BIOLOGICAL DENITRIFICATION OF HIGH NITRATE INDUSTRIAL STREAMS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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To my parents, Peter Korath and Jessy Peter, and to Marun

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The purpose of this project was to develop a biological denitrification system for high nitrate industrial streams. High nitrate samples obtained from an industrial source and synthetic nitrate solution prepared using nitric acid and DI water were used in the experiments. The goal was to obtain a denitrification rate of 2 mg NO_3-N/L/min. Attached growth systems and continuous stirred tank reactors were used for the study. The reactors were kept in anoxic conditions in order to maintain denitrification conditions. Effects of micronutrient deficiency, volatile contaminants high nitrate concentration, and sodium on denitrification rates were studied.

From the study, it was concluded that the industrial stream was treatable; however, volatile contaminants present in the feed solution must be aerated out and metals must be removed by ion exchange prior to denitrification. It was also concluded that micronutrients should be added directly to the reactor in order to avoid its precipitation in the feed and deficiency in the reactor. Studies comparing sodium and potassium neutralized nitrate solutions did not show any significant differences in denitrification rates. Thus, it was concluded that sodium did not affect the denitrification process considerably. In the suspended growth system, denitrification rate of

4 mg NO_3-N/L/min was achieved with synthetic nitrate solution as the feed, and a denitrification rate of 2 mg NO_3-N/L/min was achieved using industrial nitrate solution.

An assay was also developed in order to test the biotreatability of high nitrate solutions. In the assay procedure, a batch test is conducted using the test sample along with a synthetic nitrate solution containing the same NO_3-N concentration. The absorbance values and denitrification rates of the test sample and synthetic nitrate solution are obtained after 4, 8, and 24 hours. Y_{NO} and μ_{maxNO} of the sample and synthetic nitrate solution are obtained from the absorbance and NO_3-N measurements. The biotreatability index, BI, of the test sample is obtained by taking the ratio of μ_{maxNO} of the sample to μ_{maxNO} of a synthetic nitrate solution containing the same nitrate concentration. A BI value of 1 means that the test sample can be successfully denitrified, and values considerably less than 1 show that the denitrification will be very difficult.

CHAPTER 1
INTRODUCTION

Biochemical Operations in Wastewater Treatment

Biochemical operations are used widely in wastewater treatment systems to pollutants that can cause harm to aquatic environments after discharge (Tchobanoglous, 2003). Pollutants in aquatic systems can cause low dissolved oxygen concentrations, eutrophication, and increased toxicity due to organic chemicals. Pollutants in wastewater may be classified by their physical characteristics, chemical characteristics, by their susceptibility to alteration by microorganisms, by their origin, and by their effects (Tchobanoglous, 2003). Many of these classifications may overlap for various components. The goal of wastewater treatment is to remove these pollutants in an efficient and economical manner by utilizing various unit operations. The unit operations can be divided into physical, chemical, and biochemical operations. Physical operations are operated based on laws of physics, chemical operations are operated by utilizing various chemical reactions to remove toxins, and biochemical operations use enzymatic catalysis of living microorganisms in order to treat the wastewater (Tchobanoglous, 2003).

Most wastewater containing biodegradable constituents can be treated biologically with proper control. The primary objective in using biological treatment in industrial wastewater is to reduce the concentrations of organic and inorganic compounds, as well as remove any nutrients present, such as nitrate or phosphorus (Tchobanoglous, 2003). Sometimes pretreatment is needed prior to biological treatment since industrial streams may contain components that are toxic to bacteria. In biochemical processes, soluble pollutants in the wastewater are converted to an inert form, such as CO_2 or N_2, or into new microbial biomass. Generally after the treatment, excess biomass is removed from treated effluent by a physical operation, such as settling tanks. Microorganisms may also entrap any insoluble organic matter present in the waste stream after

preliminary treatment, and it is removed through physical operation that follows. Thus, the effluent from the physical operation is relatively clean, and generally no additional treatment is required (Tchobanoglous, 2003).

Biochemical operations in wastewater treatment may be classified according to the biochemical transformation, the biochemical environment, and the bioreactor configuration (Grady, 1999). Biochemical transformations include removal of soluble organic matter, stabilization of insoluble organic matter, and conversion of soluble inorganic matter. Removal of soluble organic matter occurs when the microorganisms use the organic matter as carbon and energy source. Part of the carbon is converted to carbon dioxide during enzymatic reactions, and the rest is used to produce more biomass. This process can be carried out in aerobic conditions and anaerobic conditions (Grady, 1999). Stabilization of insoluble organic matter occurs when the particulates are entrapped within the biomass, and is converted to stable end products. Conversion of soluble inorganic matter is utilized in biological nutrient removal processes, generally to reduce phosphorus and nitrogen concentrations in wastewater. Phosphates in wastewater are converted ultimately to orthophosphates through microbial activity, and are then taken up by specialized bacteria that store large quantities of phosphates in granules within the cell (Grady, 1999). Nitrogen can be present as ammonium or nitrate in wastewater. Ammonium is converted to nitrate in aerobic environments, and nitrate is converted to inert nitrogen gas in anaerobic environments.

Biochemical environment where microbial activity takes place is classified mainly according to the terminal electron acceptor during energy production. Three main types of electron acceptors are oxygen, inorganic compounds, and organic compounds (Grady, 1999). When dissolved oxygen is present in high concentration, it becomes the primary electron

acceptor, and the environment is aerobic. When nitrate or nitrite is present in high concentrations compared to DO levels, the environment is anoxic, and nitrate or nitrite serves as the terminal electron acceptor. Sometimes organic compounds, carbon dioxide, and sulfate may act as the terminal electron acceptor, in which the environment is anaerobic (Grady, 1999). Wide ranges of microbial species thrive in aerobic environments, while anoxic and anaerobic processes are carried out by a limited number of organisms.

Biological Denitrification

Denitrification is used widely in wastewater treatment plants in order to treat nitrate and ammonia, in combination with nitrification. Nitrate concentrations in effluent streams are highly regulated by EPA. Since nitrate can act as a fertilizer, aquatic weeds, grasses and algae can grow excessively. This can lead to eutrophication and depleted oxygen levels in receiving waters, causing fish kills (Kanow, 1993). Therefore, unless the water is properly diluted or sufficient flow of water is employed to prevent accumulation, high nitrate concentrations can cause ecological imbalance. Nitrate concentrations in contaminated water have also been linked to outbreaks of methemoglobinemia in infants, and various health problems in animals (Barber, 2000).

The standard for total nitrate concentration in effluent water is $\leq$ 10 mg/L, or less than the concentration that exceeds nutrient criteria (US EPA, 2005). Various methods exist for nitrate reduction in wastewater, including air stripping, break point chlorination, ion-exchange systems, fluidized bed systems, expanded bed systems, and biological denitrification (Kanow, 1993). Most of the chemical methods used for denitrification can be costly, and the chemicals involved may release toxic compounds to the environment (Lee, 2006). Biological denitrification is

comparatively stable and reliable, and can have higher potential removal efficiency and easy process control (Barber, 2000).

In domestic wastewater plants, biological denitrification is coupled with nitrification. Nitrification converts ammonia to nitrate, and in denitrification, nitrate is converted ultimately to nitrogen gas. Two modes of nitrate removal can occur in effluent streams: assimilating and dissimilating nitrate reduction (Tchobanoglous, 2003). In assimilating nitrate reduction, nitrate is converted to ammonia for cell synthesis. This usually occurs when low NH_4^+-N is available, and the process does not depend on dissolved oxygen concentrations. In dissimilating nitrate reduction, the nitrate reduction is associated with respiratory electron chain, where nitrate or nitrite ions are used as terminal electron acceptor for the oxidation of electron donors (Tchobanoglous, 2003). The bacteria that are used for denitrification are facultative aerobic; they are able to switch from aerobic mode to anoxic mode (Grady, 1999). In aerobic conditions, bacteria use oxygen as the terminal electron acceptor. In the presence of nitrate in anoxic environments, nitrate is used as the terminal electron acceptor. In the absence of dissolved oxygen, nitrate reductase enzyme in the electron transport chain is induced, which leads to the transfer of electrons to nitrate. Presence of oxygen can suppress the activity of the enzyme (Tchobanoglous, 2003). Therefore, low oxygen concentrations and high nitrate concentrations are preferable for high denitrification rates.

Heterotrophic bacteria such as paracoccus denitrificans, thiobacillus denitrificans, and other pseudomonas are usually used for denitrification processes (Tchobanoglous, 2003). The mechanism for biological denitrification is given in Figure 1-1 (Wasser, 2002). The nitrate content in the waste stream is converted to an inert form. The enzymes involved in the

denitrification process are nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Grady, 1999).

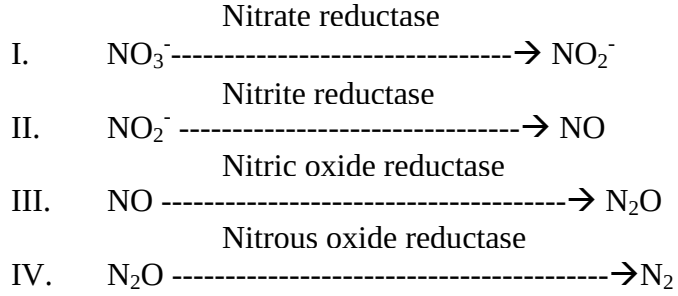

Figure 1-1. Denitrification reactions

Heterotrophic bacteria use organic carbon as the energy source for metabolism. The amount of carbon in the stream is measured using chemical oxygen demand. Chemical oxygen demand measures electrons available in an organic compound. The COD test is based on the fact that organic compounds can be fully oxidized into carbon dioxide and water with a strong oxidizing agent. The COD is expressed as the amount of oxygen required to accept the electrons from the organic compound after complete oxidation (Grady, 1999). Three sources of carbon may be used in denitrification: COD in the influent wastewater, COD produced during endogenous decay, or COD from an exogenous source (Tchobanoglous, 2003). Generally, methanol or acetate is used when exogenous carbon source is needed. Along with carbon, other nutrients are also required for bacterial metabolism and growth, including phosphorus, sulfur, potassium, calcium, iron, sodium, chlorine, and magnesium (Tchobanoglous, 2003). Domestic waters often contain necessary nutrients; for industrial wastewaters, nutrients are added externally. The energy source used for growth is called the substrate due to the extensive role of enzymes in microbial metabolism.

Kinetics of Denitrification

Bacterial metabolism can be described as a series of oxidation-reduction reactions. The substrate is consumed during growth, and additional cells are produced by binary fission, asexual mode, or by budding (Tchobanoglous, 2003). In binary fission, the cell becomes two organisms. The time required for binary fission is called generation time, which can range from less than 20 minutes to several days. In water treatment systems, the generation of new cells is limited by availability of substrate and nutrients. The generalized equation for microbial growth is given in Equation 1-1 (Tchobanoglous, 2003):

Carbon source + energy source + electron acceptor + nutrients -------→ biomass + CO_2 + reduced acceptor + end products (1-1)

For a balanced growth equation, carbon utilization and biomass growth are coupled. Therefore, the growth yield, Y, is expressed as units of biomass produced per unit of substrate removed. When the reactions are coupled, the rate of substrate utilization for synthesis and the rate of biomass growth are proportional, with Y as the proportionality constant. The actual amount of biomass formed always less than Y, since a portion of energy is used for maintenance purposes. This yield is referred to as the observed yield (Y_{obs}) (Grady, 1999).

Bacterial growth in anoxic conditions, as in denitrification, with nitrate as the nitrogen source and terminal electron acceptor, and acetate as the carbon source, follows the oxidation reduction reactions as given in Table 1-1 (Grady, 1999). The general formula used for biomass is $C_5H_7O_2N$.

Table 1-1. Oxidation-reduction reactions for denitrification

Reaction for bacterial cell synthesis with nitrate as the nitrogen source:	$1/28\ C_5H_7O_2N + 11/28\ H_2O = 1/28\ NO_3^- + 5/28\ CO_2 + 29/28\ H^+ + e^-$
Reaction for electron acceptor (with nitrate as the terminal electron acceptor):	$1/10\ N_2 + 3/5\ H_2O = 1/5\ NO_3^- + 6/5\ H^+ + e^-$
Reaction for electron donor (with acetate as the carbon source):	$1/8\ CH_3COO^- + 3/8\ H_2O = 1/8\ CO_2 + 1/8\ HCO_3^- + H^+ + e^-$

The overall molar-based equation for bacterial growth can be obtained by using the Equations 1-2a and 1-2b (Grady, 1999).

$$R = R_d - f_e*R_a - f_s*R_c \quad (1\text{-}2a)$$

$$f_s + f_e = 1 \quad (1\text{-}2b)$$

R_d is the equation for electron donor, R_a is the equation for electron acceptor, R_c is the equation for bacterial cell synthesis, f_s is the fraction of transferred electrons used for cell synthesis, and f_e is the fraction of electrons used for energy. The negative terms are inverted when used in the equation. The fraction of electrons used for synthesis can be obtained from the growth yield. The carbon for synthesis is shifted to biomass and the carbon used for energy converted to carbon dioxide during the oxidation-reduction reactions. When industrial carbon source is used, the formula for the electron donor can be estimated by analyzing the elemental compositions. Also, when nitrate is the predominant nitrogen form, the nitrogen must be reduced from +V state to –III state before it can be used as the nitrogen source for cell synthesis (Grady, 1999).

The equation for microbial growth is often written also with COD basis in terms of true growth yield. The general formula is given in Equation 1-3,

$$S_s + (-(1-Y_H))\ S_N \text{ ----------> } Y_H X_{BH} \quad (1\text{-}3)$$

where S_s is amount of substrate COD, S_N is the amount of terminal electron acceptor in COD units, Y_H is the growth yield of active heterotrophic bacteria, and X_{BH} is the active heterotrophic biomass in COD units (Grady, 1999).

Bacteria grow exponentially in favorable conditions. When there is a limiting nutrient present, the specific growth rate coefficient of the bacteria, μ, will depend on the concentration of the nutrient. The limiting nutrient can be the carbon source, nutrients, or other factor needed

by the organisms for growth. The growth rate coefficient increases initially with increasing substrate concentration, and asymptotically approaches a maximum called the maximum specific growth rate, μ_max (Grady, 1999). A general equation used to characterize bacterial growth is the Monod equation (Equation 1-4). This equation is developed strictly on empirical basis (Grady, 1999).

$$\mu = \mu\_max * (S_s/(K_s + S_s)) \qquad (1\text{-}4)$$

In the Monod equation, S_s is the substrate concentration and K_s is the half-saturation coefficient. K_s is the substrate concentration where the specific growth rate of the bacteria is equal to half of the maximum growth rate. It can used to predict how fast μ_max is obtained. The Monod equation can be extended to consider the effects of various substrates when multiple limiting nutrients are present, as shown in Equations 1-5 and 1-6 (Grady, 1999). Equation 1-5 is used when both growth limiting nutrients are required for biomass growth and they can influence the specific growth rate at the same time. Then the specific growth rate is calculated by taking the product of the Monod terms for the two nutrients. Equation 1-6 is used when both nutrients are required for biomass growth, but only one nutrient will limit the growth at a given time. Then the specific growth rate is obtained by taking the lowest value obtained from separate single-substrate models.

$$\mu = \mu\_max * (S_{s1}/(K_{s1} + S_{s1})) * (S_{s2}/(K_{s2} + S_{s2})) \qquad (1\text{-}5)$$

$$\mu = \min (\mu\_max * (S_{s1}/(K_{s1} + S_{s1}))), (\mu\_max(S_{s2}/(K_{s2} + S_{s2}))) \qquad (1\text{-}6)$$

The pH range of the system should also be considered during the denitrification. The pH of the system is generally increased during denitrification since OH^- ions are produced. The optimal pH range for the bacterial culture can be determined using various batch tests, and the

bacterial growth can be recorded. Generally, mild alkaline conditions are preferred for denitrification (Tchobanoglous, 2003).

Bioreactor Configuration for Denitrification

Two general anoxic models are used for denitrification processes: suspended growth systems and attached growth systems (Grady, 1999). In attached growth systems, microbes are grown attached to a packing material, generally rocks, sand, or other synthetic materials. The wastewater flows upward or downward, coming into contact with the attached biofilm, and the treated water flows out of the system. In anoxic systems, the packing material can be completely submerged, with gas space above the biofilm liquid layer (Grady, 1999). Excess biomass that may exit with the effluent can be separated using a clarifier and can be removed for further processing.

In suspended growth systems, microbes are kept in a suspended state by appropriate mixing. Suspended growth systems can be operated in batch mode or continuous mode. In batch systems feed is added into the reactor with suspended biomass and is allowed to react to completion. Reaction conditions and growth environment change with time. In continuous systems feed solution is continuous pumped into the reactor and an effluent is pumped out at the same flow rate. The concentrations of components in effluent are the same as the concentrations of components in the reactor. One example of a continuous suspended system is the continuous stirred tank reactor (CSTR). In a CSTR, the liquid volume is kept constant and sufficient mixing is added to keep the conditions uniform throughout the reactor. A physical operation, such as sedimentation, may be implemented in order to separate biomass from the effluent. The overflow from the sedimentation basin will have very small concentration of biomass. The underflow will have very high concentration of biomass, most of which is recycled to the bioreactor. A portion of the concentrated slurry is wasted, and is further treated before

discharging it to the environment. In several operations, flexibility is implemented by placing several CSTRs in a series. The conditions in various stages of the system can vary, thus allowing for completion of various transformations. Recycle may also be employed at desired stage of the system or through the entire chain (Grady, 1999).

