4. C:N ratio of 2:1 was chosen for all column reactors as a controlling factor.

Table 2.4: Experimental conditions for Biowish, nitrogen source and carbon source in experiment 3

	C:N	Biowish	N source	C source
			NaNO_2 (NO_2^- as N)	$\text{C}_6\text{H}_{12}\text{O}_6$
Control A	2:1	-	1.6 g (15 ppm)	1.67 g
Control B	2:1	-	1.6 g (15 ppm)	1.67 g
Reactor A	2:1	222 ml (50 ppm)	1.6 g (15 ppm)	1.67 g
Reactor B	2:1	222 ml (50 ppm)	1.6 g (15 ppm)	1.67 g

2.4 Experiment 4 – Treatment of Ammonia / Nitrate

The experiment was conducted in 4 ft and 6 ft column reactors. The initial solution contained a mixture of 20 ppm of NO_3^- , 15 ppm of NH_3 and 50 ppm of Biowish; except for the control reactor, in which Biowish was not added. Each 4 ft column was then duplicated and small samples were taken at each column reactor's port at the beginning of the experiment, as well as after 7.5, 19, 29, 48 and 72 hours to quantify the

presence of NH_3 , NO_3^- and NO_2^- . Table 2.5 shows the initial conditions in each column reactor.

Table 2.5: Experimental conditions for Biowish, nitrogen source and carbon source in experiment 4

	Height	C:N	Biowish	N source		C source
				NH_4OH (ammonia as N)	KNO_3 (NO_3^- as N)	$\text{C}_6\text{H}_{12}\text{O}_6$
Control A	4 ft	1:1	-	313 ml (15 ppm)	3.20 g (20 ppm)	1.94 g
Control B	4 ft	1:1	-	313 ml (15 ppm)	3.20 g (20 ppm)	1.94 g
Column Reactor 1	4 ft	1:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	1.94 g
Column Reactor 2	4 ft	1:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	1.94 g
Column Reactor 3	6 ft	1:1	333 ml (50 ppm)	470 ml (15 ppm)	4.80 g (20 ppm)	2.92 g
Column Reactor 4	4 ft	2:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	3.89 g
Column Reactor 5	4 ft	2:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	3.89 g
Column Reactor 6	4 ft	3:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	5.82 g
Column Reactor 7	4 ft	3:1	222 ml (50 ppm)	313 ml (15 ppm)	3.20 g (20 ppm)	5.82 g

2.5 Experiment 5 – Urea Hydrolysis

This experiment was held in 500 mL Erlenmeyer flasks containing 100 ppm of urea in each flask and different concentrations of Biowish with control flasks containing 100 ppm of urea and no Biowish as shown in Table 2.6. Also, each concentration of Biowish was tested in duplicate flasks. Every flask was sampled at time 0, 6.5, 17.5, 24, 44 and 72 hours.

Table 2.6: Experimental conditions for Biowish, nitrogen source and carbon source in experiment 5

	C:N	Biowish	N source Urea (as N)	C source $C_6H_{12}O_6$
Control	2:1	-	64 mg (100 ppm)	0.15 g
Flask 1	2:1	3 mL (50 ppm)	64 mg (100 ppm)	0.15 g
Flask 2	2:1	3 mL (50 ppm)	64 mg (100 ppm)	0.15 g
Flask 3	2:1	4.5 mL (75 ppm)	64 mg (100 ppm)	0.15 g
Flask 4	2:1	4.5 mL (75 ppm)	64 mg (100 ppm)	0.15 g
Flask 5	2:1	6 mL (100 ppm)	64 mg (100 ppm)	0.15 g
Flask 6	2:1	6 mL (100 ppm)	64 mg (100 ppm)	0.15 g

CHAPTER 3

Results

3.1 Experiment 1 – Ammonia Removal

This experiment explores the capabilities of Biowish to enhance the ammonia removal process. Table 3.1 shows the various C:N ratios prepared for the experiment.

Table 3.1: Experimental conditions for ammonia removal

	C:N	Biowish
Control 1	-	Yes
Control 2	4:1	No
Flask 1	2:1	Yes
Flask 2	4:1	Yes
Flask 3	6:1	Yes

To verify no ammonia volatilization occurred and to confirm growth of Biowish organisms in this experiment, the pH and OD values were monitored and shown in Figure 3.1. All flasks remained below pH of 7.6 which means that most of the ammoniacal N is in the non-volatile form of ammonium (NH_4^+). In the OD graph, only C:N ratio of 2:1 showed significant increase in OD value (from 0 to 0.3 in 96 hours) indicating the most bacterial growth followed by C:N ratio of 4:1.

Table 3.2 provides all the raw data obtained in this experiment while Figure 3.2 shows the NH_3 , NO_3^- and NO_2^- content as a function of time for flasks with different C:N ratios. Figure 3.3 shows similar plots for two flasks that have the same C:N ratio (4:1); one does not have Biowish in the mixture (Control 2) and the other one contains 75 ppm of Biowish in the mixture. With Biowish being the only variable, the results suggest Biowish organisms were responsible for the removal of ammonia.

In Figure 3.2, the concentration of NH_3 remained nearly unchanged in the control flask. This shows that neither assimilation, nor evaporation of NH_3 occurred in that flask. On the contrary, in the flasks that contained both Biowish and glucose, the concentration of NH_3 slowly decreased; though it never reached a concentration lower than 7 ppm after 96 hours from the start of the experiment. It is interesting to note that while the concentration of NH_3 decreased as time elapsed, the levels of NO_3^- and NO_2^- stayed constant at 0 ppm. This indicates that nitrification of the ammonia present in the solution did not occur. Table 3.3 shows the zero order k values obtained in the ammonia removal experiment.

Table 3.2a: NH_3 , NO_3^- , NO_2^- , pH and OD data for control samples for ammonia removal experiment

Time (hrs)	Control 1 (No glucose)					Control 2 (No BW, C:N = 4:1)				
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	OD_{600}	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	OD_{600}
0	15.55	0.130	0.000	7.14	0.004	16.45	0.114	0.000	7.21	0.000
24	14.63	0.125	0.000	7.14	0.002	15.28	0.117	0.000	7.20	0.002
48	15.22	0.124	0.000	7.49	0.006	15.35	0.124	0.000	7.63	0.003
72	15.28	0.141	0.000	7.39	0.004	15.84	0.125	0.000	7.55	0.000
96	13.86	0.130	0.000	7.20	0.003	15.69	0.119	0.000	7.48	0.000

Table 3.2b: NH_3 , NO_3^- , NO_2^- , pH and OD data for samples with C:N ratios of 2:1, 4:1 and 6:1 for ammonia removal experiment

Time (hrs)	C:N = 2:1					C:N = 4:1					C:N = 6:1				
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	OD_{600}	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	OD_{600}	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	pH	OD_{600}
0	14.01	0.116	0.000	7.04	0.006	15.82	0.077	0.000	7.23	0.004	14.19	0.117	0.000	7.27	0.004
24	14.18	0.130	0.000	7.13	0.008	13.13	0.077	0.000	7.12	0.004	13.93	0.116	0.000	7.48	0.004
48	13.99	0.029	0.132	6.21	0.046	11.61	0.037	0.143	6.28	0.030	13.93	0.117	0.000	7.52	0.005
72	12.36	0.000	0.097	6.62	0.213	10.35	0.059	0.164	6.24	0.068	14.82	0.121	0.000	7.42	0.002
96	7.86	0.000	0.000	6.44	0.295	7.76	0.000	0.097	6.33	0.051	12.60	0.122	0.000	6.98	0.010

(a)

(b)

Figure 3.1: (a) pH and (b) OD as a function of time for every flask in the ammonia removal experiment

Figure 3.2: Ammonia removal experiment results showing NH_3 , NO_3^- and NO_2^- concentrations as a function of time for (a) control flask (no glucose), (b) C:N = 2:1 and Biowish, (c) C:N = 4:1 and Biowish, and (d) C:N = 6:1 and Biowish

Ctrl 2 (No Biowish, C:N = 4:1)

(a)

C:N = 4:1 with Biowish

(b)

Figure 3.3: Ammonia removal experiment results showing NH_3 , NO_3^- and NO_2^- concentrations as a function of time for a C:N ratio of 4:1. (a) Control flask without Biowish and (b) flask with Biowish

Table 3.3: Zero order K values for ammonia removal experiment

Flask	Zero order K_{NH_3} (ppm/day)
Control 1	-
Control 2	-
C:N = 2:1	3.06
C:N = 4:1	1.89
C:N = 6:1	0.23

Figure 3.4: Zero order K values for ammonia removal experiment as a function of C:N ratio

Figure 3.4 shows that C:N ratio of 2:1 achieved the highest zero order k value (3.06) for the removal of ammonia, followed by C:N ratio of 4:1 (1.89).

3.2 Experiment 2 – Denitrification

This experiment explores the capabilities of Biowish in the denitrification process. This experiment started on April 5, 2011 and it was carried out for 53 hours. Table 3.4 illustrates the different C:N ratios and column reactor heights used in this experiment.

Table 3.4: Experimental conditions for denitrification

	C:N	Height
Control	-	4ft
Column Reactor 1	2:1	4ft
Column Reactor 2	4:1	4ft
Column Reactor 3	6:1	4ft
Column Reactor 4	6:1	6ft
Column Reactor 5	6:1	7ft

Table 3.5 provides the nitrate and nitrite results for the control column reactor which is plotted in Figure 3.5 with NO_3^- and NO_2^- concentrations as a function of time. Both nitrate and nitrite remained constant throughout the experiment. Data for reactors 1

to reactor 5 is recorded in Table 3.6 through Table 3.10 and plotted in Figure 3.6 to Figure 3.11. In the 4 ft column with C:N ratio of 2:1, nitrate and nitrite reduced to zero at every depth within 20 hours and 44 hours respectively. In the 4 ft column with C:N ratio of 4:1, nitrate reduced to zero in 44 hours while nitrite took 53 hours. The last 4 ft column with C:N ratio of 6:1, showed complete reduction of nitrate in 30 hours and reduction of nitrite in 44 hours. The 6 ft column reactor with C:N ratio of 6:1 displayed nitrate removal within 44 hours and nitrite within 53 hours. Finally, the 7 ft column with C:N ratio of 6:1 exhibited complete nitrate and nitrite reduction in 44 hours.

