AUTOTROPHIC DENITRIFICATION OF GROUNDWATER IN A GRANULAR SULFUR-PACKED UP-FLOW REACTOR

by

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ABSTRACT

AUTOTROPHIC DENITRIFICATION OF GROUNDWATER IN A GRANULAR SULFUR-PACKED UP-FLOW REACTOR

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Autotrophic denitrification is an effective treatment technique for nitrate removal from groundwater. Six basic elements are required for the growth of autotrophic denitrifiers: (1) electron donor, (2) electron acceptor, (3) active bacteria, (4) anoxic/anaerobic environment, (5) micronutrients, and (6) optimum pH and temperature. In this research, granular sulfur is an electron donor; nitrate is an electron acceptor; anoxic and anaerobic environment was maintained in the reactor, and micronutrients were added; pH was controlled between 6 and 9, and temperature was maintained at the room temperature ($20 \pm 2\ ^{o}C$). Batch reactor and continuous up-flow reactor experiments were carried out to investigate the denitrification rate, and reaction rate kinetic constants. The observed nitrate removal corresponded to the first order reaction kinetic. The data correlation between alkalinity destruction and nitrate nitrogen reduction was linear with a slope of 3.09 mg-$CaCO_3$ alkalinity destroyed per mg-$NO_3^- - N$ removed. The data correlation between sulfate production and nitrate nitrogen reduction was linear with a slope of 6.91 mg-SO_4^{2-} produced per mg-$NO_3^- - N$ removed. Based on the biologically mediated half-reaction equations, the overall reaction equations were developed. Based on the experimental data, the energy coefficients and the stoichiometry of autotrophic denitrification were developed. Finally, an analytical model based on conjugate reaction kinetic was utilized. The reaction rate constants k_1 and k_2 were determined from the experimental data. The model provides an analytical tool to predict the nitrate and nitrite concentrations in the effluent from the up-flow column. An example is presented to illustrate the design procedure of a sulfur-packed up-flow column. In this example a sulfur-packed up-flow column is designed to treat a given flow rate and influent concentration of nitrate nitrogen to achieve a desired degree of treatment.

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CHAPTER 1

INTRODUCTION

1.1 Background

High concentration of nitrate in drinking water is associated with serious health risks. It causes methemoglobinemia or “blue-baby syndrome” in infants. Nitrate is reduced to nitrite in saliva or in the gastrointestinal tract. Nitrite oxidizes the hemoglobin to methemoglobin causing a loss in oxygen carrying capacity of the blood. Methemoglobinemia occurs mainly in infants (Challis, 1973). Under abnormal conditions, some circumstantial evidences have shown that ingestion of nitrate may cause gastric cancer due to the formation of nitrite (Mirvish, 1991).

Research on nitrate removal technologies from municipal water supplies is increasing because of nitrate contamination. Power and Schepers (1989) indicated that in North America, 90 percent of the rural population and 50 percent of the total population depend on groundwater as their water supply source. The major sources of groundwater contamination from nitrate are due to excessive application of agricultural fertilizers and the septic tanks effluent (Exner and Spaulding, 1990). A 1985 survey of American Water Works Association (AWWA) indicated that about 23 percent of violations of Primary Drinking Water Standards in the United States are due to excessive nitrate levels (Bouwer and Crowe, 1988). A 1995 survey of AWWA showed that 20 percent of shallow private wells in the farming areas of the United States violate the federal drinking water standard and one percent of public water supply wells exceed the Maximum Contaminant Level (MCL) due to excessive nitrate concentration. Bouchard et al. (1992) indicated that the nitrate-N concentration exceeded the 10 mg/L MCL level in more than 20 percent of the wells sampled in Iowa. Davidson and Ridgeway (1995) reported that over 250,000 acre-feet of the groundwater basin in Orange County, California contains nitrate nitrogen concentrations exceeding the MCL.