Objective of the Project

The objective of this study was to develop a feasible denitrification process for treating an industrial nitrate stream. Attached growth bioreactors and suspended growth bioreactors were used for this study. Initial inoculum for the reactors were obtained from the denitrification basins at University of Florida Water Reclamation Facility. The optimal pH for denitrification was determined to be between 8 and 9.5 in previous studies. Since industrial nitrate stream does not contain enough COD to support denitrification, potassium acetate was used as an exogenous carbon source. The goal of the project was to obtain a denitrification rate of 2 mg NO_3-N/L/day.

CHAPTER 2
DENTRIFICATION OF HIGH NITRATE INDUSTRIAL STREAMS USING GROWTH BIOREACTORS

Initial attempts to denitrify the high nitrate industrial stream were performed in attached growth bioreactors, Bios2 and Bios3. The submerged attached growth bioreactors allow for short hydraulic residence times with high solids retention times, and low solids waste after denitrification. A packed bed bioreactor with upward flow and a recycle stream was used to conduct the experiments. Rock media obtained from Adventus® was used to pack the reactor, which provided high surface area for the attached growth of bacterial cells. The reactor was kept anoxic in order to allow for proper denitrification.

Materials and Methods

Setting up Bios2

A 3.78 L HDPE bottle was used to construct Bios2. The set up of the bioreactor is shown in Figure 2-1.

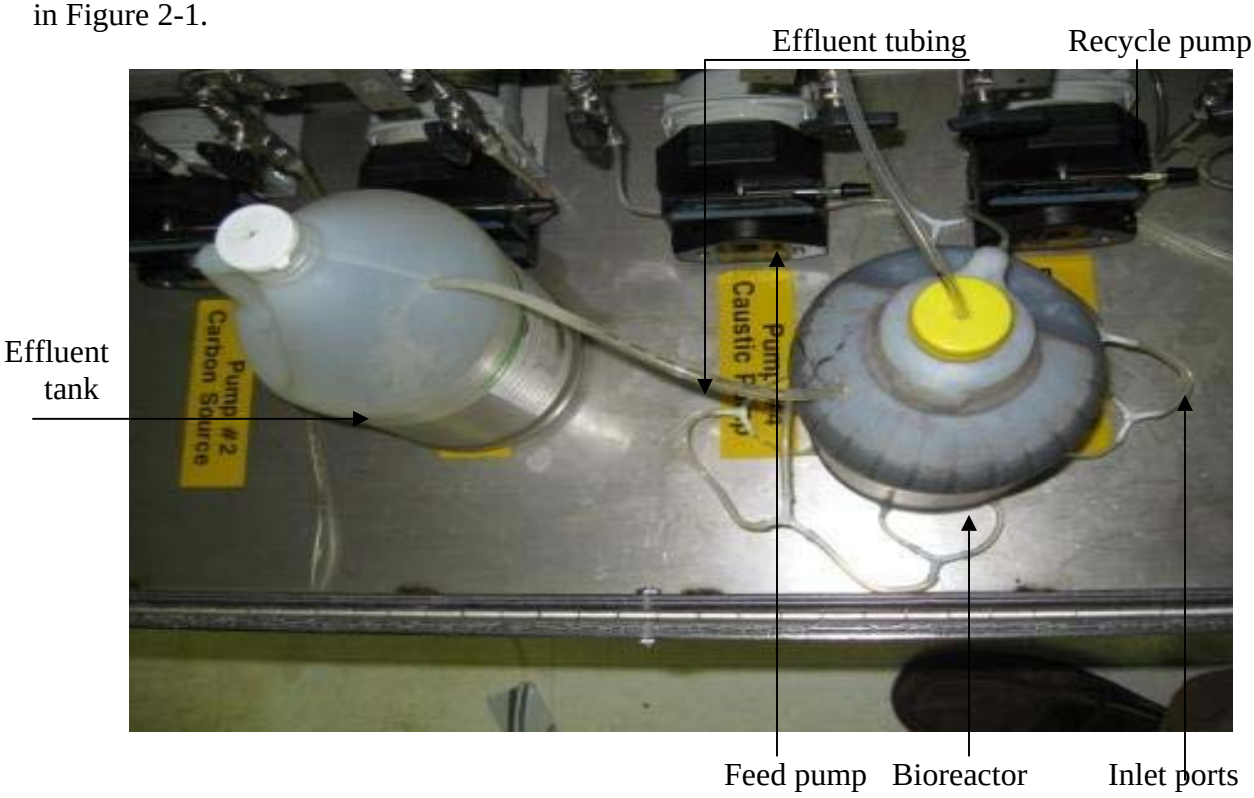

Figure 2-1. Attached growth bioreactor

The bioreactor contained eight feed inlet ports at the bottom and an outlet port at the top. This allowed for the feed to enter from all sides of the bioreactor, which allowed for better contact with the denitrifying bacteria inside. An outlet port was connected back to the feed stream as the recycle, and another outlet port was used to collect the overflow into a receiving bottle. A sample port was also constructed along the recycle outlet tubing. The feed stream was pumped from the feed tank and was allowed to mix with the recycle. This allowed for the regulation of pH of the stream entering the bioreactor. Feed and recycle streams flowed through peristaltic pumps, and pump flow dials were used to monitor the flow rates. Feed and recycle pumps were standardized and calibration curves were used to obtain the flow rates with respect to pump dials.

The mixed stream flowed through the eight inlet ports, and was allowed contact with the bacteria as it flows upward and out the bioreactor. The top portion of the bioreactor was used as the gas separator. Tubing was attached to the lid of the bioreactor to allow the gas to escape. Cole Parmer® L/S 13 tubing was used for all the connections in the bioreactor. The bioreactor was filled with stone media from Adventus® and 1.5 L of inoculum from UF WRF. The working volume of the bioreactor was 3 L.

Feed Preparation and Sampling for Bios2

Acetate was added as the carbon source for the industrial stream that was fed to the reactor in the form of sodium acetate. The stream was pre-neutralized in order to increase the pH, and had a concentration of approximately 1300 mg/L NO_3-N. The carbon source for Bios2 feed was added in order to obtain 8:1 COD-NO_3-N ratio. In order to obtain an 8:1 COD-NO_3-N ratio, 10400 mg COD/L was required in the feed stream. The initial COD was assumed to be 6000 mg/L, and thus 4.4 g/L of COD was needed in the feed solution. Thus 6.445 g/L of acetate

was added to the feed solution. In order to increase the denitrification rate of the bioreactor, approximately 0.01 g/L of yeast extract and 0.3 ml/L molybdenum (as 1.1 g/L molybdic acid) were also added to the feed stream, providing nutrients to the bacterial culture.

The initial feed flow for Bios2 was turned on to 0.57 mL/min and the recycle was turned on to 6.67 mL/min. The recycle flow to feed flow ratio was 11.57. The inlet pH of the feed solution was maintained at 5. Concentrated hydrochloric acid was added to the feed tank in increments of 10 mL if the feed stream was measured to be high.

During sampling, it was noticed that the sample port was in fact allowing a mixture of the feed stream and recycle stream to flow through the valve, and thus the nitrate concentrations measured through the valve were not a true representation of the NO_3^-N of the effluent stream of the bioreactor. Thus the sample port was moved to the recycle outlet, and sample was obtained by disconnecting the recycle tubing. This allowed for measuring the pH and NO_3-N of both inlet stream and outlet stream of the bioreactor. A small volume of the sample was discarded initially each time in order to flush out the tubing and the sample port valve. The pH and NO_3^-N tests were conducted three times a day, and the flow rates were adjusted accordingly. NO_3-N was measured using HACH® NitraVer test kits and measured using a spectrophotometer. In general, the sample was diluted to 20 times in order to obtain the nitrate concentration.

Setting up Bios3

The main purpose of Bios3 was to compare the effects of sodium and potassium on denitrification. The construction and set-up for Bios3 was identical to that of Bios2. The reactor was filled with rock media from Adventus® and 1.1 L of inoculum from UF WRF was added. The working volume of the reactor was 3 L. Eight inlet ports were still used for feed flow, along with a recycle outlet, gas outlet, and a sample port for the inlet stream. Computer Controlled

pumps were used for Bios3. The pumps were calibrated by measuring the effluent accumulated in the receiving bottle for a certain period of time.

Feed Preparation and Sampling for Bios3

Bios3 was initially started with sodium hydroxide neutralized industrial stream (sample A)-the same feed as Bios2. The feed was then switched to potassium hydroxide neutralized industrial stream (sample C). Potassium acetate was used as the carbon source for the feed. The nitrate-nitrogen concentration of sample C was 2100 mg/L, which was much higher than Bios2 feed. 15 g/L of potassium acetate was added to the feed in order to ensure excess carbon, along with 0.024 g/L potassium phosphate as a source for phosphorus, 0.01 g/L yeast extract, and 3 mL of 1.1 g molybdenum in 1000 mL molybdic acid. The feed pH was maintained at 5, and 10 mL increments of hydrochloric acid were added to the feed if high pH was observed. Nitrate and pH measurements of the effluent were taken three times daily. Nitrate-nitrogen measurements were taken using HACH® NitraVer Test 'N Tubes and a spectrophotometer. A small amount of the effluent was flushed out of the tubing before samples were taken. Two samples were collected for each measurement, one corresponding to the effluent stream collected from the recycle outlet, the other sample corresponding to the inlet stream collected from the sample port valve. The bioreactor was started with a feed flow of 0.5 mL/min and a recycle flow rate of 2 mL/min.

Results and Discussion

Bios2 Results

As shown in Figure 2-2A, Bios2 initially had high denitrification rates; however, the denitrification rate began to decrease eventually. A large amount of gas bubbles were noticed in the reactor throughout its operation. The reactor was agitated periodically to release the gas bubbles. It was noticed that when the reactor was agitated, the denitrification rates improved temporarily. However, the nitrate concentration in the reactor increased again. High

denitrification rate was also observed after the addition of micronutrients to the feed and to the reactor. 40 drops of micronutrients were added to the feed and 5 drops were added directly to the reactor. The denitrification briefly increased after the addition of micronutrients; however, the results did not stay positive for long. The highest flow rate achieved by Bios2 was 0.8 mL/min, with a denitrification rate of 95.96%. This corresponded to a denitrification rate of 0.354 mg NO_3-N/L/day. Thus, bioreactor failed to denitrify the industrial stream at the desirable rate, which was 2 mg NO_3-N/L/min.

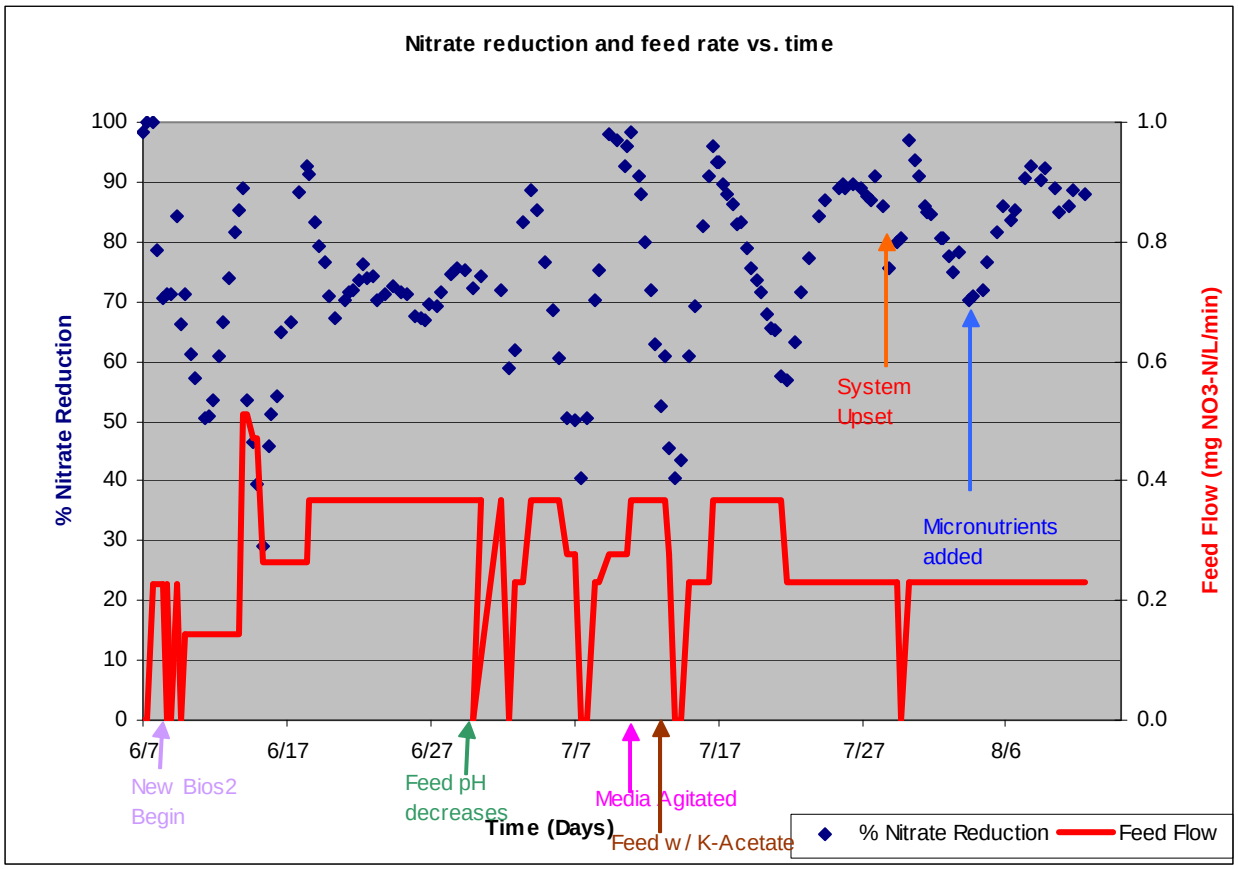

Figure 2-2A. Bios2 denitrification and feed flow rate vs. time. As shown in the figure, a slight increase in denitrification was observed when the media was agitated. However, it still continued to perform poorly. An increase in denitrification was also shown immediately after the addition of micronutrients. Also, as shown in the figure, when the pH of the effluent was measured to be higher than 9.5, the pH of the feed was decreased by the addition of concentrated HCl to the feed tank. Target feed rate for the reactor was 2 mg NO3-N/L/min.

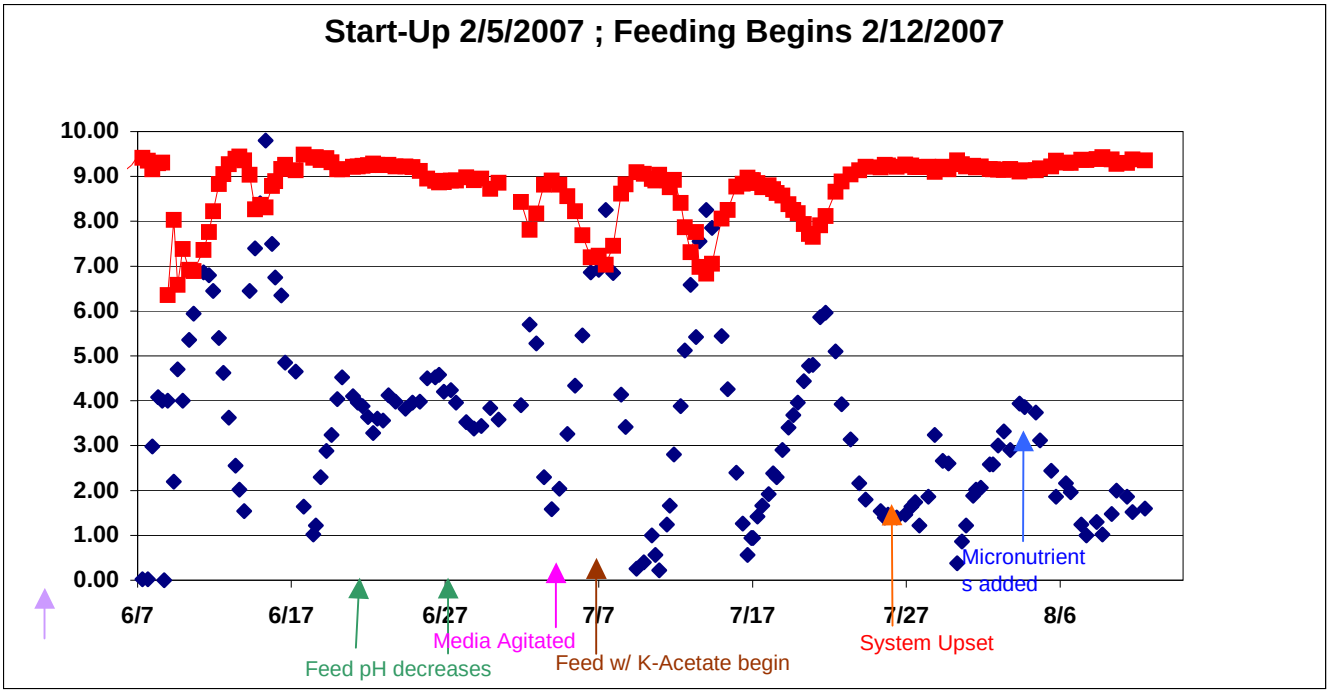

Figure 2-2B. Bios2 pH and nitrate concentrations vs. time

One factor that was taken into account after Bios2 failed to denitrify was the effect of sodium on denitrification. It was hypothesized that sodium contribute significantly to the osmotic pressure across the cell membrane, which would affect the metabolism and denitrification of the bacteria. An alternate to using sodium salt for neutralization and as carbon source was to use potassium salts. Even though potassium ions contribute to the osmotic pressure also, their effects would be smaller than those of sodium ions. Therefore, a new bioreactor was started in order to examine if potassium would lead to high denitrification rates of the industrial stream samples.