Table 3.5: NO_3^- and NO_2^- data for control column reactor (no organic carbon source) for denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	7.987	0.000	7.412	0.000	7.701	0.000	7.009	0.000
6	7.942	0.000	7.636	0.000	8.073	0.000	7.259	0.000
20	8.095	0.000	7.571	0.208	8.028	0.000	7.453	0.000
30	8.014	0.081	6.290	0.234	7.554	0.074	7.337	0.000
44	7.587	0.000	6.282	0.188	7.325	0.055	7.453	0.168
53	8.029	0.047	6.803	0.240	7.704	0.064	7.114	0.316

Table 3.6: NO_3^- and NO_2^- data for C:N = 2:1 column reactor for denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	8.355	0.000	6.659	0.103	7.803	0.049	7.353	0.000
6	7.832	0.000	7.207	0.000	6.686	0.000	7.147	0.046
20	0.022	5.665	0.000	2.309	0.089	2.905	0.074	3.151
30	0.000	1.457	0.000	1.128	0.000	1.100	0.000	1.323
44	0.089	0.000	0.030	0.000	0.024	0.000	0.000	0.000
53	0.000	0.054	0.000	0.000	0.030	0.000	0.000	0.000

Figure 3.5: NO_3^- and NO_2^- concentrations as a function of time for 4 ft control column reactor (no Biowish) for denitrification experiment

*Col. refers to column reactors. For port/depth correlation refer to Figure 2.4

Figure 3.6: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor with C:N ratio of 2:1 for denitrification experiment

Table 3.7: NO_3^- and NO_2^- data for C:N = 4:1 column reactor for denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	7.761	0.000	7.594	0.000	7.022	0.000	6.917	0.000
6	7.269	0.163	6.117	0.326	4.814	0.163	5.437	0.068
20	4.653	1.263	3.322	0.845	3.785	0.639	3.841	0.818
30	1.561	2.105	1.191	1.759	1.039	1.534	1.354	1.708
44	0.033	0.515	0.033	0.317	0.000	0.385	0.019	0.409
53	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000

Table 3.8: NO_3^- and NO_2^- data for C:N = 6:1 column reactor for denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	7.398	0.000	7.356	0.034	8.861	0.000	8.218	0.000
6	6.806	0.000	7.105	0.200	6.612	0.000	5.701	0.124
20	2.221	2.109	1.881	1.645	2.218	1.794	1.703	1.795
30	0.087	2.371	0.048	1.698	0.095	1.896	0.066	1.645
44	0.094	0.000	0.000	0.000	0.057	0.000	0.079	0.000
53	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3.7: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor with C:N ratio of 4:1 for denitrification experiment

Figure 3.8: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor with C:N ratio of 6:1 for denitrification experiment

Table 3.9: NO_3^- and NO_2^- data for C:N = 6:1 column reactor for denitrification experiment in 6ft reactor

Time (hrs)	Surface		Port 1		Port 2	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	7.956	0.000	7.878	0.000	7.518	0.000
6	7.595	0.000	7.581	0.000	7.129	0.000
20	4.036	2.414	4.813	1.966	4.462	1.597
30	0.772	4.938	1.251	4.460	0.874	4.845
44	0.073	2.018	0.000	2.403	0.000	2.544
53	0.000	0.000	0.000	0.000	0.000	0.158

Time (hrs)	Port 3		Port 4		Port 5	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	6.117	0.000	7.310	0.000	6.037	0.000
6	7.260	0.000	6.874	0.000	7.391	0.000
20	1.173	1.987	4.009	1.878	4.385	1.814
30	0.797	5.021	0.585	4.838	0.840	4.754
44	0.000	2.443	0.000	2.157	0.000	2.210
53	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3.9: NO_3^- and NO_2^- concentrations as a function of time for 6 ft column reactor with C:N ratio of 6:1 for denitrification experiment

Table 3.10: NO_3^- and NO_2^- data for C:N = 6:1 column reactor for denitrification experiment in 7 ft reactor

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	7.911	0.000	7.851	0.000	7.913	0.000	6.339	0.000
6	7.663	0.000	7.351	0.000	7.222	0.000	7.163	0.000
20	4.974	1.530	5.620	1.003	5.146	0.975	4.937	0.279
30	1.110	3.067	1.797	2.629	2.405	1.852	0.829	2.399
44	0.000	0.000	0.038	0.200	0.022	0.000	0.000	0.000
53	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Time (hrs)	Port 4		Port 5		Port 6	
	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	6.021	0.000	6.431	0.000	6.062	0.000
6	7.168	0.000	6.893	0.000	7.358	0.000
20	5.380	1.138	5.265	1.142	5.078	0.802
30	1.117	3.425	1.317	3.153	1.657	3.009
44	0.000	0.000	0.027	0.111	0.019	0.000
53	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3.10: NO₃⁻ and NO₂⁻ concentrations as a function of time for 7 ft column reactor with C:N ratio of 6:1 for denitrification experiment

Figure 3.11: NO_3^- and NO_2^- concentrations as a function of time for 7 ft column reactor with C:N ratio of 6:1 for denitrification experiment

From Figure 3.5, it can be seen that no denitrification occurred at the control column reactor; an expected result because no organic carbon was present in the mixture. In all other column reactors, the concentration of NO_3^- dropped down to zero by the end of the experiment. Also, the concentration of NO_2^- rose from zero as the concentration of NO_3^- decreased, and after it reached its peak value, it started decreasing until virtually eliminated. This clearly indicates that denitrification occurred in all those column reactors. Table 3.11 shows the amount of time it took to completely remove NO_3^- from the solution, the total time each column reactor needed to finish the denitrification process, as well as its rate of denitrification. Figure 3.12a plots the zero order rate coefficient ($k_{\text{NO}_3^-}$) as a function of C:N ratio and Figure 3.12 plots $k_{\text{NO}_3^-}$ as a function of depth for C:N ratio of 6:1.

Table 3.11: Summary of results from denitrification experiment

	Time needed to completely remove NO_3^- (hours)	Zero order $K_{\text{NO}_3^-}$ (ppm/day)*	Specific denitrification rate for NO_3^- (mg-N/g-VSS d)**	Total time for denitrification (hours)	Rate of complete denitrification (ppm/day)
Control	-	0.21	4.2	-	-
4 ft C:N = 2:1	20	9.58	191.6	44	4.56
4 ft C:N = 4:1	44	3.49	69.8	72	2.59
4 ft C:N = 6:1	30	5.94	118.8	44	4.83
6 ft C:N = 6:1	44	5.01	100.2	72	2.65
7 ft C:N = 6:1	44	4.19	83.8	44	4.32

* To obtain zero order $K_{\text{NO}_3^-}$ values refer to appendix A.2

** Specific denitrification rate is calculated by dividing $K_{\text{NO}_3^-}$ by initial Biowish concentration

Figure 3.12: (a) K values as a function of C:N ratio for 4 ft column reactors (b) K values as a function of depth for C:N = 6:1

Dissolved oxygen (DO) concentration and oxidation reduction potentials (ORP) were monitored intermittently throughout the denitrification experiment. Table 3.12 and Figure 3.13 illustrate the dissolved oxygen profile as a function of depth for all column reactors used in this experiment. The graphs show that DO concentrations for all Biowish inoculated columns vary along the depth of each reactor within the range of 0.3 to 1.4 ppm with no particular trend observed (Figure 3.13). However, the control column reactor exhibited significantly higher DO levels. Figure 3.14 shows positive ORP values only in the control column reactor and negative ORP in the Biowish inoculated columns.

Table 3.12a: DO Results from denitrification experiment at 25 hrs

Depth (ft)	Dissolved Oxygen (ppm)				
	4 FT Columns			6 FT Column	7 FT Column
	Control	C:N = 2:1	C:N = 4:1	C:N = 6:1	C:N = 6:1
1	1.92	0.36	0.48	0.50	0.43
2	1.94	0.55	0.88	0.65	0.42
3	1.83	1.33	1.13	0.84	1.24
4	---	0.92	0.67	0.85	0.85
5	---	---	---	0.77	1.38
6	---	---	---	0.83	0.78
7	---	---	---	---	0.49

Table 3.12b: DO Results from denitrification experiment at 45 hrs

Depth (ft)	Dissolved Oxygen (ppm)				
	4 FT Columns			6 FT Column	7 FT Column
	Control	C:N = 2:1	C:N = 4:1	C:N = 6:1	C:N = 6:1
1	1.50	0.37	0.42	0.44	0.41
2	1.27	0.47	0.55	0.59	0.43
3	1.36	0.89	0.31	0.98	0.86
4	---	0.85	0.67	0.83	1.00
5	---	---	---	0.82	1.01
6	---	---	---	0.72	0.62
7	---	---	---	---	0.50

Table 3.12c: DO Results from denitrification experiment at 72 hrs

Depth (ft)	Dissolved Oxygen (ppm)				
	4 FT Columns			6 FT Column	7 FT Column
	Control	C:N = 2:1	C:N = 4:1	C:N = 6:1	C:N = 6:1
1	1.30	0.29	0.50	0.38	0.28
2	1.55	0.41	0.80	0.48	0.34
3	1.48	0.73	0.54	0.44	0.59
4	---	0.69	0.39	0.52	0.85
5	---	---	---	0.60	1.37
6	---	---	---	0.71	1.42
7	---	---	---	---	0.48

Table 3.13: ORP Results from denitrification experiment at 72 hrs

Depth (ft)	Oxidation Reduction Potential (mV)				
	4 FT Columns			6 FT Column	7 FT Column
	Ctrl	C:N = 2:1	C:N = 4:1	C:N = 6:1	C:N = 6:1
1	51.5	-161.5	-181.0	-230.2	-190.8
2	55.7	-161.3	-190.0	-233.6	-176.4
3	63.2	-166.0	-185.6	-249.8	-185.7
4	---	-163.3	-192.3	-246.3	-194.7
5	---	---	---	-224.9	-177.2
6	---	---	---	-236.4	-169.6
7	---	---	---	---	-210.1

Figure 3.13: DO data for all columns as a function of depth for denitrification experiment at 25, 45 and 72 hours. Control column contains no Biowish

Figure 3.14: ORP data for all columns as a function of depth for denitrification experiment at 72 hours. Control column contains no Biowish

Figure 3.15 shows DO and ORP values plotted as a function of depth for the denitrification experiment at 72 hours. From the graphs, it is observed that the ORP values track with the DO measurements for all reactors except for two of the points which are considered anomalies due to possible measurement error in the 7 ft column reactor with C:N ratio of 6:1.