A proven and an economically effective method for nitrate removal is biological denitrification. It is carried out by heterotrophic or autotrophic organisms. Heterotrophic denitrification is the most common method used in wastewater treatment. An external carbon source is needed to denitrify the drinking water source. In drinking water, carbon is undesired as it is a precursor for trihalomethane formation, and excessive amounts may increase the microbial growth in the distribution system resulting in taste and odor problems, and accelerated pipe corrosion (Gantzer, 1995). Because of these problems, the autotrophic denitrification is considered a better alternative for nitrate removal. The benefits of autotrophic denitrification over heterotrophic denitrification are: (1) does not need organic carbon as a carbon and energy source, (2) uses inorganic carbon dioxide as a carbon source, and (3) uses inorganic mineral as an energy source. Under an anaerobic condition, autotrophic organisms oxidize inorganic minerals while reducing nitrate to nitrogen gas.

1.2 Purpose of the Study

Many researchers have studied autotrophic denitrification using different sources of electron donors. The inorganic electron donors include: S^o, $S_2O_3^{2-}$, S^{2-}, H_2, HS^-, NO_2^-, and Fe^{+2} (Batchelor, 1978b; Kurt et al. 1987; Hiscock et al. 1991). Granular sulfur is chosen in this investigation as an electron donor because it is stable and insoluble, a low cost mineral, and is easy to handle. Only few researchers have studied the feasibility of using granular sulfur as an electron donor. There is a need to determine the performance of autotrophic denitrifican using granular sulfur as an electron donor. Effective design and operation of such a system requires an understanding of basic principles governing substrate utilization, alkalinity destruction, sulfate production, and denitrification theory and design.

1.3 Research Objectives

The objectives of this research are: (1) to develop the performance criteria of autotrophic denitrifiers in a granular sulfur-packed up-flow reactor, (2) to develop stoichiometry of autotrophic denitrification, and (3) to develop a model to express the nitrate and nitrite concentrations in the finished water as a function of influent nitrate concentration, and true hydraulic retention time.

1.4 Research Scope

The research objectives were achieved by conducting experimental studies using a batch reactor, and a continuous up-flow sulfur-packed column. The scope of this research is summarized below:

1.4.1 Batch Reactor Study

Batch reactor study was conducted to develop the preliminary information needed to design and operate a continuous up-flow sulfur-packed reactor. The batch reactor study was used to:

- Develop autotrophic denitrifying culture for use in the continuous up-flow reactor.
- Develop the preliminary information on relationships of alkalinity destruction, sulfate production, and gas production with respect to nitrate nitrogen removal.

1.4.2 Continuous Granular Sulfur-Packed Up-flow Column Study

The sulfur-packed up-flow column study was utilized to

- Develop the ratios of alkalinity destruction per unit of nitrate nitrogen removal, and sulfate production per unit of nitrate nitrogen removal.
- Develop biological kinetic constants, half reaction energy coefficients, and stoichiometry.
- Develop an analytical model to predict the effluent concentrations of nitrate and nitrite as a function of influent nitrate concentration and true hydraulic retention time.

1.5 Research Organization

The research work presented in this report is organized to enhance the readability. The literature review provides the state-of-the-art survey of the published material on autotrophic denitrification. The experimental work and results of the batch reactor and continuous flow reactor study are presented separately followed by the discussion, conclusions and recommendations.

The major findings are reported in the main body of this report. The supporting material and bulk data are arranged in seven Appendixes.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Municipal and industrial discharges and extensive use of fertilizers in agriculture has resulted in nitrate contamination in the surface and groundwater resources. Because of the adverse health effects associated with nitrate in drinking water, the U.S. Environmental Protection Agency (EPA) has set a 10 mg/L $NO_3^- - N$ under Primary Drinking Water Standards (Pontius, 1993). The feasible methods for removing nitrate from water supply are: ion exchange, reverse osmosis, electrodialysis, catalytic denitrification, and biological denitrification. Of these methods, the biological denitrification is the most cost effective method (Kapoor, 1997). Currently, ion exchange is the most commonly used method for removing nitrate from small water supply system in the United States (Clifford and Liu, 1993). Conventional ion exchange is economically not feasible for large applications because ion exchange technology removes all anions and produces brine rich in nitrate, chloride, and sulfate. The production of concentrated waste brines creates serious wastewater disposal problems.

The biological treatment of nitrate is preferred over physicochemical methods because of selective removal capability and formation of harmless end products. Biological denitrification has two benefits: (1) the nitrate is destroyed rather than merely concentrated, and (2) it does not produce large quantities of waste products such as regeneration brines (Delanghe et al., 1994a).