Bios3 Results

Bios3 had initial high denitrification rates; however, on the whole, it had the same results as Bios2, as shown in Figure 2-3A. It was noticed that Bios3 recovered faster from system upsets than Bios2, possibly due to the presence of potassium instead of sodium. In general, no

significant changes were observed from Bios2 results. An increase in denitrification was observed after adding micronutrients to the feed solution (4 drops/L) and directly to the reactor (5 drops). However, the high denitrification rate was temporary. Gas bubbles were also observed in the walls of the reactor. Agitation of the reactor failed to increase its performance. The highest flow rate that was achieved by Bios2 was 1.2 mL/min, with an overall denitrification of 98.7%. This corresponded to a denitrification rate of 0.612 mg NO_3-N/L/day. Therefore, Bios3 also failed to meet the target denitrification rate of 2 mg NO_3-N/L/day. The results showed using potassium salts did not have any significant impact on denitrification.

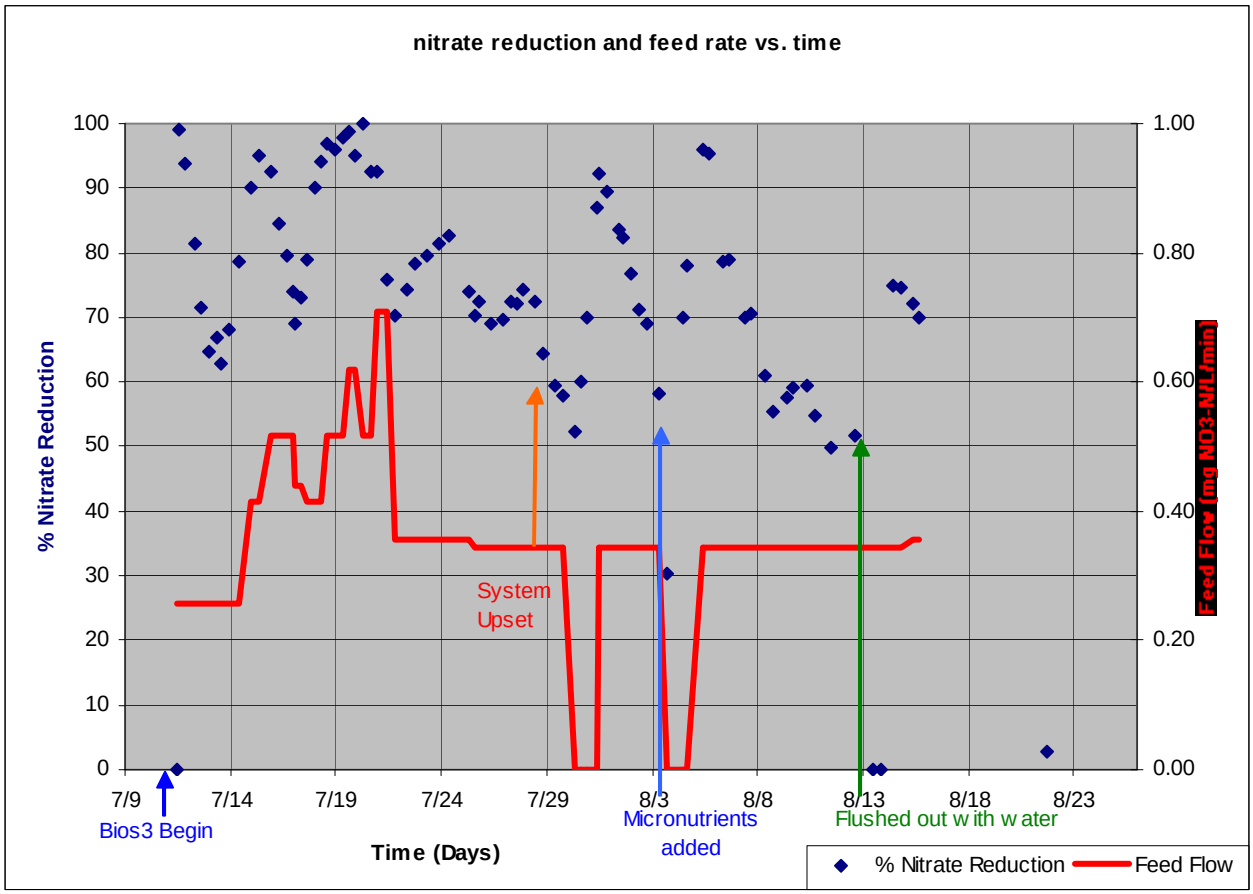

Figure 2-3A. Bios3 denitrification and feed flow rate vs. time. Bios3 results did not vary significantly from Bios2 results, even though potassium hydroxide was used to neutralize the feed rather than sodium hydroxide. Notice that an increase in denitrification was also observed in Bios3 immediately after the addition of micronutrients.

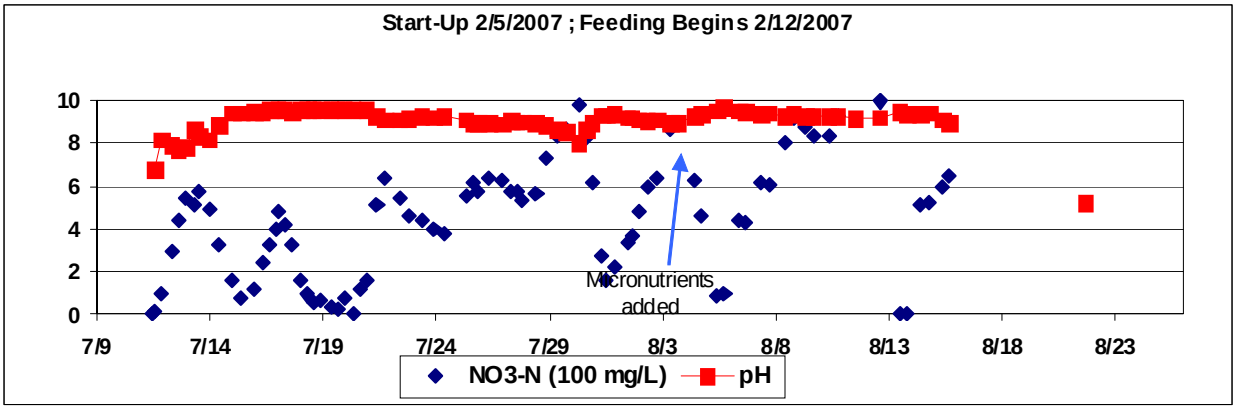

Figure 2-3B. Bios3 pH and nitrate concentration vs. time

After Bios2 and Bios3 failed to denitrify industrial nitrate streams properly, several factors were considered that might have resulted in low performance of the reactors. One possibility was the presence of metals in high concentrations in the feed, which can affect bacterial metabolism. A metals analysis of Bios2 feed, Bios3 feed, and Bios3 effluent was conducted using Inductively Coupled Plasma (ICP). However, very low concentrations of metals were found in all samples. Another factor was the high nitrate concentration of the industrial stream. The effects of high nitrate concentration of the feed on denitrification were not studied. Also, clogging of the tubing could have occurred due to various inlet ports and low flow rate of the feed and recycle. Clogging in the pores of the rock media could have occurred also, due to high concentration of biomass, which can lower the surface area for the attached growth of bacterial growth. Channeling and short-circuiting inside the reactor is also possible, and proper contact of the feed stream with the bacteria can be diminished. Another factor considered was the presence of toxic materials in the feed affecting bacterial growth. It was also possible that toxic substances present in the feed accumulated on the rock media, which can affect the metabolism of the attached bacteria and cause low denitrification.

At this point, it was concluded that the low denitrification was attributed to some possible factors related to reactor design and some possible factors related to the feed. In order to distinguish the causes, a standard suspended growth reactor was started. The suspended growth reactor used was Continuous Stirred Tank Reactor (CSTR).

CHAPTER 3
DENITRIFICATION OF HIGH NITRATE INDUSTRIAL STREAMS USING SUSPENDED GROWTH BIOREACTORS

Suspended growth bioreactors consist of reactor vessels containing biomass growing in a suspended state rather than attached to a media. Continuous stirred tank reactor (CSTR) is a suspended growth bioreactor where the contents in the reactor are stirred continuously in order to keep the conditions homogeneous within the reactor and the culture suspended. The stream to be treated is fed into the reactor, and the treated liquid mixed with biomass is pumped out as the effluent. The fractions of constituents in the effluent are identical to those inside the reactor. Many processes have a recycle stream where most of the biomass is recycled into the reactor.

Materials and Methods

Setting up Suspended Growth Bioreactor

The assembly of the continuous stirred tank is given in Figure 3-1.

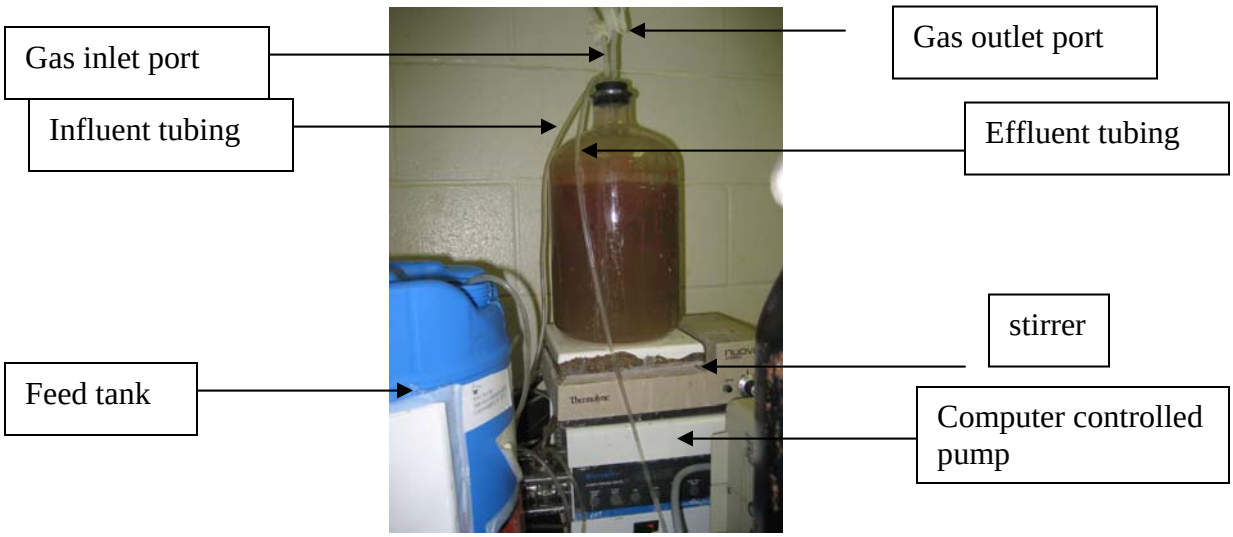

Figure 3-1. Continuous stirred tank reactor

As shown in Figure 3-1, the continuous stirred tank reactor was constructed using a 4.2 L flask. The flask was filled 10% with feed solution, and 90% with inoculum from UF WRF denitrification basin. The reactor was then set in a stir plate with the stirrer inside and was

stirred continuously. A rubber stopper was used to close the reactor and it was secured to the reactor using parafilm. The rubber stopper had a feed inlet port, an effluent port, a gas inlet port, and a gas outlet port. A nitrogen tank was setup beside the reactor and nitrogen was bubbled through the reactor twice daily for 10 minutes to strip off any oxygen that is present. Initially, the reactor was started up in the batch mode. Samples for the nitrate tests were taken through the inlet gas port. The reactor was operated in batch mode until the pH and absorbance increased, and then it was switched to continuous mode. For the continuous mode, a computer controlled pump was set up near the reactor with the same inlet and outlet flow rates. The inlet tubing was secured into the feed port in the rubber stopper, and the outlet tubing was attached to the effluent port. Cole Parmer® L/S 13 tubing was used for feed and effluent connections. The effluent from the reactor was collected and measured at a specific time period in order to calibrate the pump.

Sampling and Data Collection

NO_3-N, pH, and absorbance measurements were taken twice daily from the effluent of the reactor. In continuous mode, samples were taken from the effluent. The reactor was purged with nitrogen gas after each sampling and the gas inlet port was then secured firmly in order to keep the anoxic conditions in the bioreactor. The gas outlet port was closed only slightly in order to vent out gas formed in the reactor. Nitrate-nitrogen concentrations were measured using HACH NitraVer Test'N Tubes® and a HACH spectrophotometer. In general, the effluent sample was filtered and diluted to 20 times before nitrate-nitrogen measurements. Absorbance measurements were representative of the bacterial concentration in the reactor, and were taken using a spectrometer. The results from nitrate-nitrogen, pH and absorbance measurements were used for adjusting the flow rates in the reactor. COD measurements were taken periodically using HACH® COD test kits. The samples were filtered and diluted to 20 times. After the

samples were added to the test tubes with the reactive material, they were placed in a COD reactor for two hours. COD measurements were then taken using a spectrophotometer.

Feed Preparation

The high nitrate industrial stream obtained was pre-neutralized to appropriate pH using either sodium hydroxide or potassium hydroxide. Potassium acetate was used as the carbon source for the bacteria. 17.5 g/L of potassium acetate was used in the feed, along with 0.02 g/L potassium phosphate. A micronutrient solution was prepared using instructions for trace element solution by Vishniac & Santer. The contents in the solution are given in Table 3-1. The micronutrient solution was added in the feed initially in the amount of 4 drops/L. Later in the experiments, micronutrients were added directly to the reactor rather than in the feed.

Table 3-1. Contents of the micronutrient solution (Vishniac and Santer, 1957)

Ingredient	Amount (per 500 mL DI water)
Ethylenediaminetetraacetic acid (EDTA)	25.0 g
$ZnSO_4$	11.2 g
$CaCl_2$	2.79 g
MnCl	2.58 g
$FeSO_4$	2.59 g
$Mo_7O_{24}(NH_3)_6$	0.6 g
$CuSO_4.5H_2O$	0.785 g
$CoCl_2.6H_2O$	0.81 g
Magnesium sulfate	2.525 g
Molybdic acid	0.55 g

Artificial nitrate solution used in the experiments was prepared using 69.6 weight percent nitric acid assay and DI water. The solution was prepared by adding 8.6 mL of HNO_3 assay per one liter of DI water to obtain the same NO_3-N concentration as sample A. The solution was neutralized by adding approximately 4 g NaOH pellets per liter of solution. The feed solution also contained 17.5 g/L potassium acetate as the carbon source and 0.024 g/L potassium phosphate. The nitrate-nitrogen concentration of the feed was approximately 1450 mg/L, which was comparable to the nitrate-nitrogen concentration of sample A.