Figure 3.15: DO and ORP data for all columns as a function of depth for denitrification experiment at 72 hours

3.3 Experiment 3 – Nitrite Removal

Designed to observe the nitrite removal capabilities of Biowish without the presence of nitrate, this experiment started on July 19, 2011 and was carried out for 68 hours. Table 3.14 shows the content and dosage in each column reactor for this experiment.

Table 3.14: Experimental conditions for nitrite removal

	C:N	NO ₂ ⁻ (ppm)	Biowish
Control A	2:1	15	No
Control B	2:1	15	No
Reactor A	2:1	15	Yes
Reactor B	2:1	15	Yes

Table 3.15 through Table 3.18 list data obtained in this experiment, and Figure 3.16 through Figure 3.19 shows the concentration of NH₃, NO₃⁻ and NO₂⁻ as a function of time. In Figure 3.16 and Figure 3.17, nitrite levels maintained around 15 ppm throughout the duration of the experiment for both control columns. In Figure 3.18, Column Reactor A inoculated with 50 ppm of Biowish showed complete reduction of nitrite (from approximately 15 ppm to 0 ppm in 68 hours) while in Figure 3.19, Column Reactor B displayed a reduction of about 12 ppm of nitrite in 68 hours. Both ammonia and nitrate levels for all column reactors remained close to 0 ppm throughout the experiment.

Table 3.15: NH_3 , NO_3^- and NO_2^- data for control Column Reactor A for nitrite removal experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	0.18	0.21	14.80	0.16	0.20	14.36	0.27	0.19	14.13	0.08	0.25	14.63
6.5	0.12	0.14	15.29	0.00	0.30	15.59	0.28	0.09	14.45	0.24	0.24	14.97
20	0.14	0.06	15.89	0.13	0.12	14.38	0.12	0.15	14.88	0.23	0.29	14.72
32.5	0.07	0.13	14.60	0.15	0.18	15.24	0.31	0.18	15.21	0.29	0.30	14.01
53	0.16	0.01	14.81	0.32	0.09	14.70	0.14	0.10	15.66	0.31	0.29	14.37
68	0.17	0.18	15.59	0.19	0.10	15.11	0.18	0.17	14.93	0.33	0.08	14.02

Table 3.16: NH_3 , NO_3^- and NO_2^- data for control Column Reactor B for nitrite removal experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	0.28	0.29	14.71	0.26	0.22	15.20	0.21	0.24	15.02	0.27	0.04	14.74
6.5	0.11	0.16	15.71	0.07	0.12	15.54	0.01	0.30	14.36	0.03	0.11	15.38
20	0.25	0.13	14.49	0.22	0.23	15.47	0.13	0.16	14.84	0.20	0.19	14.21
32.5	0.12	0.19	14.56	0.12	0.19	15.40	0.25	0.12	14.40	0.26	0.32	14.62
53	0.21	0.09	15.21	0.25	0.09	15.63	0.00	0.09	15.81	0.23	0.13	14.44
68	0.09	0.15	14.83	0.04	0.20	14.33	0.01	0.24	14.79	0.32	0.29	15.05

Figure 3.16: NH_3 , NO_3^- and NO_2^- concentrations as a function of time for control reactor A (no Biowish) for nitrite removal experiment

Figure 3.17: NH_3 , NO_3^- and NO_2^- concentrations as a function of time for control reactor B (no Biowish) for nitrite removal experiment

Table 3.17: NH_3 , NO_3^- and NO_2^- data for Reactor A for nitrite removal experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	0.10	0.21	14.87	0.10	0.18	15.89	0.13	0.09	15.71	0.12	0.15	13.93
6.5	0.10	0.17	14.83	0.14	0.19	15.14	0.21	0.18	15.20	0.15	0.03	14.55
20	0.08	0.15	11.51	0.16	0.11	13.55	0.09	0.00	10.72	0.08	0.06	12.17
32.5	0.06	0.04	9.18	0.03	0.00	9.14	0.11	0.02	9.37	0.05	0.00	9.36
53	0.05	0.04	3.44	0.04	0.05	3.99	0.03	0.06	4.01	0.12	0.05	4.04
68	0.03	0.00	0.34	0.02	0.04	0.41	0.03	0.03	0.43	0.06	0.00	0.35

Table 3.18: NH_3 , NO_3^- and NO_2^- data for Reactor B for nitrite removal experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	0.19	0.17	15.57	0.29	0.18	15.64	1.08	0.17	13.73	1.17	0.16	15.15
6.5	0.65	0.02	14.13	0.21	0.13	14.66	0.18	0.16	14.65	0.14	0.00	14.08
20	0.41	0.12	13.24	0.14	0.21	12.65	0.11	0.02	13.96	0.09	0.15	14.23
32.5	0.04	0.00	12.49	0.06	0.03	12.90	0.13	0.00	13.77	0.04	0.05	13.72
53	0.08	0.05	7.82	0.09	0.08	9.64	0.07	0.00	7.65	0.07	0.00	7.65
68	0.03	0.05	3.09	0.03	0.00	3.03	0.03	0.00	3.00	0.03	0.15	2.87

Figure 3.18: NH₃, NO₃⁻ and NO₂⁻ concentrations as a function of time for Column Reactor A for nitrite removal experiment

Figure 3.19: NH_3 , NO_3^- and NO_2^- concentrations as a function of time for Column Reactor B for nitrite removal experiment

Table 3.19 summarizes the kinetic results for nitrite removal. No noticeable reduction of nitrite was observed in control column reactors while Column Reactor A and B showed zero order k values of 5.55 ppm/day and 5.64 ppm/day respectively.

Table 3.19: Summary of kinetic results
for nitrite removal experiment

Column reactor	Height	Total reduction of NO_2^- (ppm)	Zero order $K_{\text{NO}_2^-}$ (ppm/day)
Control A	4ft	-	-
Control B	4ft	-	-
Nitrite A	4ft	14.72	5.55
Nitrite B	4ft	12.03	5.64

3.4 Experiment 4 – Treatment of Ammonia / Nitrate

In the previous experiments, the initial solution only contained either NH_3 or NO_3^- and the effects of Biowish on ammonia assimilation and denitrification were observed. In this experiment, the initial solution will contain both NH_3 and NO_3^- to simulate a more realistic scenario. The experiment started on July 17, 2011 and it ended after 72 hours. Table 3.20 shows the various C:N ratios and column reactor heights used.

Table 3.20: Experimental conditions for treatment of ammonia / nitrate

	C:N	Height	Biowish
Control A	1:1	4 ft	No
Control B	1:1	4 ft	No
Column Reactor 1	1:1	4 ft	Yes
Column Reactor 2	1:1	4 ft	Yes
Column Reactor 3	1:1	6 ft	Yes
Column Reactor 4	2:1	4 ft	Yes
Column Reactor 5	2:1	4 ft	Yes
Column Reactor 6	3:1	4 ft	Yes
Column Reactor 7	3:1	4 ft	Yes

Table 3.21a and Figure 3.20 show the NH_3 , NO_3^- and NO_2^- concentrations as a function of time for control reactor A, and Table 3.21b and Figure 3.21 show the same plots for control reactor B. From Figure 3.20 and Figure 3.21, it can be noticed that the concentration of NH_3 , NO_3^- and NO_2^- remained constant at about 18 ppm, 10 ppm and 0 ppm respectively in the control reactors. This means that neither ammonia assimilation, nor denitrification occurred in any of those reactors. Since the control reactors did not contain Biowish, this observation is anticipated. Furthermore, no significant evaporation was observed throughout the entire experiment.

Table 3.22, Figure 3.22 and Figure 3.23 plots correspond to the 4 ft column reactors with a C:N ratio of 1:1. Table 3.23 and Figure 3.24 plots corresponds to the 6 ft column reactor with the same C:N ratio. In the 4 ft and 6 ft column reactors with a C:N ratio of 1:1, the concentration of NO_3^- decreased an average of 9.14 ppm. Also, the NO_2^- levels started increasing at the 30th hour for reactor A, and at the 20th hour for both reactor B and the 6 ft reactor. This signifies the occurrence of denitrification in both reactors. Moreover, a slight decrease in the concentration of NH_3 can be observed throughout the sample period, which indicates the occurrence of ammonia assimilation.

Table 3.24, Figure 3.25 and Figure 3.26 show the NH_3 , NO_3^- and NO_2^- plots for the 4 ft column reactors with a C:N ratio of 2:1. In both reactor A and B, NO_3^- levels decreased to zero (close to zero for reactor B) by the 44th hour and NH_3 levels decreased an average of 4.2 ppm by the end of the experiment. NO_2^- levels increased from the

beginning of the experiment, but started decreasing and reached zero by the 72nd hour, which indicates there was almost no lag period in denitrification.