2.2 Microbiology and Stoichiometry of Biological Denitrification

Biological nitrate reduction is an oxidation-reduction process through microbial metabolic activity. Microorganisms obtain energy through oxidizing electron donors (either organic or inorganic) and releasing electrons. Nitrate is an electron acceptor and will be reduced upon receiving the electrons. There are two types of biological nitrate reduction. One is assimilative nitrate reduction, where nitrate is reduced to ammonia for use as a nitrogen source for growth, and the other is dissimilative nitrate reduction, where nitrate is used as an electron acceptor and is reduced to N_2 in energy generation. Brock et al. (1994) reported five biological denitrification properties. These are: (1) under most conditions, the end product of dissimilative nitrate reduction is N_2 or N_2O, (2) the first product of nitrate reduction is nitrite and nitrate reductase is the enzyme to catalyze the reaction, (3) nitrite reductase is responsible for the next step to reduce nitrite into nitrogen gas, (4) in general, assimilative nitrate reductases are ammonia repressed, whereas dissimilative nitrate reductases are repressed by O_2 and synthesized under anoxic conditions, and (5) the process of denitrification is strictly an anaerobic/anoxic process, whereas assimilative nitrate reduction can occur quite well under fully aerobic conditions.

Cizinska et al. (1992) reported that biological nitrate reduction is currently the predominant method for nitrate removal from wastewater. Four basic elements are required for denitrification to occur. These are electron donor, electron acceptor, active bacteria, and anoxic condition. Biological denitrification is accomplished either by heterotrophic denitrification or by autotrophic denitrification. Heterotrophic process is conducted by microorganisms which require an organic substrate for carbon source. Autotrophic process is conducted by microorganisms which require inorganic carbon for carbon source. In both processes the microorganisms obtain energy via reduction of nitrate and oxidation of suitable substrate.

Many bacteria can reduce nitrogen oxides into harmless nitrogen gas in the anoxic environment. The most common heterotrophic denitrifiers are various species of *Pseudomonas* and *Bacillus* (Brezonik, 1977). Davies (1973) isolated denitrifican bacteria from settled wastewater, using methane as a sole carbon source and nitrate as the terminal electron acceptor. The identified genera included *Alcaligence, Achromobacter, Bacillus, Methanomonas, and Pseudomonas*.

The autotrophic organisms oxidize inorganic mineral and release the electrons to reduce nitrate; nitrogen gas is released. Many researchers have shown that *Thiobacillus denitrificans (T. denitrificans)* and *T. thioparus* can effectively remove nitrate from water by using a reduced sulfur compound as the electron donor and nitrate as the electron acceptor (Gayle et al, 1989). The typical reduced sulfur compounds are sulfides and elemental sulfur (S^{2-}, $S_2O_3^{2-}$, and S^o). The *determinative bacteriology* of Bergey's Manual indicated that *T. denitrifican* is the responsible microorganism for autotrophic denitrification. This bacterium can reduce nitrate into nitrogen gas while oxidizing elemental sulfur (Bergey et al, 1974). Other major autotrophic bacteria are *Micrococcus denitrificans* and *Paracoccus denitrificans* which oxidize H_2 using nitrate as the terminal electron acceptor (Kurt et al., 1987). *Paracoccus denitrificans* and *Thiobacillus denitrificans* can denitrify using hydrogen and reduced-sulfur compound, respectively. However, both can also grow heterotrophically if an organic carbon source is present (Waki et al, 1980; Kurt et al. 1987; Claus and Kutzner, 1985b). The advantage of autotrophic growth is that there is no need for organic substrate for energy and cell synthesis. Some aerobic autotrophic bacteria can completely oxidize the inorganic sulfur for their energy source. *Thiobacillus thiooxidans* is a good example. A comprehensive research on the respiration process of *T. denitrifican* using thiosulfate as the electron donor has been presented by Justin and Kelly (1978a).

Lubchenoko (1996) reported six physiological properties of autotrophic denitrifying bacteria. These are: (1) growth is in purely mineral medium but in the presence of an inorganic substance such as sulfur that can be oxidized, (2) the vital activity of microorganisms is closely related to the presence of oxidizable inorganic substance, (3) oxidation of inorganic substance is the sole source of energy, (4) organic substance is not needed as a source of carbon and energy, (5) autotrophic organisms are unable to decompose organic substances; the presence of organic matters slows down the development of autotrophic organisms, and (6) carbon dioxide is the sole source of carbon.