Results and Discussion

CSTR1 Results

CSTR1 was started in order to denitrify sample A neutralized with NaOH in the tote. The reactor was started in batch mode, and was switched to continuous mode at a flow rate of 1 mL/min once the pH and absorbance increased. Micronutrients were added in the feed of the reactor in the amount of 4 drops/L. The results of the reactor are shown below:

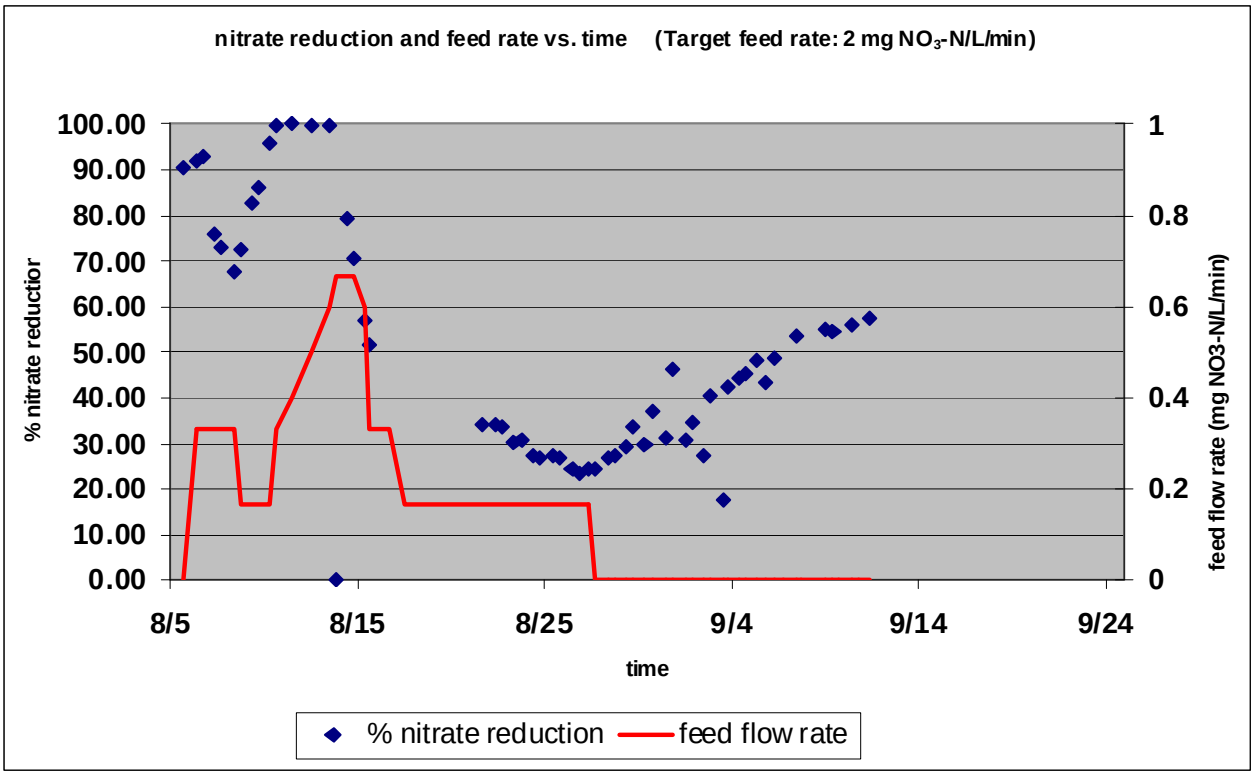

Figure 3-2. CSTR1 nitrate reduction and feed flow rate vs. time

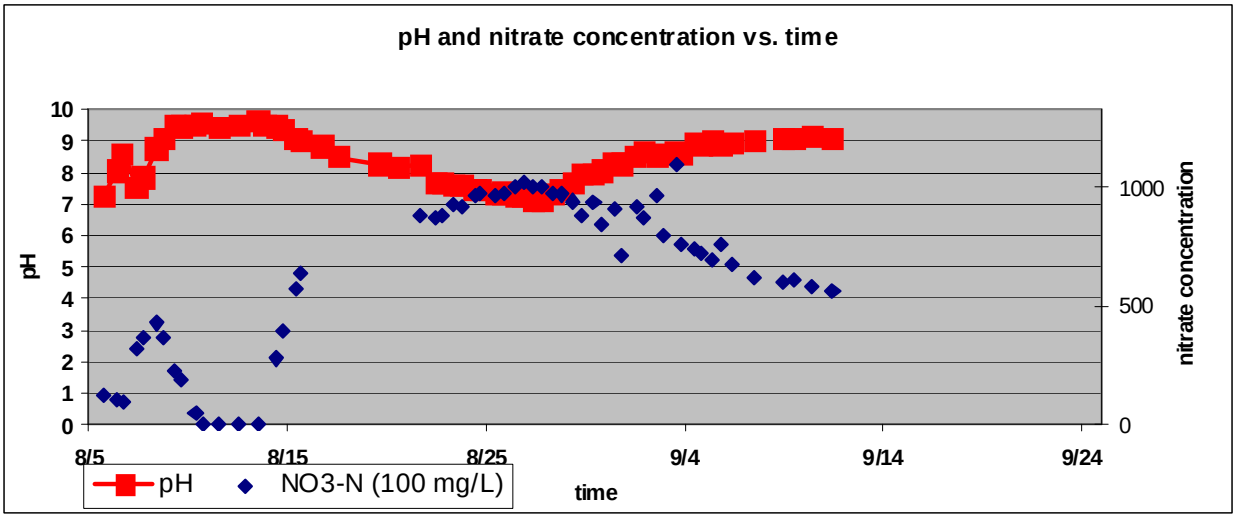

Figure 3-3. CSTR1 pH and nitrate concentrations vs. time

As shown in Figure 3-2, the denitrification rate of CSTR1 was initially high. However, it started to decrease after 8 days and continued to decrease without possibility of recovery. The pH began decreasing and nitrate concentration started decreasing with time, as shown in Figure 3-3. The reactor failed to recover even after switching to batch mode.

After CSTR1 failed, possible causes that might have contributed to the low denitrification of the industrial stream were considered. It was hypothesized that a toxic component in the sample might be accumulating in the reactor and was not consumed during the metabolism of the bacteria. Possible candidates were sodium buildup, high nitrate buildup, or accumulation of some other toxic component in the industrial solution. An analysis was done to predict the concentration of a toxic compound that might be accumulating in the reactor after a certain time period.

Analysis of CSTR1 Results

After the hypothesis that the accumulation of a constituent is causing low denitrification, a modeling analysis was done on CSTR1 results in order to predict the accumulation period of the constituent. A mass balance was done on the reactor as shown in Equation 3-1.

$$d/dt\,(VC) = FC_f - FC_o - V\,r \qquad (3\text{-}1)$$

at constant volume,

$$V\,dC/dt = FC_f - FC_o - V\,r \qquad (3\text{-}2)$$

$$dC/dt = (F/V)\,C_f - (F/V)\,C_o - r \qquad (3\text{-}3)$$

$$(F/V) = D \qquad (3\text{-}4)$$

(D = dilution rate)

$$dC/dt = D\,C_f - D\,C_o - r \qquad (3\text{-}5)$$

Developing Equations

When accumulation is considered, the reaction is neglected; then

$$dC/dt = D\,C_f - D\,C_o \qquad (3\text{-}6)$$

Since dilution rate changes with flow rate and thus with time,

$$dC/dt + D(t)\,C_o = D(t)\,C_f \qquad (3\text{-}7)$$

Using integrating factor, $IF = \exp(\int D(\tau))\,d\tau$

$$D(\tau) = \alpha\tau + D_o \qquad (3\text{-}8)$$

$$\exp(\int(\alpha\tau + D_o)\,d\tau = \exp(\alpha\tau^2/2 + D_o\tau) \qquad (3\text{-}9)$$

Multiplying by IF gives

$$\exp(\alpha\tau^2/2 + D_o\tau)\,dC/dt + \exp(\alpha\tau^2/2 + D_o\tau)\,C_o = \exp(\alpha\tau^2/2 + D_o\tau)\,C_f \qquad (3\text{-}10)$$

$$\int_t d/dt(\exp(\alpha\tau^2/2 + D_o\tau)\,C) = (\exp(\alpha\tau^2/2 + D_o\tau)\,D(t)\,C_f \qquad (3\text{-}11)$$

$$C\exp(\alpha\tau^2/2 + D_o\tau) - C_o = \int D(\tau)\,C_f \exp(\alpha\tau^2/2 + D_o\tau)\,d\tau \qquad (3\text{-}12)$$

$$C\exp(\alpha\tau^2/2 + D_o\tau) - C_o = \int (\alpha\tau + D_o)\,C_f \exp(\alpha\tau^2/2 + D_o\tau)\,d\tau \qquad (3\text{-}13)$$

$$C(t)=Co\exp -(\alpha t^2/2 + D_o t)+ \exp -(\alpha t^2/2 + D_o t)\int (\alpha\tau + D_o)\,C_f \exp(\alpha\tau^2/2 + D_o\tau)\,d\tau \qquad (3\text{-}14)$$

At constant dilution rate, $\alpha = 0$, then

$$C(t) = Co\exp -(D_o t) + C_f \exp -(D_o t)\int (D_o)\exp(D_o\tau)\,d\tau \qquad (3\text{-}15)$$

$$C(t) = C_o\exp(-D_o t) + C_f\,(1-\exp(-D_o t)) \qquad (3\text{-}16)$$

When dilution rate is not constant, it is assumed to change linearly with time; then $D = \alpha\tau + D_o$. Substituting gives

$$(\alpha\tau + D_o)d\tau = d\,(\alpha\tau^2/2 + D_o\tau) \qquad (3\text{-}17)$$

$$C_f \int_0^t (\alpha\tau + D_o)\exp(\alpha\tau^2/2 + D_o\tau)\,d\tau = C_f \int_0^t \exp(\alpha\tau^2/2 + D_o\tau)\,d\,(\alpha\tau^2/2 + D_o\tau) \qquad (3\text{-}18)$$

$$C_f \int_0^t \exp(\alpha\tau^2/2 + D_o\tau)\, d(\alpha\tau^2/2 + D_o\tau) = C_f (\exp(\alpha\tau^2/2 + D_o\tau) - 1) \quad (3\text{-}19)$$

$$C(t) = C_o \exp(-(\alpha\tau^2/2 + D_o\tau)) + C_f \exp(-(\alpha\tau^2/2 + D_o\tau)) (\exp(\alpha\tau^2/2 + D_o\tau) - 1) \quad (3\text{-}20)$$

$$C(t) = C_o \exp(-(\alpha\tau^2/2 + D_o\tau)) + C_f (1 - \exp(-(\alpha\tau^2/2 + D_o\tau))) \quad (3\text{-}21)$$

Applying CSTR1 Results

The results were analyzed over three time periods. As shown in Figure 3-4, time period 1 was taken from t_o to t_1, time period 2 was taken from t_1 to t_2, and time period 3 was taken from t_2 to t_3. It was assumed that time period 1 and time period 2 had constant dilution rate, and time period 3 had dilution rate that changed linearly with time. At $t = t_o$, C_o was taken to be zero; thus it was assumed that no toxins were present in the system initially. The results of the analysis are shown in Tables 3-2, 3-3, and 3-4.

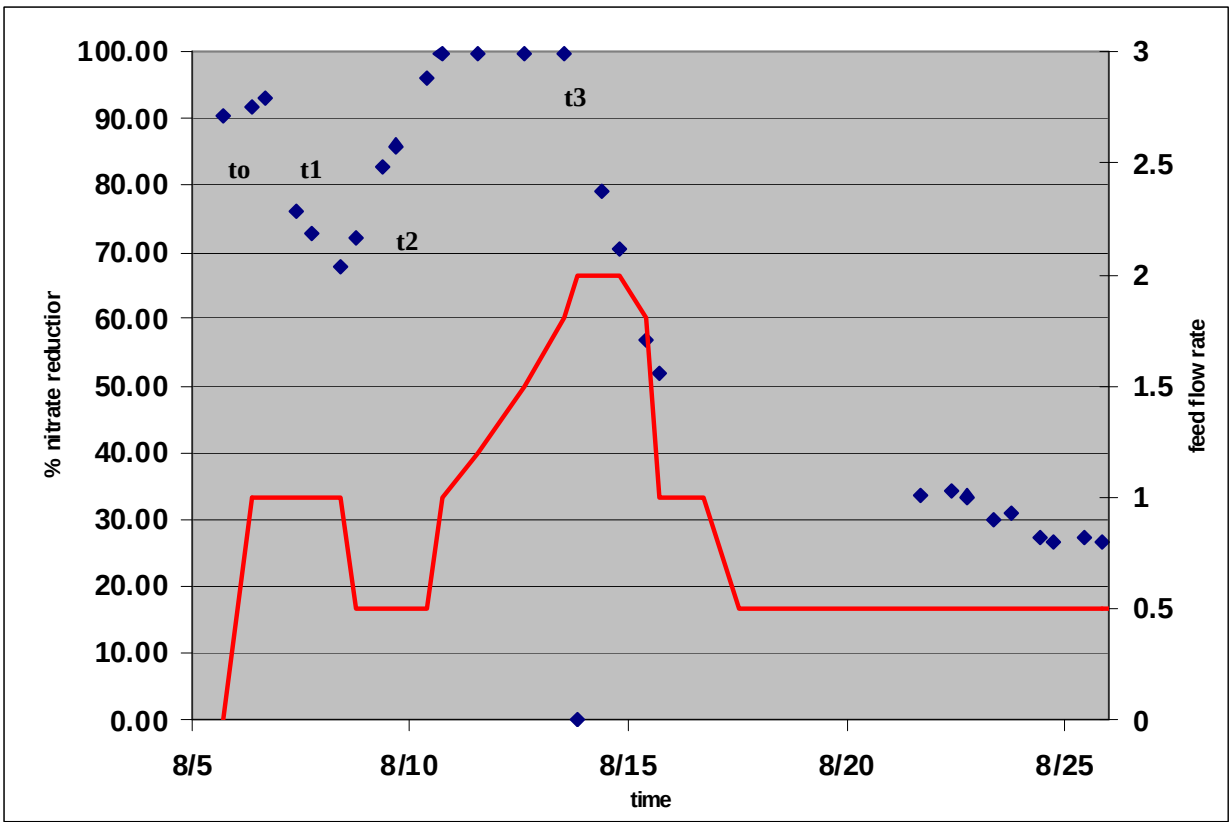

Figure 3-4. Time periods used for CSTR1 data analysis

Table 3-2. Concentration after the first time period for CSTR1

After the first time period:

$C(t) = C_o \exp(-D_o t) + C_f (1-\exp(-D_o t))$

$C_o = 0$

$C_1(t) = C_f (1- \exp(-D_o t))$

t = 3420 minutes

Do (dilution rate) = F/V = (1 mL/min)/(4000 mL) = 0.00025 min^{-1}

$C_1(t) = C_f (1- \exp((-0.00025/min)(3420 min))$

$C_1(t) = 0.575\ C_f$

Thus, after the first time period, 57.5% of the initial toxin concentration will have accumulated

Table 3-3. Concentration after the second time period for CSTR1

After the second time period:

$C_2(t) = C_1(t) \exp(-D_o t) + C_f (1-\exp(-D_o t))$

t = 3365 minutes

D_o = F/V = (0.5 mL/min)/(4000 mL) = 0.000125 min^{-1}

$C_2(t) = 0.575\ C_f \exp((-0.000125/min)(3365 min))+C_f (1- \exp(-0.000125/min)(3365 min))$

$C_2(t) = 0.721\ C_f$

Thus, after the second time period, 72.1% of the initial toxin concentration will have accumulated in the reactor.

Table 3-4. Concentration after the third time period for CSTR1

After the third time period:

Dilution changes linearly with time. Thus $D(t) = \alpha\tau + D_o$

t = 4500 minutes

α and Do were determined from the plot of dilution rate vs. time, as shown in Figure 3-5

From the plot, α = 5.4E-8

Do = 0.0002

$C_3(t) = C_o \exp(- \alpha t^2/2 + D_o t) + C_f (1- \exp(- \alpha t^2/2 + D_o t))$

$Co = C_2(t) = 0.721\ C_f$

$\exp(-\alpha t^2/2 + D_o t) = \exp\{(-5.4E\text{-}8*(4500 min)^2/2)+((0.000241 min^{-1})(4500 min))\} = 0.196$

$C_3(t) = 0.721\ C_f (0.196) + C_f (0.804)$

$C_3(t) = 0.946\ C_f$

After the third time period, 94.6% Co will have accumulated in the reactor.

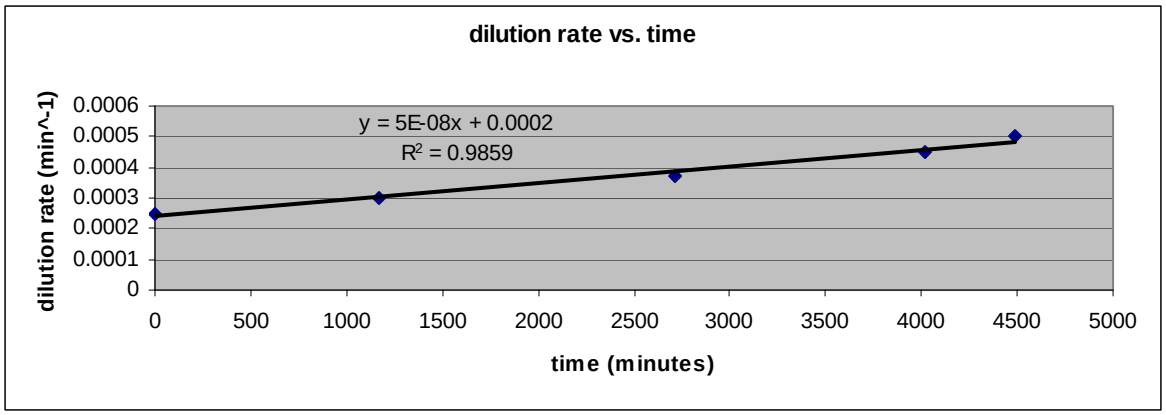

Figure 3-5. Dilution rate of CSTR1 with respect to time. From the linear regression, α was calculated to be 5E-08 and Do was obtained to be 0.0002.

The analysis showed that after 57 hours, 57.5% of the initial toxic component would have accumulated in the reactor. The model predicts that at the time of failure (8/15/2007), approximately 94.6% of the feed concentration of the toxin would have accumulated in the reactor, which can cause significant impact on the denitrification rates. Therefore, a new reactor was started using synthetic nitrate solution to investigate whether the accumulating toxin is a component present in the industrial feed or the sodium ions added to the feed for neutralization.