Finally, Table 3.25, Figure 3.27 and Figure 3.28 show nitrogen content for the 4 ft column reactors with C:N ratio of 3:1. In reactor A and B, NO_3^- concentrations reduced to zero within 40 hours and 52.5 hours respectively, while NH_3 levels reduced an average of 3.1 ppm. NO_2^- started appearing from the 12th hour for reactor A and 22.5th hour for reactor B, and it was completely eliminated by the 52.5th hour for reactor A. Table 3.26 and Table 3.27 summarize the figures of merit pertaining this experiment: column reactors with C:N ratio of 2:1 and 3:1 follow zero order kinetics; reactors with C:N ratio of 1:1 appear to behave as first order reactions.

Table 3.21a: NH_3 , NO_3^- and NO_2^- results in control column reactor A for treatment of ammonia / nitrate

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	11.45	18.42	0.00	11.50	18.42	0.00	11.26	18.60	0.00	11.26	18.35	0.00
7.5	10.76	18.28	0.00	11.07	18.48	0.00	11.35	18.56	0.00	11.03	18.72	0.00
19	10.67	18.46	0.07	10.72	18.67	0.00	11.28	18.64	0.06	10.90	19.18	0.00
29	9.84	18.45	0.00	10.27	18.60	0.00	10.40	18.77	0.00	10.23	18.69	0.00
48	8.93	17.92	0.24	9.57	18.34	0.14	9.38	18.36	0.28	9.34	19.25	0.07
72	9.62	18.06	0.26	9.89	18.55	0.26	9.97	18.44	0.32	10.01	19.47	0.04

Table 3.21b: NH_3 , NO_3^- and NO_2^- results in control column reactor B for treatment of ammonia / nitrate

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	11.59	18.59	0.00	11.64	18.67	0.00	11.74	18.87	0.00	11.16	16.89	0.00
7.5	11.17	18.51	0.00	11.54	18.09	0.00	11.40	18.07	0.00	11.40	18.48	0.00
19	10.95	19.24	0.00	11.00	18.69	0.15	11.14	18.70	0.11	10.90	18.79	0.00
29	10.60	18.49	0.00	10.44	17.38	0.13	10.60	17.47	0.22	10.52	18.07	0.00
48	9.53	19.23	0.00	9.89	18.12	0.22	10.14	18.27	0.27	10.01	19.36	0.05
72	9.89	19.20	0.04	9.85	18.23	0.23	9.66	18.06	0.26	9.66	19.11	0.08

Figure 3.20: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft control reactor A for treatment of ammonia / nitrate

Figure 3.21: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft control reactor B for treatment of ammonia / nitrate

Table 3.22a: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 1:1 A for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	11.31	18.40	0.00	11.45	18.56	0.00	11.31	18.79	0.00	10.36	16.25	0.00
7.5	11.35	17.97	0.08	11.63	18.29	0.10	11.63	18.57	0.00	11.07	17.46	0.00
19	10.45	17.80	0.00	11.04	18.71	0.00	10.86	17.48	0.24	10.32	17.21	0.08
29	9.61	16.63	0.18	9.73	16.72	0.30	9.24	16.29	0.32	8.96	16.87	0.32
48	8.71	11.36	2.37	8.85	11.59	2.44	8.78	11.43	2.57	8.67	11.19	2.36
72	9.85	9.26	2.36	9.81	9.43	2.78	9.85	9.22	2.73	9.58	9.11	2.54

Table 3.22b: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 1:1 B for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	11.21	18.51	0.00	11.07	18.31	0.00	11.31	18.75	0.00	10.98	18.43	0.00
7.5	11.30	17.23	0.30	11.26	17.53	0.21	10.80	18.63	0.00	10.45	18.40	0.00
19	10.36	16.72	0.26	10.10	17.14	0.44	10.27	17.37	0.31	9.72	17.84	0.24
29	8.92	12.71	2.20	8.89	12.17	2.62	9.13	12.38	2.34	9.17	12.70	2.50
48	9.26	9.42	2.92	9.00	9.13	2.94	9.30	9.14	2.81	9.30	9.45	2.92
72	9.42	8.05	2.31	9.35	8.65	2.77	9.42	8.33	2.57	9.35	8.90	2.76

Figure 3.22: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 1:1 A for treatment of ammonia / nitrate

Figure 3.23: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 1:1 B for treatment of ammonia / nitrate

Table 3.23: NH_3 , NO_3^- and NO_2^- results in 6 ft column reactor C:N = 1:1 for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	8.30	15.20	0.00	8.23	15.54	0.00	9.93	16.85	0.00
7.5	9.75	16.85	0.00	9.87	16.99	0.00	9.83	17.60	0.00
19	8.66	17.28	0.34	9.00	16.98	0.57	9.00	16.71	0.37
29	9.21	10.86	2.44	9.10	10.41	3.14	9.13	10.17	2.94
48	9.19	8.12	2.91	9.30	8.03	2.94	9.30	8.13	2.81
72	10.10	7.61	2.61	10.05	7.12	2.34	10.50	7.24	2.24

Time (hrs)	Port 4			Port 5			Port 6		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	10.14	17.48	0.00	11.26	19.27	0.00	11.54	21.12	0.00
7.5	10.16	17.30	0.08	10.24	17.65	0.00	10.58	20.67	0.27
19	8.70	16.53	0.34	8.44	16.34	0.58	8.96	16.06	1.76
29	9.28	10.22	2.77	8.78	10.66	2.98	9.32	8.19	4.83
48	9.22	7.87	2.73	9.15	8.07	2.73	9.19	6.69	3.66
72	10.30	7.26	2.29	10.14	7.53	2.57	10.30	6.75	2.75

Figure 3.24: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 6 ft reactor C:N = 1:1 for treatment of ammonia / nitrate

Table 3.24a: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 2:1 A for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	10.51	17.65	0.00	12.19	19.13	0.00	12.53	19.26	0.00	11.72	19.18	0.00
6.5	11.58	16.57	0.52	11.49	17.78	0.61	12.05	17.16	0.62	10.22	17.01	0.66
18	11.26	12.42	2.93	11.58	11.98	2.98	11.35	12.40	3.18	11.72	11.46	3.02
24	11.35	8.58	4.99	11.40	9.12	5.05	11.49	8.46	5.07	11.44	8.27	5.14
44	7.90	0.00	6.65	8.16	0.09	7.24	7.96	0.09	7.18	8.00	0.05	6.67
72	7.71	0.00	0.00	7.50	0.00	0.00	7.34	0.00	0.00	7.34	0.00	0.00

Table 3.24b: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 2:1 B for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	10.97	17.51	0.00	11.40	17.75	0.00	11.05	17.97	0.00	12.33	17.52	0.06
6.5	10.38	15.59	0.62	10.26	16.04	0.69	11.27	16.68	0.51	10.22	17.26	0.58
18	12.74	11.03	2.82	12.39	12.17	3.89	11.49	11.69	3.02	12.85	11.77	4.10
24	11.35	8.06	4.61	11.40	7.89	4.49	11.44	8.30	5.10	11.53	7.85	5.74
44	8.13	0.97	5.42	8.23	1.10	5.16	7.80	0.98	5.51	7.71	1.07	5.29
72	7.40	0.00	0.00	7.32	0.00	0.00	7.34	0.02	0.00	7.37	0.00	0.00

Figure 3.25: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 2:1 A for treatment of ammonia / nitrate

Figure 3.26: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 2:1 B for treatment of ammonia / nitrate

Table 3.25a: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 3:1 A for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	10.52	8.36	0.00	11.03	9.41	0.00	11.37	8.59	0.00	9.73	7.80	0.00
12	9.97	7.81	0.08	9.97	8.05	0.16	10.01	8.18	0.17	10.64	8.35	0.05
22.5	8.62	5.69	2.83	8.65	5.40	2.89	9.09	5.82	2.70	8.94	6.51	2.64
42.5	7.55	0.00	1.15	7.55	0.07	1.26	7.38	0.00	1.19	7.38	0.00	1.25
52.5	7.22	0.00	0.00	7.50	0.00	0.00	7.82	0.00	0.00	7.75	0.00	0.10

Table 3.25b: NH_3 , NO_3^- and NO_2^- results in 4 ft column reactor C:N = 3:1 B for treatment of ammonia / nitrate experiment

Time (hrs)	Port 1			Port 2			Port 3			Port 4		
	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)	NH_3 (ppm)	NO_3^- (ppm)	NO_2^- (ppm)
0	11.98	9.16	0.00	11.93	9.25	0.00	11.87	8.70	0.00	12.29	7.91	0.00
12	10.98	7.71	0.21	10.77	8.00	0.00	10.90	8.04	0.12	10.90	8.38	0.00
22.5	9.74	8.63	0.33	10.10	9.63	0.12	9.86	8.90	0.32	10.27	9.49	0.08
42.5	7.91	1.78	2.80	8.09	1.98	2.92	8.02	1.82	2.95	7.98	1.89	2.92
52.5	8.61	0.14	1.49	8.68	0.50	1.63	8.97	0.16	1.59	8.82	0.00	1.38

Figure 3.27: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 3:1 A for treatment of ammonia / nitrate

Figure 3.28: NH_3 , NO_3^- and NO_2^- concentrations as a function of time in 4 ft reactor C:N = 3:1 B for treatment of ammonia / nitrate

Table 3.26: Summary of kinetics results for nitrate from treatment of ammonia / nitrate

Column reactor	Height	Average reduction of NO_3^- (ppm)	Zero order $K_{\text{NO}_3^-}$ (ppm/day)	First order $K_{\text{NO}_3^-}$ (1/day)	Average complete denitrification rate (ppm/day)
Control A	4 ft	-	-	-	-
Control B	4 ft	-	-	-	-
C:N = 1:1 A	4 ft	9.14 (50%)	3.30	0.31	*
C:N = 1:1 B	4 ft	10.46 (57%)	3.82	0.31	*
C:N = 1:1	6 ft	7.59 (50%)	4.11	0.34	*
C:N = 2:1 A	4 ft	17.65 (100%)	10.50	-	5.88
C:N = 2:1 B	4 ft	17.51 (100%)	9.45	-	5.84
C:N = 3:1 A	4 ft	8.54 (100%)	5.02	-	3.91
C:N = 3:1 B	4 ft	8.56 (98%)	4.30	-	3.91

*Denitrification rate does not apply because the concentration of NO_2^- did not reduced to 0 ppm by the end of the experiment (72 hour period)

Table 3.27: Summary of kinetics results for ammonia from treatment of ammonia / nitrate

Column reactor	Height	Average reduction of NH ₃ (ppm)	Zero Order K _{NH₃} (ppm/day)
Control A	4 ft	1.5 (13%)	0.60
Control B	4 ft	1.7 (15%)	0.65
C:N = 1:1 A	4 ft	1.3 (12%)	0.69
C:N = 1:1 B	4 ft	1.7 (16%)	0.64
C:N = 1:1	6 ft	-	-
C:N = 2:1 A	4 ft	4.3 (36%)	1.62
C:N = 2:1 B	4 ft	4.1 (36%)	1.55
C:N = 3:1 A	4 ft	2.9 (28%)	1.57
C:N = 3:1 B	4 ft	3.4 (28%)	1.69

Figure 3.29 shows zero order k values for NO_3^- and NH_3 at different C:N ratios. From these graphs, it can be observed that C:N ratio of 2:1 has the highest zero order k values followed by C:N ratio of 3:1. Also, C:N ratio of 1:1 yields the lowest zero order k values in the treatment of ammonia / nitrate.