The morphological and physiological properties of *Thiobacillus denitrifican* are clearly described in the *Systematic Bacteriology* of Bergy's Manual (1986). The properties are described as follows: *Thiobacillus denitrifican* is colorless sulfur bacteria, which can be found in any place where reduced sulfur compounds are present. The presence of colorless sulfur bacteria can be observed through the naked eye by the appearance of copious white deposits of sulfur, streamers, veils, or films. *Thiobacillus* is a chemolithoautrotrophic microorganism (obligate or facultative) growing below 55 ^{o}C, gram-native, rod-shaped, motile with polar flagella, or nonmotile. No intracellular sulfur is visible under the light microscope, but these may contain finely dispersed sulfur, visible under the electron microscope after staining with silver salts. Many *T. denitrifican* may deposit sulfur in the colonies after prolonged incubation in the presence of sulfide, making the colonies (yellowish) white and opaque.

Many stoichiometric relationships for heterotrophic and autotrophic denitrification have been proposed by several investigators. The stoichiometric constants and relationships are summarized in Tables 2.1 and 2.2. The general equations are presented in Appendix A.

2.2.1 Nutritional Requirements for Growth

Nutrients are necessary for cell growth and development. The nutrients include C, H, O, N, P, and S that are needed in large amounts. Minerals such as K, Na, Mg, Ca, and Fe are needed in small amount. Some metals such as Mn, Zn, Cu, Co, and Mo are also required in trace amount for biological cell synthesis (Champ et al., 1979). Most groundwater contains adequate concentrations of the necessary minerals and trace metals to support biosynthesis. Gaudy and Gaudy (1980) indicated that a microorganism could not reproduce if an essential element is totally unavailable.

Table 2.1 Summary of Stoichiometric Relationship for Heterotrophic Denitrification

Electron Acceptor	Electron Donor	Carbon Source	Product	Alkalinity Status	References
1 NO_3^- (nitrate)	1.08 CH_3OH (methanol)	Same as electron donor	0.47 N_2 0.76 CO_2 2.44 H_2O 0.065$C_5H_7O_2N$	1 H^+ consumed	McCarty (1969)
12 NO_3^-	5 C_2H_5OH (ethanol)	Same as electron donor	6 N_2 10 CO_2 9 H_2O	12 OH^- produced	Richard et al. (1980)
8 NO_3^-	5CH_3COOH (acetate)	Same as electron donor	4 N_2 10 CO_2 6 H_2O	8 OH^- produced	Frick and Richard (1985)
8 NO_3^-	5 CH_4 (methane)	Same as electron donor	4 N_2 5 CO_2 14 H_2O	8 H^+ consumed	Barrenstein et al. (1986)
6 NO_3^-	5 CH_3OH (methanol)	Same as electron donor	3 N_2 5 CO_2 7 H_2O	6 OH^- produced	Sherrard (1988)
2 NO_3^-	5 CO (carbon mono oxide)	Same as electron donor	1 N_2 5 CO_2 1 H_2O	2 H^+ consumed	Frunzke and Meyer (1990)
6 NO_3^-	5 CH_3OH (methanol)	Same as electron donor	3 N_2 5 CO_2 7 H_2O	6 OH^- produced	Metcalf and Eddy (2003)
10 NO_3^-	$C_{10}H_{19}O_3N$ (wastewater)	Same as electron donor	5N_2 10CO_2 3H_2O NH_3	10 OH^-	Metcalf and Eddy (2003)
8 NO_3^-	5CH_3COOH (acetate)	Same as electron donor	4N_2 10CO_2 6H_2O	8 OH^-	Metcalf and Eddy (2003)