CSTR2 Results

CSTR2 was started using synthetic nitrate solution prepared using nitric acid and DI water. Cole Parmer® L/S 13 tubing was used to pump the feed into the reactor, and Cole Parmer® L/S 14 tubing was used to pump out the effluent. Once the pH and the absorbance of the reactor increased, the reactor was switched to continuous mode. Two computer controlled pumps were placed near the reactor; one pump was used for feed and the second pump was used for the effluent. Two pumps were required because of the difference in feed and effluent tubing sizes. Micronutrients were added to the feed in the amount of 4 drops/L. The results of the reactor are shown in Figure 3-6.

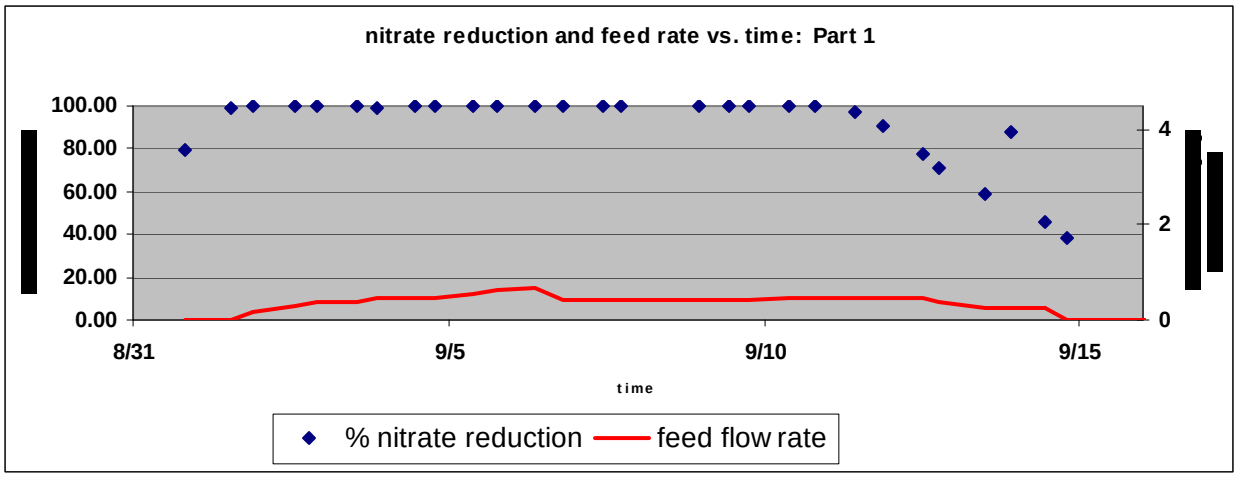

Figure 3-6. CSTR2 denitrification and feed flow rate vs. time, part I

Initially on continuous mode, the reactor was started at 0.5 mL/min. The reactor was denitrifying very well at this flow rate. The flow rate of the feed was increased up to 2 mL/min and the reactor continued to denitrify at high rates, as shown in Figure 3-6. However, the flow rate was increased without allowing the reactor to reach stable conditions. Increasing the feed flow required an increase in effluent flow rate as well, and increasing the flow prematurely can lead to washout conditions in the reactor. Therefore, the feed flow rate was decreased to 1.2 mL/min in order for the reactor to stabilize. The initial positive results seen in the reactor were brief, however, and the nitrate concentration began to increase again in the reactor. The reactor was switched to batch mode after very low denitrification rates were observed.

It was noticed that denitrification rate was high in the reactor for approximately 265 hours (11 days). The low denitrification rates and low absorbance values were observed later on. An analysis was done on the accumulation of toxins in the reactor using Equation 3-21. The initial concentration of the toxin, Co, is assumed to be zero. The ratio of C(t)/Cf was plotted against time. The results are shown in Figure 3-7

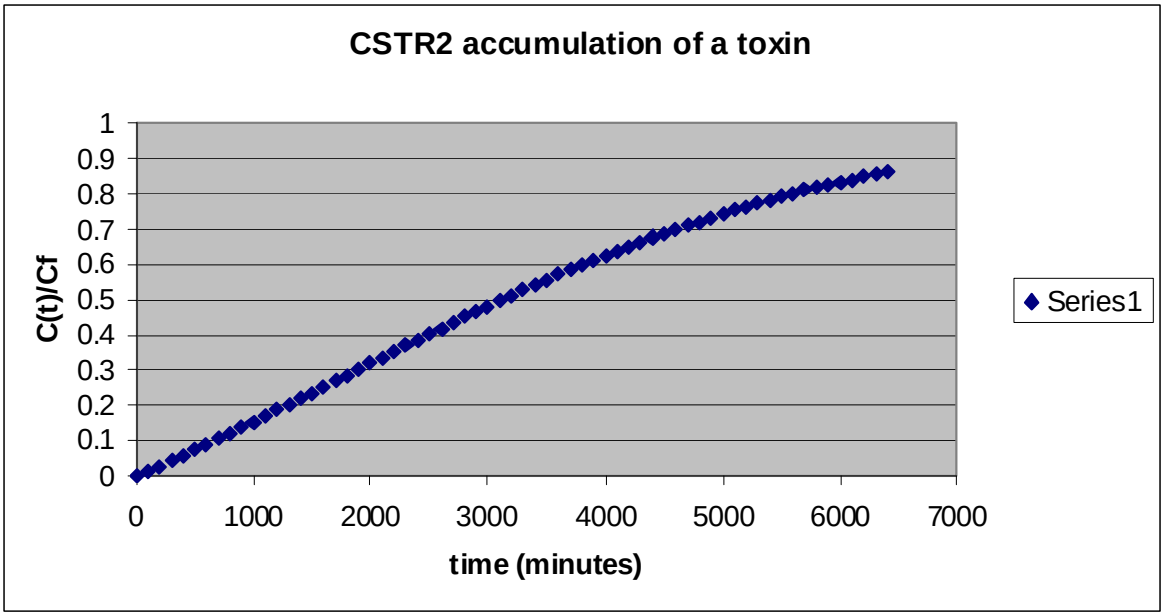

Figure 3-7. Ratio of $C(t)/C_f$ vs. time for component accumulation in CSTR2

As shown in the Figure 3-7, within after a time period of approximately 6400 minutes, the ratio $C(t)/C_f$ has reached the value of 0.86. Thus, a deterioration of the bioreactor performance should have been observed by this time. After 188 hours (9/9/2007), the toxic component concentration would have reached 99% of the feed concentration. However, as shown in Figure 3-6, the reactor continued to perform well for two more days. Thus, accumulation of a toxin was not causing the low denitrification rates in the reactor. It was then suspected that deficiency of a key constituent might be causing the low denitrification. Thus, the results were analyzed in order to predict the time that deficiency of a component might occur.

Analysis was done for the first 6400 minutes of the operation of the reactor, where a linear increase in flow rate, and thus dilution rate, was observed. The dilution rate was calculated using the relationship given in Equation 3-8, where D(t) is equal to $\alpha t + Do$. α and Do were obtained from the plot of dilution rate vs. time, as shown in Figure 3-8.

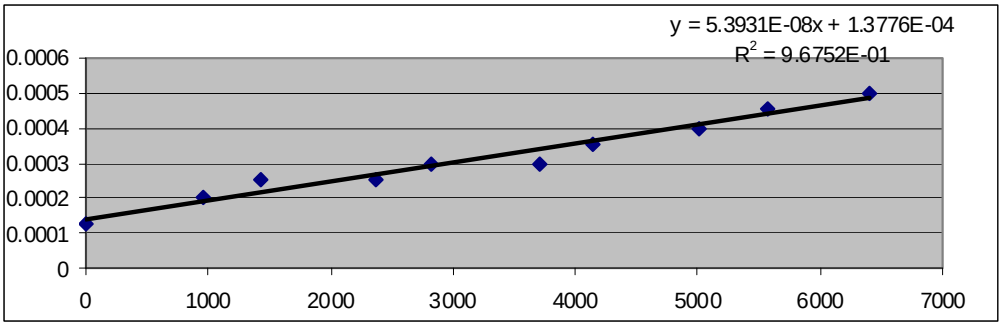

Figure 3-8. Plot of dilution rate vs. time for CSTR2

Another plot of C(t)/Co vs. time was generated in order to quantify the depletion of a component initially present in the system. Analysis done using the data obtained from the reactor showed that after approximately 107 hours, the concentration of a component added initially to the reactor would have reached approximately 13% of the feed concentration. The results are shown in Figure 3-9.

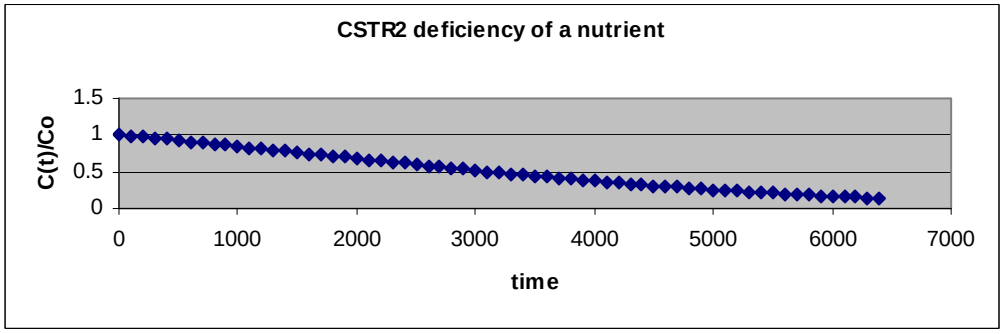

Figure 3-9. Ratio of C(t)/C(o) vs. time for component deficiency in CSTR2

Since the reactor was performing very well up to this point, it was noted that the ingredient that is exhausted is needed only in very small amounts for denitrification. Further analysis of the data showed that at the time of the failure (9/11/2007), the amount of the nutrient will have reached 0.6% of its initial concentration. One component that was required for bacterial growth in very small amounts was the micronutrients. It was noted that attached film bioreactors had started to recover previously when micronutrients were added to the feed and directly to the

reactor. However, the effect was short-lived and the reactors deteriorated again. This led to the conjecture that micronutrients added to the feed may have precipitated, as suggested by Ben Koopman. Thus, most of the micronutrients added to the feed of the CSTR would have been precipitated out. Taking this into consideration, micronutrients were added directly to the reactor everyday in proportion to the flow rate of the feed. Generally, 1 mL of micronutrients was added to the reactor when the feed flow rate is 1 mL/min. Magnesium was also added to the feed solution in the form of magnesium sulfate heptahydrate in order to ensure its availability.

After the micronutrients were added, an increase in the reactor performance as well as bacterial growth was observed, as shown in Figure 3-6. These results showed that it was indeed the deficiency of micronutrients that caused low denitrification in the reactor previously. It was also determined that the addition of micronutrients to the feed can cause precipitation, and thus, it must be added directly to the reactor. The amount of potassium phosphate added to the feed was also increased to 0.5 mg/L in order to avoid phosphorus deficiency.

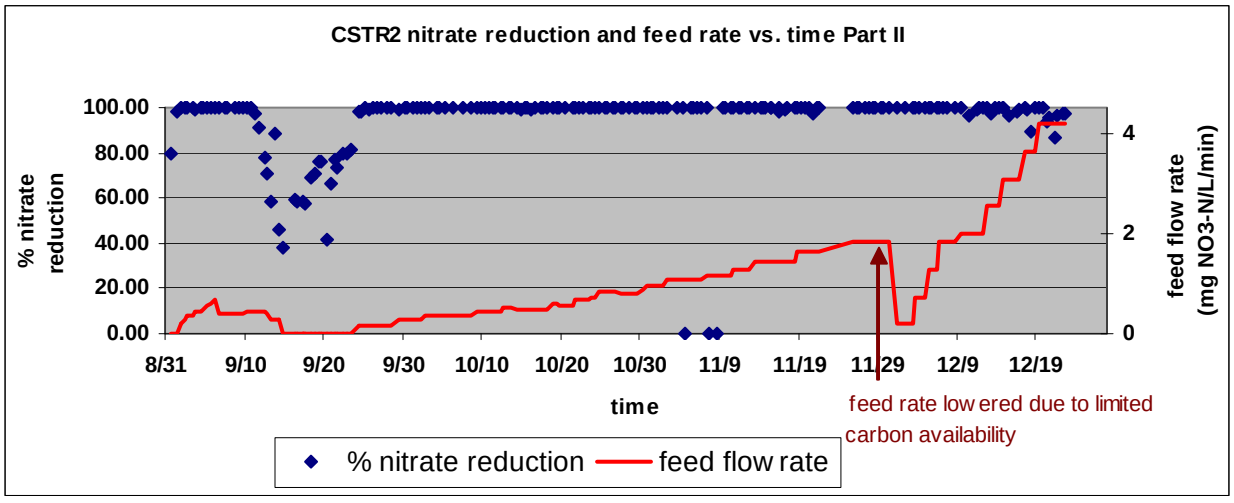

Figure 3-10. CSTR2 denitrification and feed flow rate vs. time, part II. As shown in the figure, a deficiency in carbon occurred during the operation, and the flow rate to the reactor was set at the minimum for 2 days. The flow rate was increased rapidly after carbon was available to reach appropriate steady state.

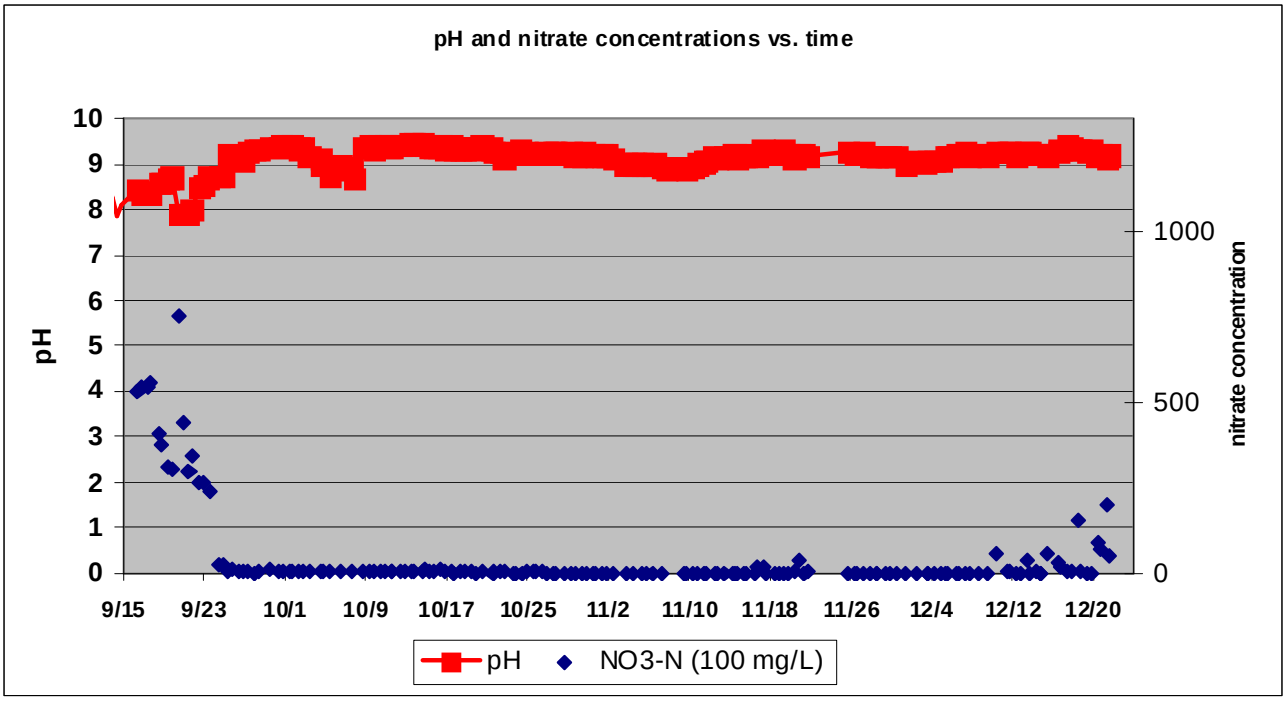

Figure 3-11. CSTR2 pH and nitrate concentrations vs. time

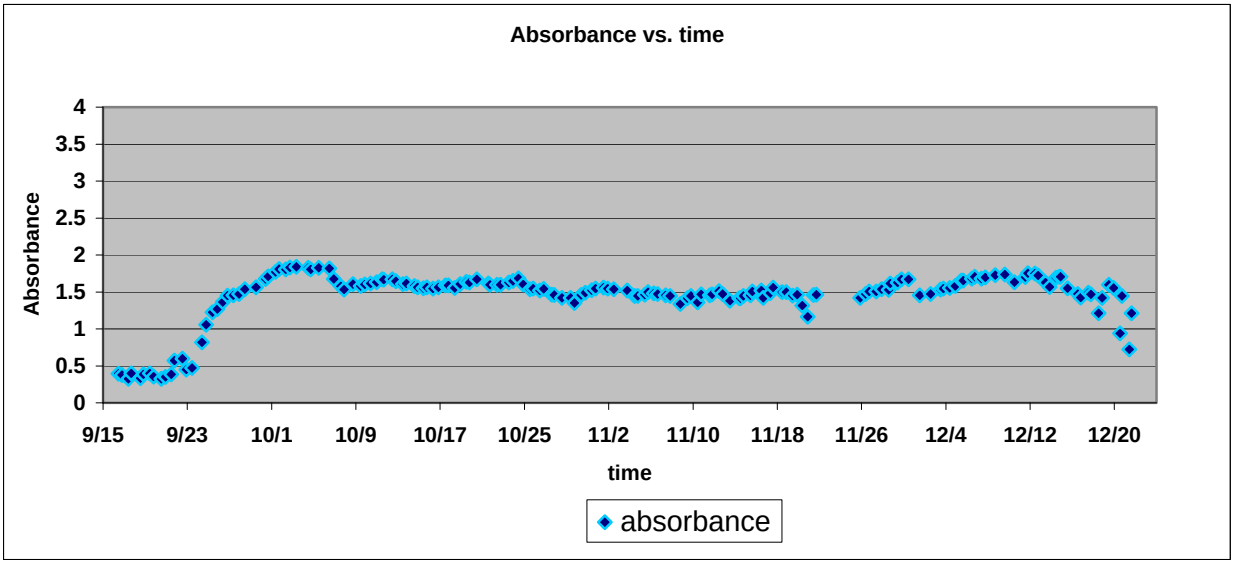

Figure 3-12. CSTR2 absorbance vs. time

After the addition of micronutrients, increase in denitrification rate and absorbance was observed in the reactor. The overall denitrification remained high with increasing flow rates, as shown in Figure 3-10. The pH of the reactor was in between 9 and 9.5 throughout its operation,

as shown in Figure 3-11. The absorbance decreased generally with an increase in feed flow rate, as expected since the effluent flow rate was also increased. The absorbance stabilized once the reactor reached steady state for each flow rate. The flow rate of the reactor was continuously increased afterwards up to a flow rate of 11.5 mL/min, as shown in Figure 3-10, which was close to twice the target flow rate, and the highest denitrification rate observed in CSTR2 was 4 mg NO3-N/L-min. From these results, it was confirmed that high nitrate concentration did not affect the metabolism and denitrification rate of the denitrifying bacteria.