Figure 3.29: K values for NO_3^- and NH_3 presented as a function of C:N ratio

Dissolved oxygen from Figure 3.30 shows the vertical DO profile at two different sample times, and Figure 3.31 presents the Oxidation-Reduction-Potential (ORP) profile at the 60th hour. It can be seen in both DO plots that Control A and Control B have a higher dissolved oxygen concentration than the samples that contain Biowish. Also from the ORP graphs, it can be observed that both control samples have positive readings (~ 300 mV), and all the samples containing Biowish have a negative reading (~ -500 mV).

Figure 3.30: DO concentration as a function of depth for treatment of ammonia / nitrate experiment

Figure 3.31: ORP data as a function of depth for treatment of ammonia / nitrate experiment

Optical density was measured intermittently throughout the treatment of ammonia / nitrate experiment to verify that bacterial growth was taking place in all Biowish inoculated column reactors. Table 3.28 to Table 3.30 and Figure 3.32 to Figure 3.34 show OD collected for the reactors with C:N ratio of 1:1, 2:1 and 3:1 respectively. Table 3.28 and Figure 3.32 illustrate OD profile for control reactors and reactors with C:N ratio of 1:1 as a function of time. Figure 3.32 shows OD for control reactors was maintained consistently below 0.05 and most Biowish inoculated columns exhibited an OD range of about 0.075 to 0.30 except for the 6 ft column at 47 hours. Considering the OD levels mentioned above, an OD value greater than 0.05 can be regarded as an indication of bacterial growth in the columns.

Table 3.29 and Figure 3.33, and Table 3.30 and Figure 3.34 show OD profile for column reactors with C:N ratio of 2:1 and 3:1 respectively as a function of time. Figure 3.33 and Figure 3.34 display similar trends of OD with Biowish column reactors where an increase in OD can be seen after the zeroth hour (average increase in OD value of 0.06 for C:N ratio of 2:1 and 0.04 for C:N ratio of 3:1) except for the measurements taken near the end of each experiment. Some OD measurements for C:N ratio of 3:1 are lower than expected, possibly attributed to instrumental inconsistencies.

Table 3.28a: OD₆₀₀ data for control reactors and reactors with C:N = 1:1 at port 1

Time (hr)	4 FT Columns				6 FT Column
	Ctrl A	Ctrl B	4ft A C:N = 1:1	4ft B C:N = 1:1	6 ft C:N = 1:1
0	0.000	0.001	0.004	0.004	0.007
7.5	0.026	0.027	0.090	0.110	0.101
19	0.021	0.027	0.114	0.121	0.135
29	0.023	0.024	0.076	0.145	0.160
48	0.036	0.036	0.232	0.080	0.054

Table 3.28b: OD₆₀₀ data for control reactors and reactors with C:N = 1:1 at port 2

Time (hr)	4 FT Columns				6 FT Column
	Ctrl A	Ctrl B	4ft A C:N = 1:1	4ft B C:N = 1:1	6 ft C:N = 1:1
0	0.000	0.001	0.007	0.005	0.005
7.5	0.030	0.037	0.097	0.104	0.118
19	0.030	0.023	0.107	0.109	0.142
29	0.027	0.026	0.088	0.188	0.161
48	0.038	0.036	0.180	0.056	0.059

Table 3.28c: OD₆₀₀ data for control reactors and reactors with C:N = 1:1 at port 3

Time (hr)	4 FT Columns				6 FT Column
	Ctrl A	Ctrl B	4ft A C:N = 1:1	4ft B C:N = 1:1	6 ft C:N = 1:1
0	0.000	0.001	0.004	0.009	0.001
7.5	0.035	0.029	0.075	0.113	0.127
19	0.026	0.026	0.120	0.131	0.146
29	0.026	0.025	0.097	0.140	0.183
48	0.041	0.034	0.264	0.053	0.061

Table 3.28d: OD₆₀₀ data for control reactors and reactors with C:N = 1:1 at port 4

Time (hr)	4 FT Columns				6 FT Column
	Ctrl A	Ctrl B	4ft A C:N = 1:1	4ft B C:N = 1:1	6 ft C:N = 1:1
0	0.000	0.002	0.008	0.006	0.003
7.5	0.033	0.033	0.088	0.118	0.119
19	0.028	0.025	0.102	0.103	0.143
29	0.024	0.021	0.101	0.111	0.150
48	0.038	0.033	0.080	0.050	0.055

Figure 3.32: OD₆₀₀ profile as a function of time for control reactors and reactors with C:N ratio of 1:1 at ports 1 through 4 (depth 1 ft to 4 ft)

Table 3.29a: OD₆₀₀ data for column reactors with C:N = 2:1 at port 1

Time (hr)	4ft C:N = 2:1 A	4ft C:N = 2:1 B
0	0.079	0.076
6.5	0.079	0.124
18	0.092	0.138
24	0.122	0.129
44	0.140	0.101
72	0.052	0.102

Table 3.29b: OD₆₀₀ data for column reactors with C:N = 2:1 at port 2

Time (hr)	4ft C:N = 2:1 A	4ft C:N = 2:1 B
0	0.074	0.077
6.5	0.101	0.142
18	0.141	0.161
24	0.121	0.139
44	0.134	0.138
72	0.064	0.075

Table 3.29c: OD₆₀₀ data for column reactors with C:N = 2:1 at port 3

Time (hr)	4ft C:N = 2:1 A	4ft C:N = 2:1 B
0	0.084	0.072
6.5	0.092	0.100
18	0.104	0.146
24	0.131	0.140
44	0.116	0.094
72	0.129	0.067

Table 3.29d: OD₆₀₀ data for column reactors with C:N = 2:1 at port 4

Time (hr)	4ft C:N = 2:1 A	4ft C:N = 2:1 B
0	0.069	0.083
6.5	0.084	0.093
18	0.098	0.110
24	0.100	0.130
44	0.113	0.133
72	0.045	0.070

Figure 3.33: OD₆₀₀ profile as a function of time for reactors with C:N ratio of 2:1 at ports 1 through 4 (depth 1 ft to 4 ft)

Table 3.30a: OD₆₀₀ data for column reactors with C:N = 3:1 at port 1

Time (hr)	4ft C:N = 3:1 A	4ft C:N = 3:1 B
0	0.060	0.065
12	0.038	0.022
22.5	0.056	0.031
52.5	0.066	0.123

Table 3.30b: OD₆₀₀ data for column reactors with C:N = 3:1 at port 2

Time (hr)	4ft C:N = 3:1 A	4ft C:N = 3:1 B
0	0.073	0.075
12	0.031	0.021
22.5	0.057	0.030
52.5	0.099	0.057

Table 3.30c: OD₆₀₀ data for column reactors with C:N = 3:1 at port 3

Time (hr)	4ft C:N = 3:1 A	4ft C:N = 3:1 B
0	0.060	0.057
12	0.038	0.017
22.5	0.060	0.038
52.5	0.122	0.122

Table 3.30d: OD₆₀₀ data for column reactors with C:N = 3:1 at port 4

Time (hr)	4ft C:N = 3:1 A	4ft C:N = 3:1 B
0	0.055	0.048
12	0.034	0.021
22.5	0.062	0.032
52.5	0.134	0.060

Figure 3.34: OD₆₀₀ profile as a function of time for reactors with C:N ratio of 3:1 at ports 1 through 4 (depth 1 ft to 4 ft)

3.5 Experiment 5 – Urea Hydrolysis

This experiment was designed to assess the capabilities of Biowish in the retardation of urea hydrolysis. Starting on July 7, 2011, this experiment lasted 72 hours using separate flasks with solutions that contained 100 ppm of urea and 50 ppm, 75 ppm and 100 ppm of Biowish as shown in Table 3.31.

Table 3.31: Experimental conditions for urea hydrolysis

	Urea (ppm)	Biowish (ppm)	C:N
Control A	100	-	2:1
Control B	100	-	2:1
Flask 1	100	50	2:1
Flask 2	100	50	2:1
Flask 3	100	75	2:1
Flask 4	100	75	2:1
Flask 5	100	100	2:1
Flask 6	100	100	2:1

Control A and B displayed a decrease in urea concentration from about 130 ppm to 80 ppm, and an increase in ammonia concentration from 0 ppm to approximately 5.5 ppm. All other flasks inoculated with Biowish showed similar trends for both urea and ammonia concentrations as illustrated in Table 3.32, Figure 3.35 and Figure 3.36.