It was furthermore speculated in the beginning that high amount of sodium can lower denitrification rates since it will lead to high osmotic pressure across the cell membrane. The effect of sodium was also tested previously using attached growth bioreactors-by operating a reactor containing sodium hydroxide neutralized industrial stream and another reactor containing potassium hydroxide neutralized industrial stream simultaneously. However, the results from the attached growth bioreactors were not reliable since the reason for poor denitrification could have been micronutrient deficiencies. Therefore, a new CSTR was started alongside CSTR2 in order to test the effects of sodium on denitrification rate. The new reactor was started using synthetic nitrate stream using DI water and 69.6% nitric acid assay, and the solution was neutralized using potassium hydroxide pellets instead of sodium hydroxide pellets. There weren't any noticeable differences between the results obtained from sodium hydroxide neutralized solution and potassium hydroxide neutralized solution. Therefore, it was concluded that sodium does not have any significant effects on denitrification.

CSTR3 Results

After it was confirmed that high nitrate concentration and sodium did not have significant effect on denitrification rate, another suspended growth reactor, CSTR3, was started again with the industrial stream as the feed solution. Effluent from CSTR2 was used as the

inoculum for the reactor. The reactor was initially started with artificial nitrate solution neutralized with potassium hydroxide pellets as the feed. The feed was later switched to ion-exchanged industrial stream that was pre-neutralized with potassium hydroxide. The feed solution contained 17.5 g/L potassium acetate as the carbon source, 0.5 g/L potassium phosphate as the phosphorus source, and 0.1 g/L magnesium sulfate heptahydrate in order to provide additional magnesium for bacterial metabolism. The results of the reactor are shown in Figures 3-13, 3-14, and 3-15.

The reactor continued to denitrify at a high rate using sample B (ion-exchanged industrial stream), as shown in Figure 3-13. The highest flow rate obtained using sample B was 0.8 mL/min with an overall denitrification of 99.97%. This corresponded to a denitrification rate of 0.315 mg NO3-N/L-min. The reactor was not able to achieve higher flow rates because of the shortage of available sample B solution.

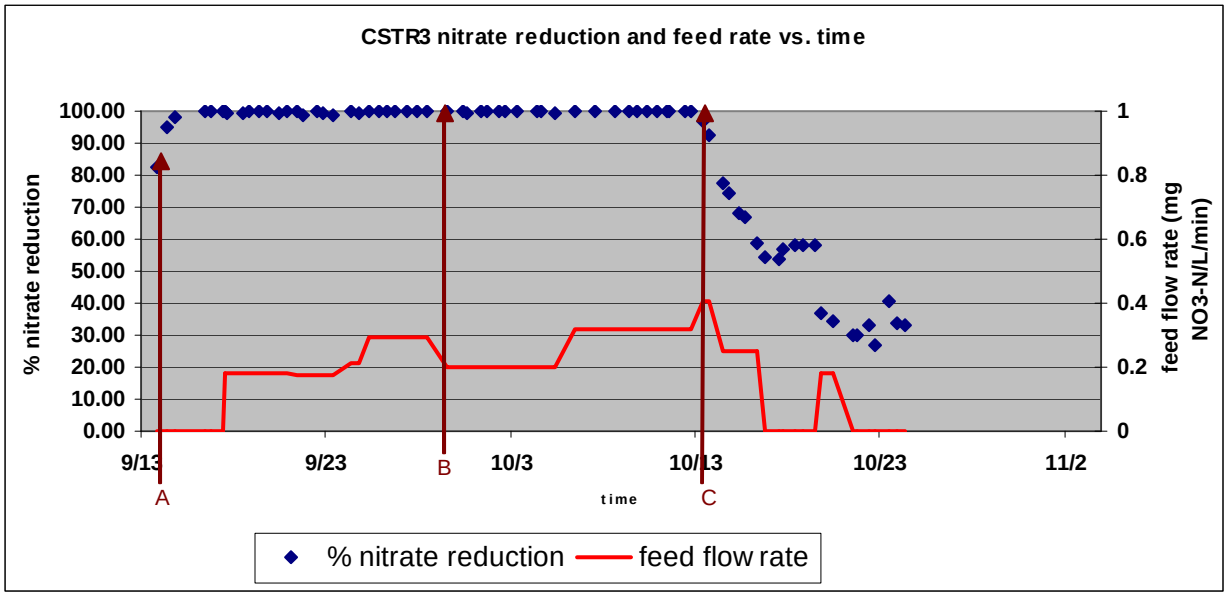

Figure 3-13. CSTR3 denitrification and feed flow rate vs. time A: HNO_3-KOH feed started; B: Switched feed to sample B, C: Switched feed to sample C

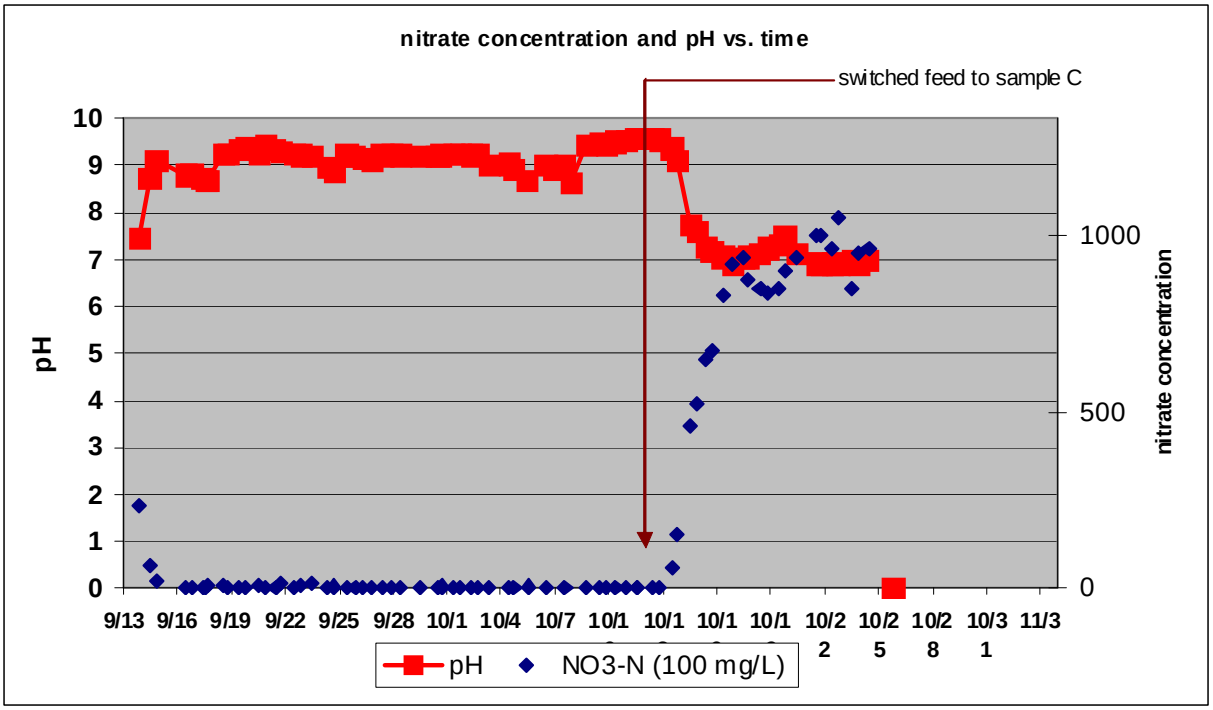

Figure 3-14. CSTR3 pH and nitrate concentration vs. time

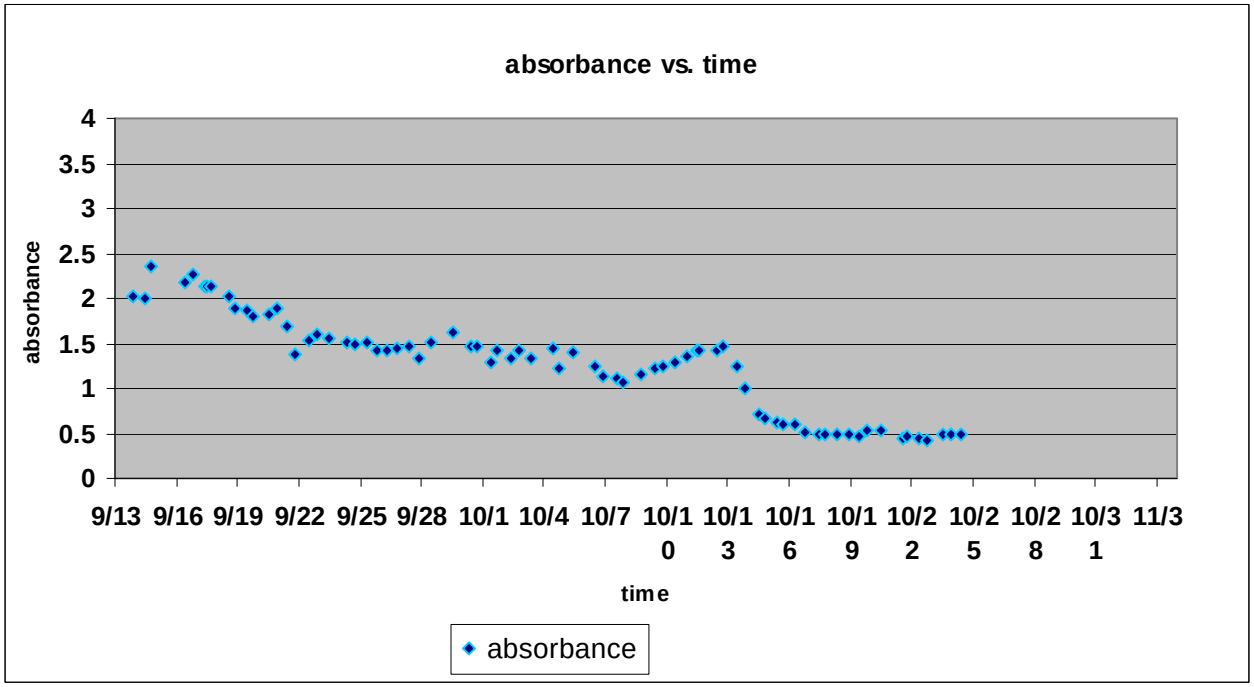

Figure 3-15. CSTR3 absorbance vs. time

After sample B was exhausted, the feed was switched to sample C, which was non-ion exchanged industrial stream neutralized with potassium hydroxide. Changes in performance of the reactor were not speculated due to this action, since it was hypothesized that it was indeed the deficiency of micronutrients that caused low denitrification of the previous industrial streams. However, as shown in Figure 3-13, the denitrification rate started to decrease as soon as the feed was switched to sample C. Figures 3-14 and 3-15 correspond to the pH and absorbance of the reactor, respectively. It can be seen clearly that the change in feed led to decrease in pH and absorbance as well. The reactor was then put on batch mode for three days and the feed was switched back to synthetic feed neutralized with potassium hydroxide. The reactor failed to recover from the low denitrification rate, however. This led to the belief that metal ions are interfering with bacterial growth and denitrification process. It was also noticed that sample C had a tint of green that was not observed in previous samples.

CSTR4 Results

After CSTR3 failed to denitrify sample C properly, other factors that may cause poor denitrification were considered. The main concern was the presence of metals in the industrial stream, which can affect bacterial metabolism and lower denitrification rates. This was further supported by the results obtained from denitrification of sample C, which was ion-exchanged. After CSTR3 was discontinued, two new batches of ion-exchanged industrial stream neutralized with KOH were received (sample D and sample E). CSTR4 was started up using sample D. The feed also contained 17.5 mg/L potassium acetate, 0.5 mg/L potassium phosphate, and 0.1 mg/L magnesium sulfate heptahydrate. Effluent from CSTR2 was used as the inoculum for the reactor. Micronutrients were added to the reactor daily in amounts proportional to the feed flow rate. The results of the reactor are shown in Figures 3-16, 3-17, and 3-18.

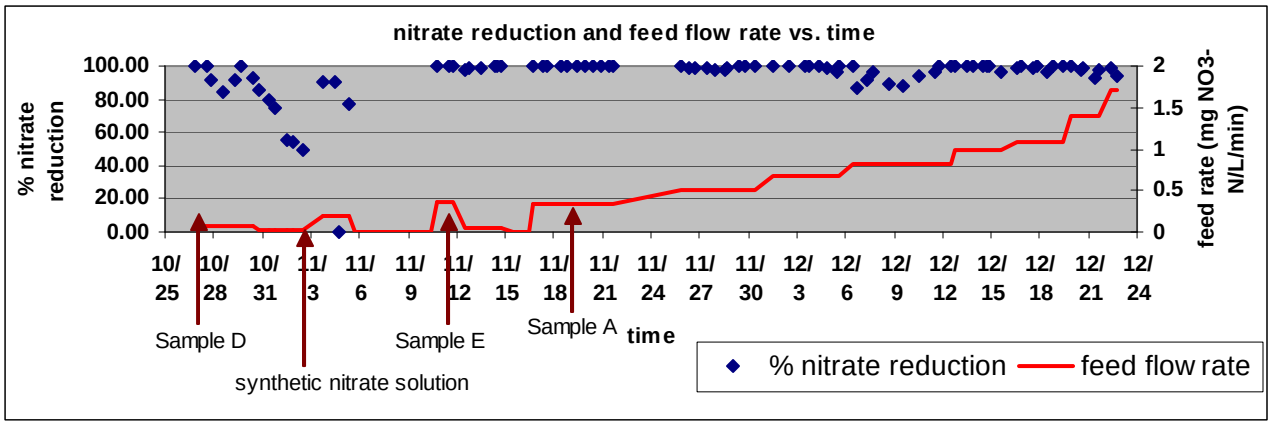

Figure 3-16. CSTR4 denitrification and feed flow rate vs. time. Low denitrification rates were observed for both ion-exchanged samples. High denitrification was observed once the feed was switched to sample A.