Table 3.32: Urea and NH₃ data for all flasks in urea hydrolysis experiment. Control A and B contain no Biowish

Time (hrs)	Control A		Control B		Flask 1 50 ppm A		Flask 2 50 ppm B	
	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)
0	122.6	0.14	132.5	0.12	83.7	0.13	98.9	0.15
6.5	133.8	0.11	132.5	0.21	90.5	0.14	108.1	0.25
17.5	121.5	1.82	138.3	2.41	104.3	2.14	98.1	3.34
24	80.6	2.30	75.5	2.80	77.0	2.68	80.3	2.93
44	127.2	2.39	142	3.49	112.9	2.29	117.8	3.52
72	89.1	2.65	79.2	5.36	88.6	3.15	83.3	4.23

Time (hrs)	Flask 3 75 ppm A		Flask 4 75 ppm B		Flask 5 100 ppm A		Flask 6 100 ppm B	
	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)	Urea (ppm)	NH ₃ (ppm)
0	109.4	0.14	92.6	0.13	127.1	0.15	100.1	0.144
6.5	121.6	0.20	100.1	0.32	108.1	0.20	112.3	0.211
17.5	102.9	2.13	118.9	3.21	127.0	2.14	99.2	2.533
24	73.3	3.17	67.5	3.12	81.4	3.05	84.6	2.888
44	113.3	2.49	136.2	3.75	144.0	2.29	117.0	3.277
72	86.2	3.65	86.2	4.84	79.6	2.93	82.9	4.699

Figure 3.35: Urea and NH_3 concentrations as a function of time for the urea hydrolysis experiment in control flasks (no Biowish) and 50 ppm Biowish flasks

Figure 3.36: Urea and NH₃ concentrations as a function of time for the urea hydrolysis experiment in 75 ppm and 100 ppm Biowish flasks

CHAPTER 4

Discussion

4.1 Experiment 1 – Ammonia Removal

There are two biological pathways to remove ammonia from wastewater. One is to nitrify, which requires an inorganic carbon source and sufficient level of aeration; and the other is to assimilate, which can happen in an organic carbon source containing medium (Hipkin 1989). As explained in Chapter 1, nitrification converts ammonia into nitrate through a biological process while assimilation allows heterotrophic microorganisms to uptake ammonium ions and synthesize glutamate for cellular metabolism.

In this experiment, while analyzing the control flask containing no Biowish, no evaporation of ammonia was observed since its level stayed constant throughout the entire experiment of 96 hours. Therefore, with pH remaining in a small range of 6.2 to

7.6 throughout the experiment, reduction of ammonia in the Biowish inoculated flasks with various C:N ratios would indicate the occurrence of assimilation or nitrification. Since nitrate and nitrite concentrations stayed closed to zero, the possibility of nitrification can be ruled out. The phenomena can only be explained with assimilation of ammonia.

It was observed that the C:N ratio of 6:1 provided the least reduction of ammonia concentration at 1.6 ppm. The flasks with C:N ratios of 2:1 and 4:1 behaved similarly with reduction of 6.2 ppm and 8.1 ppm, respectively. A high glucose to nitrogen ratio reduces the level of GS enzymatic activity resulting in a decrease in the rate of nitrogen assimilation (Mikes 1994). Therefore, considering the possible inhibition of GS activity, the calculated k_{NH_3} value, and cost effectiveness, the C:N ratio of 2:1 appears to be the optimal ammonia removal condition.

4.2 Experiment 2 – Denitrification

The objective of this experiment was to explore the effects of Biowish in the denitrification process while optimizing the growth conditions and environment for the Biowish organisms. C:N ratios of 2:1 and 6:1 resulted in similar rates of complete denitrification at 4.6 ppm/day and 4.8 ppm/day respectively. Although C:N ratio of 6:1 showed a slightly higher rate of complete denitrification, the rate of nitrate reduction is also an important factor to consider in deciding the conditions for optimal performance. The column reactor prepared with a C:N ratio of 2:1 eliminated all of the nitrate from the solution before the 20th hour while the C:N ratio of 6:1 produced the same effect by the

44th hour which means the C:N ratio of 2:1 achieved the fastest nitrate reduction rate at 10 ppm/day followed by C:N ratio of 6:1 at 7 ppm/day. From literature, C:N ratio of 2:1 was shown to be the minimum requirement in a growth media for complete denitrification (Her 1995). Furthermore, C:N ratios tested above 2:1 do not accelerate the process of denitrification (Her 1995). Also, higher carbon content in the media could allow the growth of other organisms that may decelerate denitrification by competing with the denitrifiers. Combining the above factors, it is fair to conclude that C:N ratio of 2:1 provides the most efficient and cost effective conditions for Biowish in the treatment of nitrate.

The original design plan for the column reactor was to produce an environment with a dissolved oxygen gradient in the vertical direction, mimicking a natural pond system. As observed in Table 3.13 and Figure 3.15, the DO fluctuated throughout the depth of the column reactors. Figure 4.1 is an example illustrating NO_3^- and NO_2^- concentrations at different depth of the same column. Various dissolved oxygen levels were thought to affect the process of denitrification resulting in different denitrifying conditions and rates. However, as seen in Figure 4.1, the results indicate that the depth in the column reactors does not have an effect on denitrification due to similar patterns observed in different ports throughout the column. Also, the ORP values measured and plotted in Figure 3.14 and Figure 3.15 verified the anoxic conditions of the Biowish inoculated reactors.

Figure 4.1: Comparison of NO_2^- and NO_3^- concentrations as a function of time at different depths of the 4 ft column reactor with C:N ratio of 2:1

Table 4.1a and Table 4.1b summarize denitrification rates reported in the literature over the past two decades. The specific denitrification rate of 191.6 mg-N/g-VSS d found in this research indicates that Biowish performed comparably to the upper range of reported data for wastewater treatment units. Similarly, the volumetric denitrification rate of 4.56 mg-N/L d signifies that Biowish outperforms published data in wetland settings.

Table 4.1a: Comparison of specific denitrification rates in wastewater treatment units

Specific denitrification rate (mg-N/g-VSS d)	Temperature (°C)	Reference
191.6*	30	This study
12.96 – 27.84	25	Yoo (1999)
45.12 – 69.12	25	Sarioglu (2001)
51.84 – 78.96	30	Sarioglu (2001)
20 – 120	20	Henze (1991)
62.88 – 105.6	15	Plaza (1991)

* Specific denitrification rate is calculated by dividing $K_{NO_3^-}$ by initial Biowish concentration

Table 4.1b: Comparison of volumetric denitrification rates in wetlands

Volumetric denitrification rate (mg-N/L d)	Temperature (°C)	Reference
4.56	30	This study
2.31	N/A	Joye (1996)
1.79	18	Smith (2000)
4.4	N/A	Forbes (2009)
3.75	20	Van Oostrom (1994)
2.82	21	Bachand (2000)

4.3 Experiment 3 – Nitrite Removal

Biowish removed on average 90% of nitrite from the water at a rate of 4.7 ppm/day without the presence of nitrate. The results confirmed that Biowish organisms are responsible for eliminating nitrite from the water which matches behavior of nitrite levels observed in the denitrification experiments. Combining the results for experiments designed for denitrification and nitrite removal, Biowish appears to be a promising denitrifying agent for the nitrogen treatment process.

4.4 Experiment 4 – Treatment of Ammonia / Nitrate

Experiment 1 and experiment 2 showed that Biowish reduced ammonia and nitrate concentrations while glucose is present. Experiment 4 was set up to serve as an environment for simultaneous ammonia removal and denitrification by introducing Biowish organisms into a mixture with both ammonia and nitrate. The column reactors originally provided aerobic conditions as shown by the Dissolved Oxygen (DO) and Oxidation-Reduction-Potential (ORP) results (higher DO concentration and positive ORP in the control). The Biowish inoculated samples displayed lower DO and negative ORP which suggests that microorganisms depleted the initial dissolved oxygen contents until the environment became anoxic. The denitrification process was facilitated by Biowish denitrifiers because of the lack of dissolved oxygen in the columns.

To select the optimal condition for simultaneous ammonia assimilation and denitrification, the experiment started with C:N ratio of 3:1 and then slowly decreased to 2:1 and 1:1. The column reactors with C:N ratio of 3:1 showed an averaged k_{NH_3} of 1.62 ppm/day and $k_{\text{NO}_3^-}$ of 4.71 ppm/day. Seeing the results for C:N = 3, the C:N ratio was adjusted down to 2:1 and the initial NO_3^- level was doubled to explore the potential of Biowish in managing nitrogen under the previously mentioned conditions. The column reactors with C:N ratio of 2:1 showed an averaged k_{NH_3} of 1.59 ppm/day and $k_{\text{NO}_3^-}$ of 5.86 ppm/day. This means that even when both events need to share the same organic carbon source to occur, a C:N ratio of 2:1 is still enough to completely eliminate all the nitrate and partially eliminate the ammonia present in the wastewater solution. Column

reactors with C:N ratio 1:1 did not show assimilation because the averaged k_{NH_3} is 0.67 ppm/day of ammonia reduction, which is similar to the averaged k_{NH_3} of 0.63 ppm/day for the control column reactors. Low glucose concentration resulted in organic carbon deficiency for Biowish organisms to eliminate the nitrogen compounds. Therefore, C:N ratio of 2:1 will provide the optimal amount of organic carbon needed for the management of nitrogen in wastewater using Biowish.

Figure 4.2 compares the concentration of ammonia, nitrate and nitrite at different depths for one of the column reactors in experiment 4. Combining Figure 3.25 with the aligning characteristic of points in the graphs throughout different depths in Figure 4.2, it shows that the nitrogen removal capabilities of Biowish remains consistent while DO is below 1.5 ppm.

4.5 Experiment 5 –Urea Hydrolysis

Comparing control and Biowish flasks, increase in ammonia concentration ranged from 2.65 to 5.36 ppm for all flasks which show no reduction in the rate of ammonia formation. This indicates flasks inoculated with Biowish exhibit no significant deceleration in conversion of urea into ammonia. Therefore, Biowish does not aid in retardation of urea hydrolysis under these conditions.

Figure 4.2: Comparison of NO_2^- , NO_3^- and NH_3 at different depths for one of the column reactors in experiment 4

CHAPTER 5

Conclusion and Future Work

Biowish is an innovative product that has the potential to be inexpensive, environmentally friendly and energy efficient in the removal of nitrogen. The results from these five experiments show that Biowish has the ability to denitrify and assimilate nitrogen contaminants: denitrification experiment showed complete removal of nitrate; nitrite removal experiment showed complete removal of nitrite; and treatment for ammonia and nitrate experiment showed complete removal of nitrate and partial removal of ammonia.

Overall, application of Biowish is a feasible nitrogen removing method concerning water with initial presence of ammonia and nitrate between 10 to 20 ppm. However, it is uncertain if Biowish will have similar results when used in wastewater with an indigenous population of nitrifying and denitrifying bacteria.

The experiments performed in this study were conducted in clean column reactors, and wastewater was simulated by mixing tap water with different amounts of ammonia and nitrates. In reality, wastewater treatment is performed in a more natural environment, such as a pond. Those treatment plants could contain a layer of mud and rocks at the bottom where bacteria can attach and grow. Also, organic content in the mud can possibly aid the nitrogen removal process. A study to evaluate the effect of a mud layer (various thicknesses) can be performed by repeating the experiments with a soil lining at the bottom of the column reactors. In the case that positive results are obtained, Biowish can be tested in a full size pond with similar procedures.

Salinity in some industrial wastewater can have an influence on the nitrogen removing characteristics of denitrifying bacteria. A pilot study conducted at Waseda University in Tokyo in 2006 showed that denitrification can still occur in saline conditions with 4 to 10% salinity yielding the highest denitrification rate (Yoshie 2006). It would be interesting to explore the possibility of inoculating Biowish in saline conditions for the removal of nitrogen.

Wastewater treatment is needed throughout the year when seasonal changes could cause drastic temperature fluctuation in which growth conditions could be altered. The temperature in all experiments for this work has been controlled at 30°C as suggested by the manufacturer, which is approximately 10°C higher than the average ambient temperature at the research facility. Investigating the effect of different temperatures,

between 20°C and 30°C, on the growth of organisms and the rate of denitrification can provide alternative heating options that are more economical and efficient.

Finally, wastewater contains an indigenous population of microorganisms that can naturally facilitate nitrogen removal. It will also be helpful to repeat the column experiments using wastewater collected from treatment plants to investigate the ability of Biowish in the denitrification process relative to the findings from experiment 2 and 4 in this work. Suggested future work is shown in Figure 5.1.

Figure 5.1: Flowchart for suggested future work

References

- Awadallah, R. M. et al. "Bacterial Removal of Nitrate, Nitrite and Sulphate in Wastewater." *Water Research*. 32.10 (2010): 3080–3084.
- Bachand, P. A. M. and Horne, A. J. "Denitrification in Constructed Free-Water Surface Wetlands: I. Very High Nitrate Removal Rates in a Macrocosm Study." *Ecological Engineering*. 14.1 (2000): 9–15.
- Beccari, M., et al. "Kinetics of Dissimilatory Nitrate and Nitrite Reduction in Suspended Growth Culture." *Water Pollution Control Federation*. 55.1 (1983): 58–64.
- BiOWiSH Technologies. "Industrial Wastewater Treatment: Synthite Industries, India." (2010).
- BiOWiSH Technologies. "Industrial Wastewater Treatment: Wickar Brewing Company, United Kingdom." (2010).
- Carrera, J., et al. "Biological Nitrogen Removal of High-Strength Ammonium Industrial Wastewater with Two-Sludge System." *Water Research*. 37 (2003): 4211–4221.
- Conley, D. J. "Controlling Eutrophication: Nitrogen and Phosphorus." *Science*. 323.5917 (2009): 1014–1015.

- Dinçer, A. R., Kargi, F. “Kinetics of Sequential Nitrification and Denitrification Processes.” *Enzyme and Microbial Technology*. 27 (2000): 37–42.
- Eckenfelder, W. W., Patoczka, J. B., and Pulliam, G. W. “Anaerobic Versus Aerobic Treatment in the U.S.A” 5th International Symposium of Anaerobic Digestion. (1988): 105–114.
- FAO. "Wastewater Treatment." <http://www.fao.org/docrep/t0551e/t0551e05.htm>. (2012).
- Foglar, L., et al. “High Nitrate Removal from Synthetic Wastewater with the Mixed Bacterial Culture.” *Bioresource Technology*. 96 (2005): 879–888.
- Forbes, D. A., et al. “Comparison of Aerated Marsh-Pond-Marsh and Continuous Marsh Constructed Wetlands for Treating Swine Wastewater.” *Journal of Environmental Science and Health Part A*. 45 (2010): 803–809.
- Forster, C. “Wastewater Treatment and Technology.” Thomas Telford (2003).
- Francis-Floyd, R., et al. “Ammonia in Aquatic Systems.” University of Florida IFAS Extension. FA-16 (2005).
- Henze, M. “Capabilities of Biological Nitrogen Removal Processes from Wastewater.” *Water Science Technology*. 23 (1991): 669–679.
- Her, J.-J., et al. “Influences of Carbon Source and C/N Ratio on Nitrate/Nitrite Denitrification and Carbon Breakthrough.” *Bioresource Technology*. 54.1 (1995): 45–51.
- Hipkin, C. R., et al. “Ammonium Assimilation by the Nitrate-Utilizing Yeast, *Candida nitratophila*.” *New Phytol*. 114 (1989): 429–434.

- Ikeda, T. P., et al. "Salmonella typhimurium Apparently Perceives External Nitrogen Limitation as Internal Glutamine Limitation." *Journal of Molecular Biology*. 259 (1996): 589–607.
- Joshi, K., et al. "Denitrification: Mass Transfer and Kinetic Studies." *Proceedings of the World Congress on Engineering and Computer Science*. (2007).
- Joye, S. B., et al. "Estimating Denitrification Rates in Estuarine Sediments: A Comparison of Stoichiometric and Acetylene Based Methods." *Biogeochemistry*. 33 (1996): 197–215.
- Kanamori, K., Weiss, R., and Roberts, J. "Role of Glutamate Dehydrogenase in Ammonia Assimilation in Nitrogen-Fixing *Bacillus macerans*." *Journal of Bacteriology* 169.10 (1987): 4692–4695.
- Kapagiannidis, A. G., et al. "Determination of Kinetic Parameters in a Pilot Scale BNR System Treating Municipal Wastewater." *Global NEST Journal*. 8.1 (2006): 68–74.
- Kim, S., et al. "Treatment of High Nitrate-Containing Wastewaters by Sequential Heterotrophic and Autotrophic Denitrification." *Journal of Environmental Engineering* 130.12 (2004): 1475–1480.
- Knowles, R. "Denitrification." *Microbiological Reviews*. 46.1 (1982): 43–70.
- Kuphal, T. "Quantification of Domestic Wastewater Discharge and Associated Nitrate Loading in Marion County, Florida." (2005).
- Magasanik, B. "Ammonia Assimilation by *Saccharomyces cerevisiae*." *Eukaryotic Cell*. 2.5 (2003): 827-829.

- Malek, Esmail, and Dudley, Lynn. "An Innovative Approach for Application of Saline Wastewater from a Coal-Fired Power Plant for Agricultural Purposes." 92nd American Meteorological Society Annual Meeting. (2012)
- Mikes, V. "Ammonia-Assimilating Enzymes in the Basidiomycete Fungus *Pleurotus ostreatus*." Microbiology. 140 (1994): 977–982.
- Nagatani, H., Shimizu, M., Valentine, R. C. "The Mechanism of Ammonia Assimilation in Nitrogen Fixing Bacteria." Arch. Mikrobiol. 79 (1971): 164–175.
- NSI. "Constructed Wetlands." <http://www.natsys-inc.com/resources/about-constructed-wetlands>. (2012).
- Plaza, E., et al. "Factors Affecting Biological Nitrogen Removal Efficiency in a Large Wastewater Treatment Plant." Water Science & Technology. 24.7 (1991): 121–131.
- Ra, ChangSix and Anthony Lau. "Swine Wastewater Treatment Using Submerged Biofilm SBR Process: Enhancement of Performance by Internal Circulation through Sand Filter." Journal of Environmental Engineering. 136.6 (2010): 585–590.
- Rabalais, N. N. "Nitrogen in Aquatic Ecosystems." AMBIO: A Journal of the Human Environment. 31.2 (2002): 102-112.
- Reed, S., et al. "Natural Systems for Waste Management and Treatment." McGraw-Hill. (1995)
- Reitzer, L. "Nitrogen Assimilation and Global Regulation in *Escherichia coli*." Annual Rev. Microbiology. 57 (2003): 155-176.

- Roy, D., Komi H., and Raj B. "Effect of Carbon to Nitrogen (C:N) Ratio on Nitrogen Removal from Shrimp Production Waste Water Using Sequencing Batch Reactor." *Journal of Industrial Microbiology & Biotechnology*. 37.10 (2010): 1105–1110.
- Sarioglu, M., Horan, N. J. "Kinetic of Denitrification Processes and Experimental Work for Denitrification." *Fresenius Environ. Bull.* 10 (2001): 545–549.
- Smith, L. K., et al. "Investigation of Denitrification Rates in an Ammonia-Dominated Constructed Wastewater-Treatment Wetland." *Wetlands*. 22.4 (2000): 684–696.
- U.S. EPA. "Drinking Water Contaminants." (2009).
- U.S. EPA. "Nitrates and Nitrites." (2006).
- U.S. EPA. "Onsite Wastewater Treatment Systems Technology Fact Sheet 9." (2012).
- U.S. EPA. "Water: Meat & Poultry." <http://water.epa.gov/scitech/wastetech/guide/mpp/final-fs.cfm>. (2004).
- USGS. "Eutrophication." <http://toxics.usgs.gov/definitions/eutrophication.html>. (2011).
- van Oostrom, A. J. and Russell, J. M. "Denitrification in Constructed Wastewater Wetlands Receiving High Concentrations of Nitrite." *Water Science Technology*. 29.4 (1994): 7–14.
- Yoo, H., et al. "Nitrogen Removal from Synthetic Wastewater by Simultaneous Nitrification and Denitrification (SND) Via Nitrite in an Intermittently-Aerated Reactor." *Water Res.* 33 (1999): 145–154.
- Yoshie, S., et al. "Characteristics of Bacteria Showing High Denitrification Activity in Saline Wastewater." *Letters in Applied Microbiology*. 42 (2006): 277-283.