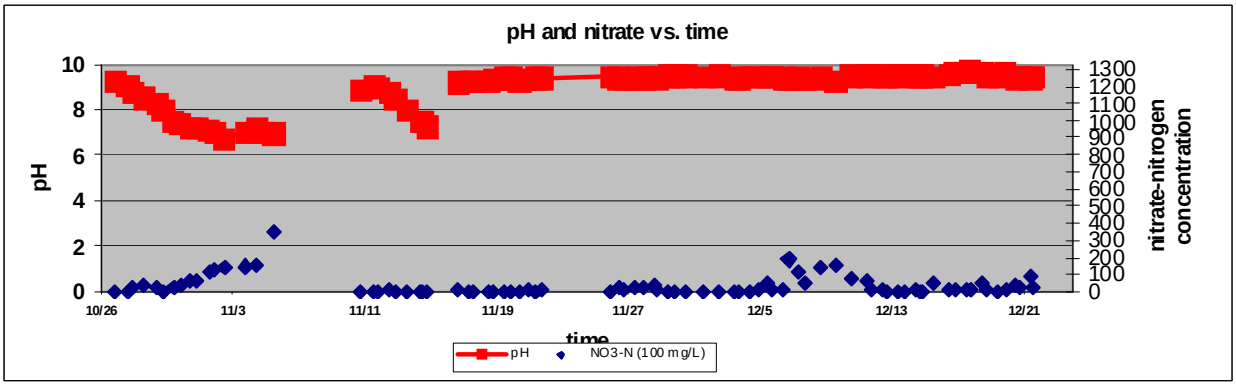

Figure 3-17. CSTR4 pH and nitrate concentrations vs. time

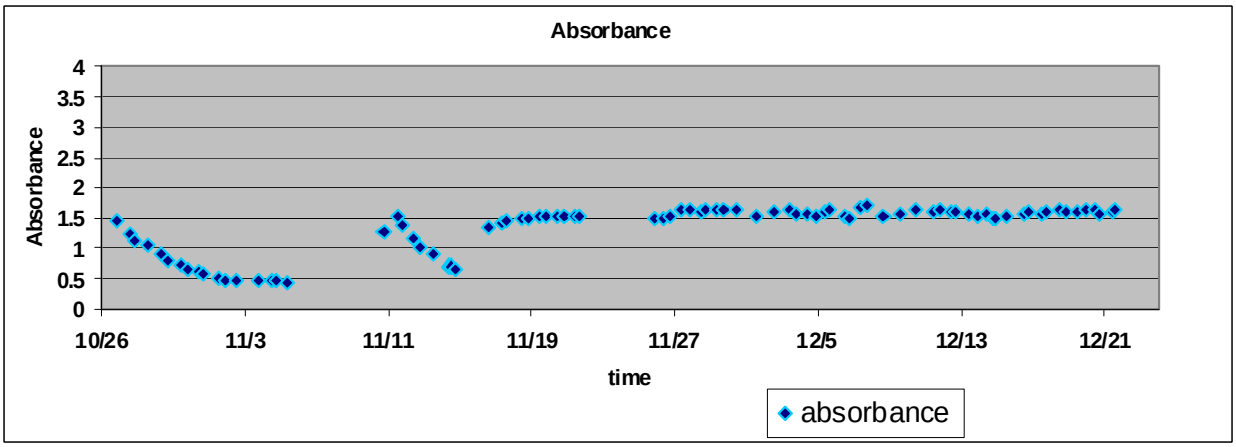

Figure 3-18. CSTR4 absorbance vs. time

CSTR4 did not have positive denitrification results initially. The pH and absorbance decreased steadily as shown in Figures 3-17 and 3-18, and the denitrification was deteriorating slowly as shown in Figure 3-16. The results were puzzling since the sample was ion-exchanged, and thus it invalidated the assumption that metal ions may be causing the problem. The reactor failed to recover after switching to synthetic feed. In order to be certain that the ion-exchange was effective, an analysis of the samples were done at the industrial site, and it was observed that one of the samples had high concentration of metal ions that might inhibit the bacterial growth and lower the denitrification rate. It wasn't positive which of the samples contained the high metal ion content. It was possible that the low denitrification of the reactor was due to presence of metal ions in the feed.

It was then decided to restart CSTR4 and observe the denitrification rates of sample E. Therefore, the reactor was restarted with synthetic feed and inoculum obtained from CSTR2 effluent. Once the reactor was in steady state with a flow rate of 1 mL/min, the feed was switched to sample E. The denitrification rate was high using this sample; however, the absorbance started decreasing. This led to the conclusion that metals were not the inhibition factor in low denitrification rates observed.

Restarting CSTR4 with Sample A

CSTR4 was restarted with sample A since it was suspected that previous low denitrification rates were caused by deficiency of micronutrients. When sample A was previously fed into the reactor, micronutrients were added in the feed solution rather than directly to the reactor. Thus, the reactor was restarted in continuous mode at 1mL/min. Effluent from CSTR2 was used as the inoculum for the reactor.

High denitrification rates were observed throughout the operation of the reactor, and pH remained steady. The reactor was able to achieve high flow rates with high overall

denitrification. The highest flow rate achieved by the reactor was 5.5 mL/min, with 97.5% denitrification, as shown in Figure 3-16. This corresponded to a denitrification rate of 1.68 mg NO_3-N/L/day, which was close to the target denitrification rate of 2 mg NO_3-N/L/day.

At this point it was concluded that the industrial stream was indeed treatable, but a component in the stream is acting as an inhibitory factor. Causes for denitrification include presence of metal ions and presence of volatile contaminants. Thus, a tool was needed to judge the treatability of the samples. Thus, a batch test was developed in order to test the treatability of high nitrate streams. It was revealed from the results of the batch tests that volatile contaminants are indeed present in the industrial stream and they might be the components contributing to the low denitrification rates and low absorbance values.

CHAPTER 4
ASSAY ON THE BIOLOGICAL TREATABILITY OF INDUSTRIAL STREAMS

A batch test procedure was developed to test the biological treatability of high nitrate water streams. The assay was inspired by studies on *Daphnia* by Gasith et. al. Since the parameter to be tested was denitrification, *Daphnia* was replaced with denitrifying bacterial culture. The denitrification results of the test solution are compared with those of the synthetic nitrate solution. The experiments were mainly conducted on ion-exchanged high nitrate industrial stream neutralized with potassium hydroxide, and non-ion exchanged industrial stream neutralized with sodium hydroxide. The batch tests primarily investigated the effects of volatile contaminants and settled particles on denitrification and bacterial growth. The general procedure developed to test the treatability of the high nitrate streams is as follows:

1. Grow denitrifying bacteria in a high nitrate stream so that they can be used for the batch tests.

2. Measure the nitrate-nitrogen concentration and pH of the test solution using HACH® NitraVer Test ‘N Tube test kits

3. Prepare synthetic nitrate solution using nitric acid with the same nitrate-nitrogen concentration as the test solution.

4. Add appropriate chemicals (given as follows) to variable and control solutions in order to support bacterial growth and metabolism:
    a. 17.5 g/L potassium acetate as carbon source
    b. 0.5 g/L potassium phosphate as phosphorus source
    c. 0.1 g/L magnesium sulfate heptahydrate as magnesium source
    d. 0.3 g/L ammonium chloride as the ammonium source

5. Neutralize the test solution and synthetic nitrate solution to pH 8 using sodium hydroxide pellets

6. Filter the solution using 2 μm filters.

7. Aerate approximately 200 mL of test solution, and keep the rest non-aerated.

8. Obtain 9 125-mL Erlenmeyer flasks; fill three flasks with 60 mL of aerated test solution, three flasks with 60 mL of non-aerated test solution, and three flasks with 60 mL of synthetic nitrate solution.

9. Add 10 mL of bacterial culture and 0.2 mL micronutrient solution-prepared using instructions for trace element solution by Vishniac & Santer to each flask.

10. Measure the nitrate-nitrogen concentration, pH, and absorbance of each flask. The nitrate-nitrogen concentration is measured using HACH test kits after filtering the sample from each flask using 0.45 μm filters, and diluting the samples to appropriate concentrations.

11. Secure all flasks with rubber stoppers and place them in an incubator at 37^oC

12. Measure NO_3-N, pH, and absorbance of one aerated flask and one non-aerated flask three times: first measurement after 3-4 hours, second measurement after 7-9 hours, and third measurement after 22-24 hours.

13. Obtain $(\mu\_max/Y'_{NO})_{sample} / ((\mu\_max/Y'_{NO})_{synthetic\ solution}$ from the data obtained in order to establish the treatability of the high nitrate stream; treatable nitrate solutions will have a value close to 1.

Developing Equations

Bacterial growth in the test flasks can be quantified since the biomass concentration is proportional to absorbance values. Biomass growth kinetics is developed assuming the presence of excess carbon and neglecting decay. The change in absorbance can be characterized by Monod equation.

$$dA/dt = \mu_{maxNO} (S_{NO}/(K_{NO} + S_{NO})) A, \text{ nitrate is the limiting component} \quad (4\text{-}1)$$

$$dS_{NO}/dt = \mu_{maxNO} (S_{NO}/(K_{NO} + S_{NO})) A \quad (4\text{-}2)$$

$$dS_{NO}/dt = (-1/Y_{NO}) (dA/dt) \quad (4\text{-}3)$$

$$A(t) = Ao + Y'_{NO} (S_{NO}(o) - S_{NO}(t)) \quad (4\text{-}4)$$

Thus, a plot of absorbance vs. nitrate concentration will have a slope of $-Y_{NO}$ and y-intercept of Ao. This plot can be obtained from the measurements of absorbance and nitrate concentration after 0, 4, and 8 hours. It was noted that K_{NO} is typically less than 1, usually for wastewater streams containing nitrate concentration around 100 mg/L. Since the nitrate concentration is much higher than 100 mg/Lin the experiments done with this assay, the ratio

($S_{NO}/(K_{NO} + S_{NO})$) is approximated to be equal to 1. Then Equation 4-2 can be rewritten as shown in Equation 4-5.

$$dS_{NO}/dt = \mu_{maxNO} [Ao + Y_{NO}(S_{NO}(o) - S_{NO}(t))] \quad (4\text{-}6)$$

$$(Ao+Y_{NO}S_{NO}(o)) = \beta \quad (4\text{-}7)$$

$$\int_{Sno(0)}^{Sno(t)} (dS_{NO}/ \beta - Y_{NO}S_{NO}) = \mu_{maxNO} \int_{0}^{t} dt \quad (4\text{-}8)$$

$$\ln(\beta - Y_{NO}S_{NO}(t)) = Y_{NO}\, \mu_{maxNO}\, t + \ln(\beta - Y_{NO}S_{NO}(o)) \quad (4\text{-}9)$$

Thus, a plot of $\ln(\beta - Y_{NO}S_{NO}(t))$ vs. $Y_{NO}*t$ will have a slope of μ_{maxNO}. Once μ_{maxNO} of the test solution is obtained, μ_{maxNO} of the test solution is compared with μ_{maxNO} of the synthetic nitrate solution. Thus, the ratio BI, given in Equation 4-10, can be considered as a treatability index. A low BI will indicate that biological denitrification of that sample will be very difficult.

$$BI=((\mu_{maxNO})_{\text{test sample}})/((\mu_{maxNO})_{\text{synthetic sample with same NO3-N concentration}}) \quad (4\text{-}10)$$

Development of the Assay

Initially the batch tests were conducted in flasks secured with rubber stoppers with a gas inlet port and a gas outlet port. The inlet and outlet ports were secured with aluminum foil after nitrogen gas was bubbled through the flasks. The first batch test was conducted to test the treatability of the ion-exchanged industrial stream neutralized with potassium hydroxide. This sample previously had poor denitrification when it was fed to the suspended growth reactor. The test was conducted alongside sodium hydroxide neutralized industrial stream, which had positive results after it was fed through the suspended growth reactor. After 24 hours, it was noticed that all the flasks had high denitrification and absorbance increase. This led to the suspicion that oxygen had entered the flasks through gas inlet and gas outlet ports, and that the absorbance increase was mainly due to aerobic growth of the bacteria. When oxygen is present, bacteria can convert the nitrate to ammonia, which is then used as the nitrogen source for bacterial growth.

In order to limit the presence of oxygen in the flasks, another batch test was conducted using flasks that were secured tightly with solid rubber stoppers. Prior to securing the flasks, nitrogen gas was bubbled through the flasks in order to purge out any oxygen. The ion-exchanged stream neutralized with potassium hydroxide was tested against artificial nitrate solution prepared using 69.6% HNO_3 assay. The results were similar to previous test, and high denitrification and absorbance increase were observed in all flasks. This did not correspond to the results obtained from suspended growth reactors. Since oxygen was purged out using nitrogen gas, the possibility of presence of any oxygen in the flasks was discarded.

It was then suspected that volatile contaminants present in the industrial stream might have caused low denitrification rates in the suspended growth reactor. It was possible that volatile contaminants may have been removed when nitrogen gas was bubbled through the flasks. Thus, another batch test was carried out using ion-exchanged sample. The flasks were again secured with solid rubber stoppers; however, nitrogen gas was not bubbled through the reactors. At the end of the batch test, it was noticed that little or no denitrification occurred in the flasks, and no bacterial growth was observed. This supported the hypothesis that volatile contaminants were causing the low denitrification rates observed previously in the CSTR, and the high denitrification in the previous batch tests were the result of removal of volatile contaminants through aeration.

In order to confirm the presence of volatile contaminants in the industrial stream, a batch test was carried out using aerated and non-aerated ion-exchanged samples. In order to avoid assimilatory denitrification, 0.3 g/L ammonium chloride was added to the flasks. The flasks containing aerated samples showed high denitrification and absorbance increase, while the flasks containing non-aerated samples had low denitrification and no absorbance increase. This

confirmed the theory that volatile contaminants were causing the low denitrification in the CSTR.

Results and Discussion

The assay was applied to test the treatability of aerated and non-aerated industrial stream samples neutralized using sodium hydroxide. Inoculum for the batch test was obtained from CSTR4 effluent. Measurements were taken after 4, 8, and 24 hours. The analyses from batch test done non ion-exchanged industrial sample are shown in Figures 4-1 through 4-6.

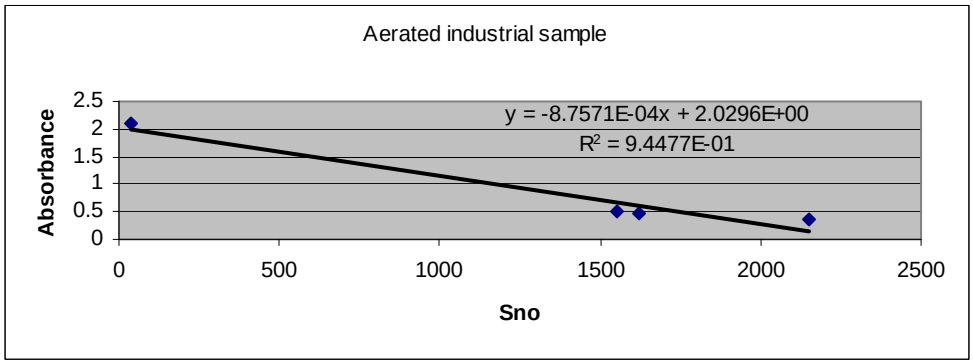

Figure 4-1. Absorbance vs. nitrate concentration, aerated sample

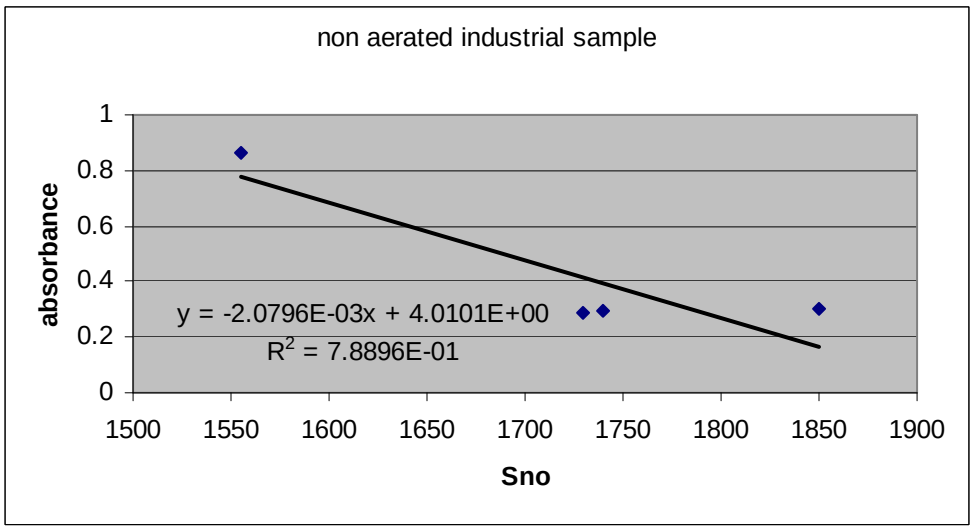

Figure 4-2. Absorbance vs. nitrate concentration, non-aerated sample

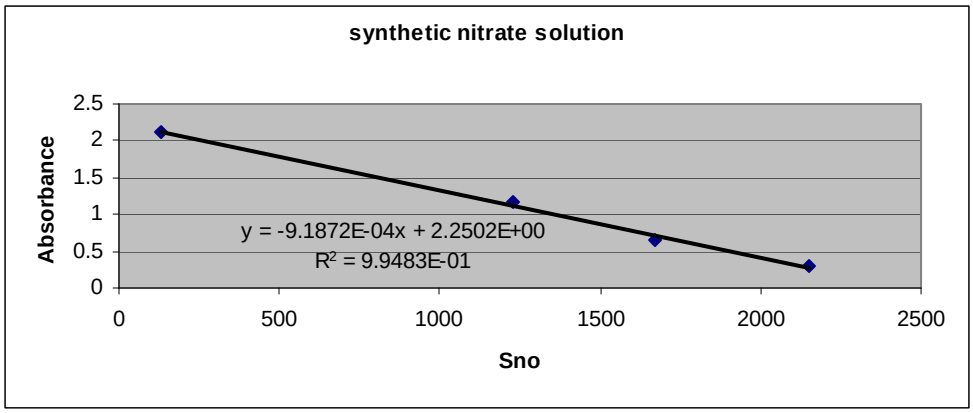

Figure 4-3. Absorbance vs. nitrate concentration, synthetic nitrate solution

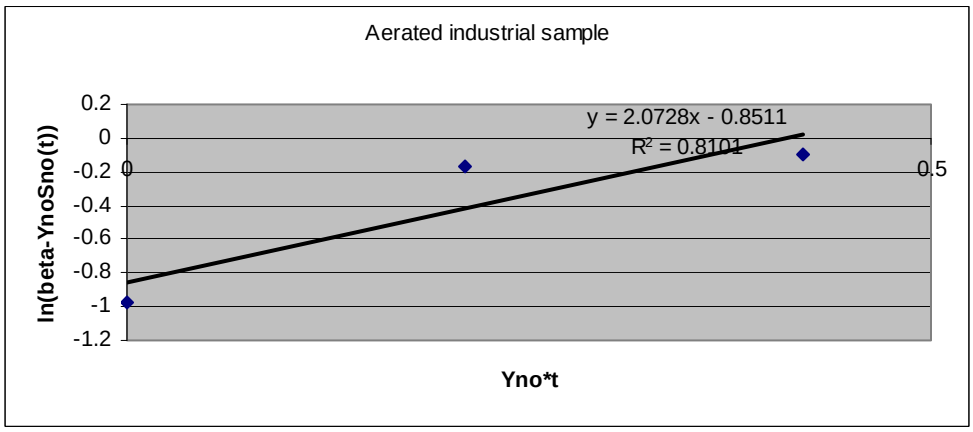

Figure 4-4. ln(β-Y_{NO}Sno(o)) vs. Y_{NO}*time, aerated sample

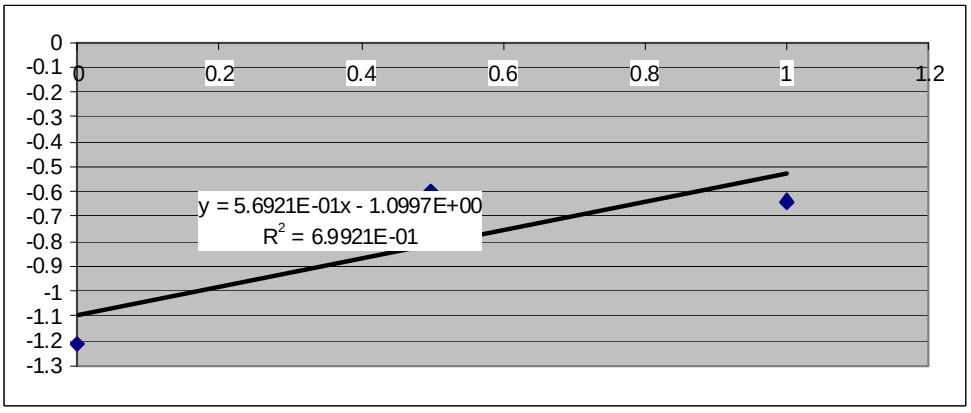

Figure 4-5. ln(β-Y_{NO}Sno(o)) vs. Y_{NO}*time, non-aerated sample

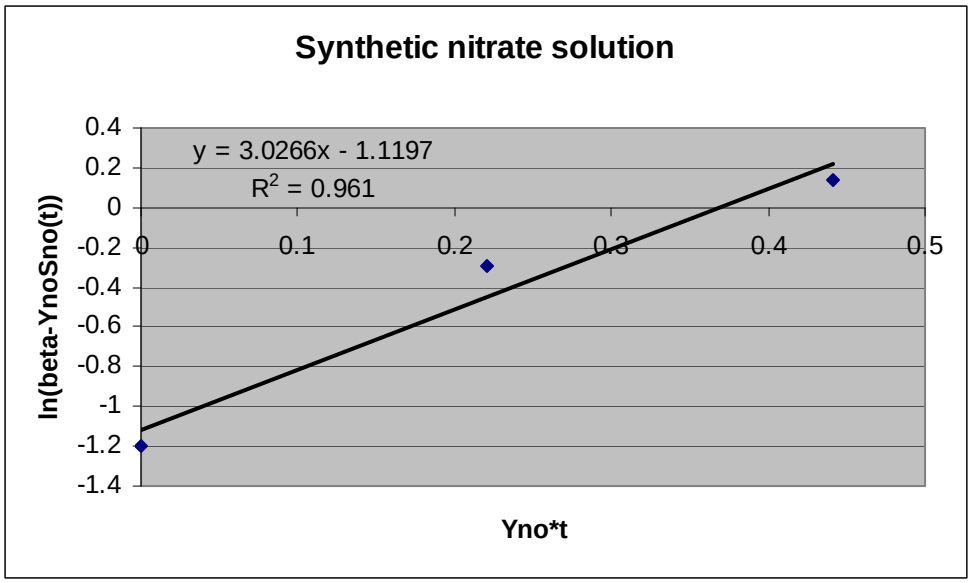

Figure 4-6. $\ln(\beta - Y_{NO}Sno(o))$ vs. Y_{NO}*time, synthetic nitrate solution

Figures 4-1, 4-2, and 4-3 show the plots of absorbance vs. nitrate concentration for aerated high nitrate industrial stream, non-aerated high nitrate industrial stream, and synthetic nitrate solution respectively. From the plots, the Y_{NO} was obtained to be 8.76E-4 for aerated stream, 2.08E-3 for non-aerated stream, and 9.19E-4 for synthetic nitrate solution. The plots in Figures 4-4, 4-5, and 4-6 were used to obtain μ_{maxNO} values for aerated sample, non-aerated sample, and synthetic nitrate solution respectively. μ_{maxNO} values were obtained to be 2.073 for aerated sample, 0.569 for non aerated sample, and 3.027 for synthetic nitrate. From μ_{maxNO} values, the biotreatability index, BI was calculated. BI for aerated sample was 0.635 and for non-aerated sample was 0.188. The assay showed that the aerated industrial stream can be treated biologically. The results were verified by using aerated industrial stream as the feed for a suspended growth reactor. High denitrification rates and absorbance values were observed. The assay was done on other industrial samples as well. The results obtained from the industrial samples are given in Figures 4-7 through 4-12. The BI values are summarized in Table 4-1.

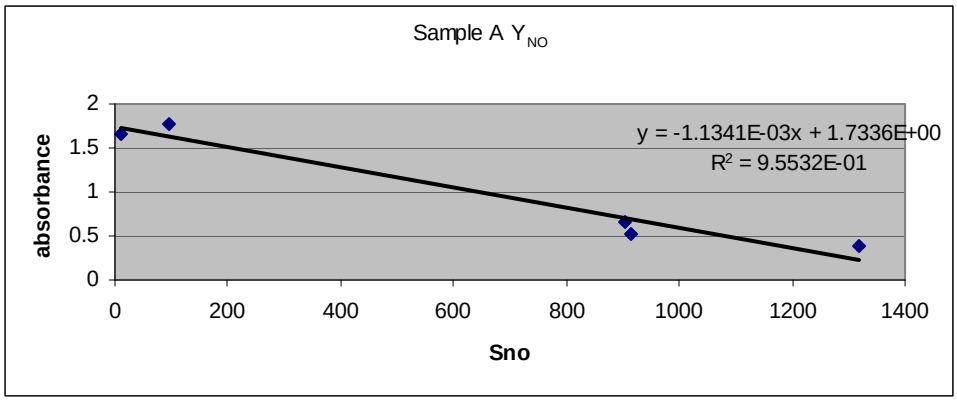

Figure 4-7. Absorbance vs. S_{NO} for sample A

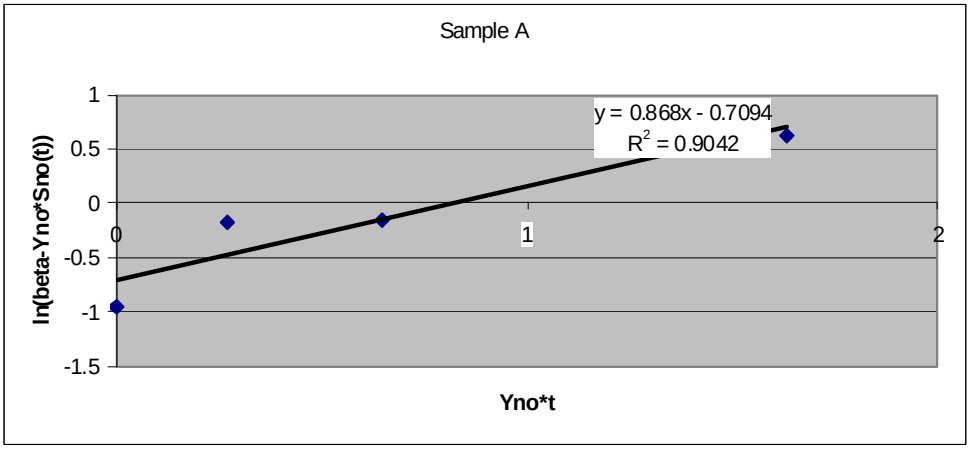

Figure 4-8. $\ln(\beta - Y_{NO}S_{NO}(0))$ vs. Y_{NO} t for sample A

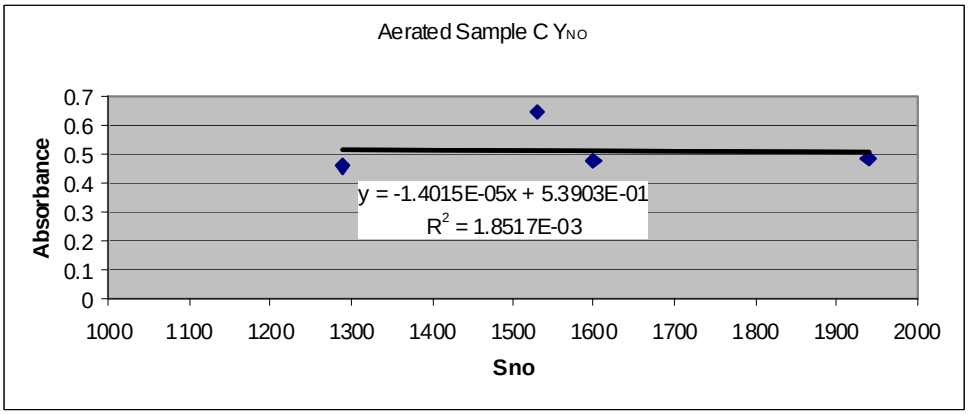

Figure 4-9. Absorbance vs. S_{NO} for aerated sample C

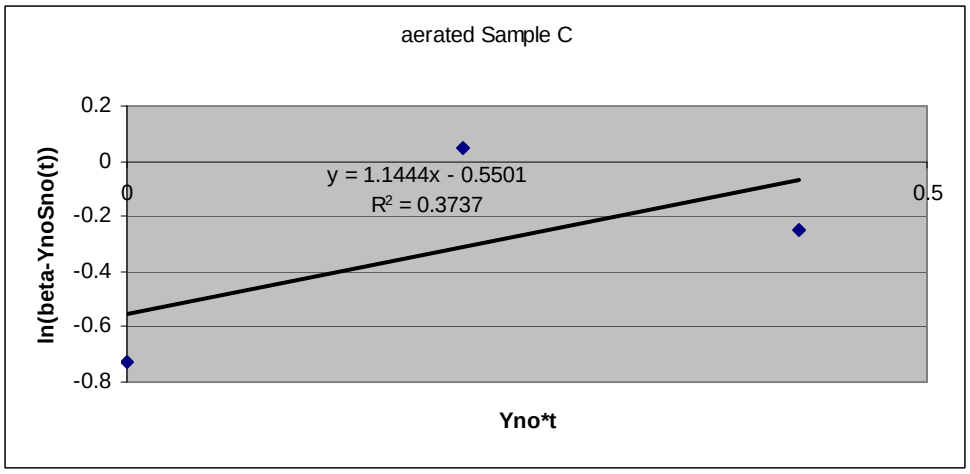

Figure 4-10. $\ln(\beta - Y_{NO}S_{NO}(0))$ vs. Y_{NO} t for aerated sample C

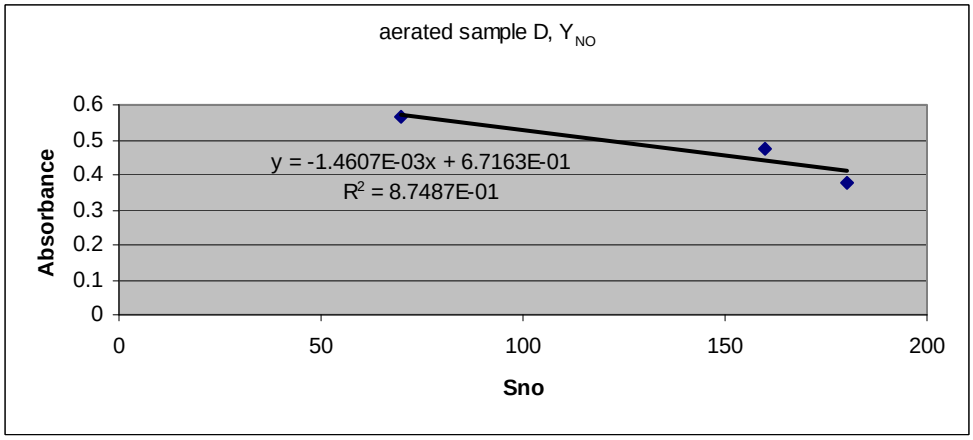

Figure 4-11. Absorbance vs. S_{NO} for aerated sample D

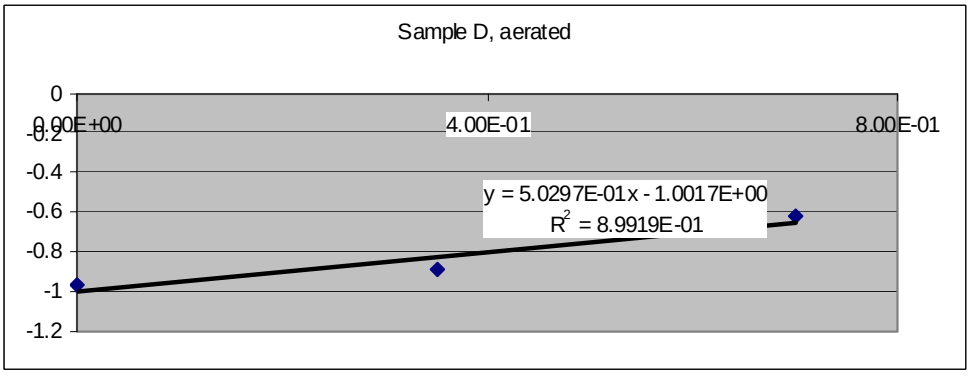

Figure 4-12. $\ln(\beta - Y_{NO}S_{NO}(0))$ vs. Y_{NO} t for aerated sample D

Table 4-1. Biotreatability assay results

Sample A	μ_{maxNO} = 0.868
Sample C aerated	BI = 0.49
Sample C non-aerated	Invalid BI value; bacteria did not grow
Sample D aerated	BI = 0.43
Sample D non-aerated	Invalid BI value; bacteria did not grow

As shown in Table 4-1, aerated sample C and sample D are treatable biologically. The non-aerated sample C and non-aerated sample D had negative Y_{NO} values, and thus gave invalid BI values. The absorbance for those samples decreased with nitrate consumption during batch test.

Conclusions

The objective of the assay is to quantitatively analyze biological treatability of high nitrate solutions. The biotreatability index, BI is used to determine if the sample can be denitrified successfully. BI is obtained from the ratio, $(\mu_{maxNO})_{\text{test sample}}/((\mu_{maxNO})_{\text{synthetic sample with same NO3-N concentration}})$. Y_{NO} values can be obtained from the plots of absorbance vs. S_{NO} observed during the batch tests, and μ_{maxNO} is obtained from the plots of $\ln(\beta - Y_{NO}S_{NO}(0))$ vs. $Y_{NO}*t$. If the value of the ratio is closer to 1, the sample is treatable. A value much lower than 1 shows that high denitrification will be very difficult to achieve for that sample.

CHAPTER 5
CONCLUSIONS AND FUTURE WORK

The objective of the project was to establish high denitrification rate for an industrial high nitrate stream using biological treatment. At the end of this study, it was concluded that the industrial stream was indeed treatable. However, volatile contaminants and metals ions present in the stream must be removed prior to biological treatment. Volatile contaminants may be removed through aeration, and metal ions can be removed through an ion exchange process. During the treatment, micronutrients should be added to the reactor directly rather than in the feed, since precipitation can occur in the feed. It was also established that neutralization with sodium did not affect denitrification rates, since the industrial stream sample neutralized with sodium hydroxide and the stream sample neutralized with potassium hydroxide had same results. CSTR with sodium hydroxide neutralized synthetic nitrate feed was able to reach a denitrification rate of 4 mg NO_3-N/L/min with a flow rate of 11.5 mL/min and an overall denitrification of 97%. This denitrification rate was twice the target denitrification rate. CSTR with the feed of sodium hydroxide neutralized industrial stream was able to reach the target denitrification rate of 2 mg NO_3-N/L/min with a flow rate of 5.5 mL/min and an overall denitrification of 98%.

The denitrification was performed using potassium acetate as the exogenous carbon source. Thus, effects of carbon limitation on denitrification were not analyzed. For the next step, an industrial carbon source may be used for the process. Studies using attached growth bioreactors can also be conducted with micronutrients added directly to the reactor instead of the feed. Attached growth bioreactors can be more feasible since sludge concentration in the reactor effluent will be very low.

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BIOGRAPHICAL SKETCH

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