Yuan J., et al. "Metabolomics-Driven Quantitative Analysis of Ammonia Assimilation in *E. coli*." *Molecular Systems Biology*. 5.302 (2009): 1-16.

APPENDIX A

A.1 Calculation of Nutrient Content

In order to prepare the solution containing 20ppm of NO_3^- as N and 15ppm of NH_3 in a 4ft column reactor with a C:N = 2:1 the following calculations are needed.

Concentration of KNO_3 :

$$20 \frac{\text{mg}}{\text{L}} \text{ as N} \times \left(\frac{101.1}{14} \right) = 144.4 \frac{\text{mg}}{\text{L}}$$

In 4ft column reactor:

$$144.4 \frac{\text{mg}}{\text{L}} \times 22.2\text{L} = 3.2\text{g of } \text{KNO}_3$$

Amount of NH_3 in 4ft column reactor:

$$15 \frac{\text{mg}}{\text{L}} \text{ as N} \times 22.2\text{L} = 333\text{mg}$$

Amount of NH_4OH :

$$333\text{mg} \times \left(\frac{35}{14} \right) \div 2660 \frac{\text{mg}}{\text{L}} = 313\text{mL}$$

To calculate the amount of glucose needed to achieve a C:N ratio of 2:1 in a column reactor containing 15ppm of NH_3 and 20ppm of NO_3^- the following procedure can be followed. The amount of nitrogen provided by 15ppm of NH_3 in the column reactor is:

$$15\text{mg/l} \times 22.2\text{l} = 333\text{mg of N}$$

The amount of nitrogen provided by 20ppm of NO_3^- in the column reactor is:

$$20\text{mg/l} \times 22.2\text{l} = 444\text{mg of N}$$

Therefore, the total amount of nitrogen present in the reactor is:

$$\text{Total N} = 333\text{mg} + 444\text{mg} = 777\text{mg}$$

Then the amount of carbon needed to obtain a C:N ratio of 2:1 is $0.777\text{g} \times 2 = 1.554\text{g}$.

$$(1.554\text{g of C}) \div \frac{6 \times 12}{6 \times 12 + 12 \times 1 + 6 \times 16} = \frac{1.554\text{g of C}}{0.4} = 3.885\text{g of C}_6\text{H}_{12}\text{O}_6$$

A.2 Rate Constant Calculations

Studies have shown that denitrification follows zeroth order kinetics (Kapagiannidis 2006) for initial nitrate concentrations above 5ppm (Dinçer 2000). However, one of the experiments conducted seem to behave as first order reactions. To obtain the rate coefficients ($k_{\text{NO}_3^-}$, $k_{\text{NO}_2^-}$, or k_{NH_3}) the following methods are employed.

Since majority of the reactions in this study contain a lag phase and exhibit a nonlinear behavior at 0.5 ppm and below, for zero order reactions, the lag phase data points and all data points after the concentration has decreased to a value below 0.5 ppm are omitted from the plots with concentration as a function of time. Figure A.1 is an example of how zero order k are extracted.

For first order reactions, the lag phase data points are removed and the natural log of the remaining points are plotted as a function of time. Figure A.2 is an illustration of how first order k are calculated.

Figure A.1: Example of zero order K extraction

Figure A.2: Example of first order K extraction

APPENDIX B

Effects of Temperature on Denitrification

B.1 Methods

To conduct this experiment, a mixture of NO_3^- and Biowish was prepared and placed in 4 ft column reactors controlled at different temperature with aquarium heaters. The nominal concentrations of NO_3^- and Biowish for the initial mixture were 10 ppm and 50 ppm respectively. Temperature was varied from 20°C to 30°C as specified in Table B.1.

Table B.1: Experimental conditions for Biowish, nitrogen source and carbon source in temperature experiment

	Height	C:N	Biowish	Temp	N source	C source
					KNO_3 (NO_3^- as N)	$\text{C}_6\text{H}_{12}\text{O}_6$
Control	4 ft	1:2	-	25°C	1.60 g (10 ppm)	0.28 g
Column Reactor 1	4 ft	1:2	222 ml (50 ppm)	20°C	1.60 g (10 ppm)	0.28 g
Column Reactor 2	4 ft	1:2	222 ml (50 ppm)	25°C	1.60 g (10 ppm)	0.28 g
Column Reactor 3	4 ft	1:2	222 ml (50 ppm)	30°C	1.60 g (10 ppm)	0.28 g

B.2 Results

Started on February 17, 2011 and lasted 66 hours, this experiment investigates the optimal temperature for denitrification using Biowish. Table B.2 illustrates the different temperatures maintained throughout the experiment.

Table B.2: Experimental conditions for temperature experiment

	C:N	Temperature
Control	1:2	25°C
Column Reactor 1	1:2	20°C
Column Reactor 2	1:2	25°C
Column Reactor 3	1:2	30°C

Table B.3 provides the nitrate and nitrite results for the column reactors which are plotted in Figure B.1 with NO_3^- and NO_2^- concentrations as a function of time.

Table B.3a: NO_3^- and NO_2^- data for control column reactor for temperature varying denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)
0	8.492	0.000	8.524	0.000	6.216	0.406	8.519	0.135
6	9.243	0.000	9.092	0.000	8.885	0.000	9.087	0.000
18	7.988	0.000	8.717	0.000	8.095	0.000	8.197	0.000
24	7.998	0.000	8.331	0.000	7.985	0.000	8.459	0.000
30	8.575	0.000	8.748	0.000	9.324	0.000	8.857	0.000
42	8.388	0.000	8.106	0.000	7.935	0.000	8.109	0.000
48	7.996	0.000	7.906	0.000	7.865	0.000	8.266	0.000
54	7.852	0.000	8.598	0.000	7.992	0.000	8.372	0.000
66	8.171	0.000	8.467	0.000	8.234	0.000	8.229	0.000

Table B.3b: NO_3^- and NO_2^- data for 20°C column reactor for temperature varying denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)
0	8.502	0.000	6.832	0.000	9.035	0.000	8.207	0.000
6	8.704	0.000	8.522	0.000	8.782	0.000	8.738	0.000
18	7.715	0.000	8.811	0.000	7.747	0.000	7.605	0.000
24	8.058	0.000	7.766	0.000	7.450	0.000	8.616	0.000
30	11.486	0.000	7.579	0.035	7.827	0.000	6.258	0.000
42	8.212	0.128	7.494	0.138	8.010	0.082	7.696	0.090
48	8.318	0.120	7.530	0.136	7.713	0.113	7.771	0.120
54	7.515	0.165	7.667	0.199	7.809	0.178	7.730	0.184
66	7.833	0.359	7.011	0.331	7.742	0.331	7.593	0.373

Table B.3c: NO_3^- and NO_2^- data for 25°C column reactor for temperature varying denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)
0	8.273	0.000	7.283	0.300	9.395	0.000	8.593	0.171
6	8.795	0.000	6.023	0.054	2.880	0.000	8.495	0.000
18	7.987	0.000	9.040	0.101	4.515	0.000	8.357	0.000
24	8.134	0.000	8.469	0.051	8.027	0.041	8.003	0.043
30	7.979	0.000	10.425	0.149	7.733	0.103	7.819	0.127
42	8.027	0.425	8.374	0.333	7.320	0.294	7.106	0.361
48	7.243	0.655	7.555	0.612	7.243	0.637	6.788	0.655
54	7.847	0.913	7.271	0.829	7.207	0.766	7.065	0.921
66	7.124	1.281	7.326	1.246	6.577	1.126	6.269	1.406

Table B.3d: NO_3^- and NO_2^- data for 30°C column reactor for temperature varying denitrification experiment

Time (hrs)	Surface		Port 1		Port 2		Port 3	
	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)	NO3 (ppm)	NO2 (ppm)
0	8.581	0.000	5.450	0.000	7.632	0.000	6.748	0.000
6	8.454	0.000	8.962	0.000	9.126	0.000	8.613	0.000
18	9.123	0.000	6.656	0.133	8.185	0.000	7.140	0.168
24	8.024	0.030	7.036	0.124	7.347	0.151	7.139	0.157
30	12.540	0.092	7.185	0.248	6.983	0.267	7.185	0.248
42	7.873	0.090	5.821	0.461	6.853	0.468	5.917	0.479
48	7.670	0.087	6.602	0.407	6.701	0.543	6.643	0.440
54	7.719	0.118	7.182	0.457	6.717	0.491	6.687	0.506
66	8.159	0.213	6.820	0.535	6.753	0.625	5.413	0.617

Figure B.1a: NO_3^- and NO_2^- concentrations as a function of time for 4 ft control column for temperature experiment

Figure B.1b: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor at 20°C for temperature experiment

Figure B.1c: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor at 25°C for temperature experiment

Figure B.1d: NO_3^- and NO_2^- concentrations as a function of time for 4 ft column reactor at 30°C for temperature experiment

Comparing control and all other column reactors at different temperatures in Figure B.1, it can be seen that all columns behave similarly with little to no reduction in NO_3^- by the end of the experiment. In some column reactors, small amounts (about 1 ppm) of NO_2^- appeared after the 54th hour.

B.3 Discussion

This experiment was set out to explore the effect temperature in the denitrification process with Biowish. Since there was no significant NO_3^- reduction and/or NO_2^- production from the results, it can be assumed that denitrification was negligible in all of the column reactors. This shows that when carbon source is limited ($\text{C:N} \leq 1:2$) denitrification will not take place despite the favorable temperature conditions given (30°C as suggested by the manufacturer).