Note: See Appendix A for additional details

Table 2.2 Summary of Stoichiometric Relationship for Autotrophic Denitrification

Electron Acceptor	Electron Donor	Carbon Source	Products	Alkalinity Status	References
1 NO_3^- ($0.08NH_4^+$ used as nitrogen source)	1.1 S^0 (elemental sulfur)	0.4 CO_2	0.5 N_2 1.1 SO_4^{2-} 0.08 $C_5H_7O_2N$	1.28 H^+ produced	Batchelor (1978c)
8 NO_3^-	5 $S_2O_3^{2-}$ (thiosulfate)	*	4 N_2 10 SO_4^{2-}	2 H^+ produced	Zumft and Cardenas (1979)
8 NO_3^-	5 $S_2O_3^{2-}$ (thiosulfate)	*	4 N_2 10 SO_4^{2-}	2 H^+ produced	Schedel and Truper (1980)
3 O_2	2 S^0 (elemental sulfur)	*	2 $H_2SO_4^{2-}$	-	Kelly (1982)
8 NO_3^-	5 $S_2O_3^{2-}$ (thiosulfate)	*	4 N_2 10 SO_4^{2-}	2 H^+ produced	Claus and Kutzner (1985)
8 NO_3^-	5 S^{2-} (sulfide)	*	4 N_2 5 SO_4^{2-} 4 H_2O	8 H^+ consumed	Barrenstein et al. (1986)
2 NO_3^-	5 H_2 (hydrogen gas)	*	1 N_2 4 H_2O	2 OH^- produced	Kurt et al. (1987)

* Carbon was not incorporated in the balanced equation. See Appendix A for additional details

Temperature and pH are also a factor, and can affect the growth. Carbon, oxygen, nitrogen, and hydrogen account for 90 percent of the cell mass. Phosphorus is required for cell mass to generate ATP. The trace elements in general, as well as potassium, calcium, and magnesium, serve as inorganic co-factors for specific enzymes as well as organic co-factors. For cultivation and isolation of *Thiobacillus denitrifican,* ammonium is required for nitrogen source. (Baalsrud and Baalsrud 1954). However, Claus and Kutzner (1985b) reported that there was no need of ammonium for the nitrogen source. They concluded that the organisms are capable of assimilatory as well as of dissimilatory nitrate reduction.

2.2.2 Natural Substrates

The natural substrates for *thiobacillus* are the soluble sulfides and other reduced sulfur compounds such as sulfur and thiosulfate (Kelly, 1982). The obligate *chemolithotrophs* reduce sulfur compounds as the only energy source, and assimilate carbon dioxide as the major carbon source. Some organic compounds can also be utilized by these organisms but only to a limited extent and in a restricted pattern. The facultative *chemolithotrophs* have a versatile physiology. They can grow autotrophically, heterotrophically, and mixotrophically (Kurt et al., 1987)

Simultaneous presence of reduced sulfur compounds and oxygen or nitrate is required for the growth of sulfur oxidizers. Many sulfur oxidizers often grow in a narrow range with the coexistence in the presence of sulfide and oxygen or nitrate. It is possible that autotrophic denitrification in aquifers can occur in the presence of inorganic species such as Mn^{+2}, Fe^{+2}, and HS^{-} (Hiscock et al., 1991). Elemental sulfur being of low cost and ease of handling is probably the most promising element for general use.

2.2.3 Inhibitors

Nitrate reductase is strongly inhibited by the presence of oxygen (Gayle et al., 1989). Hiscock et al. (1991) indicated that in autotrophic denitrification, oxygen is an important inhibitor in the energy metabolism of cells. Rittmann and Huck (1989) reported that oxygen concentrations ranging from 0.1 to 0.2 mg/L have inhibitory effect on denitrification. Skerman and MacRae (1957) demonstrated that nitrate reduction was not observed at an oxygen concentration above 0.2 mg/L. In the competition between oxygen and nitrate for an electron donor, nitrate can take over oxygen only in the presence of enzymes that are required for nitrate respiration. According to Claus and Kutzner (1985b), sulfate in excess of 5000 mg/L can inhibit denitrification. Nitrite inhibition was also noted when its concentration was over 200 mg/L. The optimal temperature was 30 ^{o}C and pH was 7.5 to 8.0. In the study of physiology of thiobacillus denitrifcan, Baalsrud (1954) reported that the optimum pH was between 6.2 and 7.0. Gauntlett and Craft (1979) indicated that denitrification is closely related to pH with an optimum in the range 7.0 - 8.0. At a low temperature, denitrification decreases markedly but is measurable between 0 and 5 ^{o}C. Meyers (1972) indicated that sulfide would depress gaseous nitrogen production but stimulated the reduction of nitrate to ammonium.

2.2.4 Energy Requirement and Bacterial Growth

Sawyer and McCarty (1978) indicated that autotrophic organisms oxidize inorganic materials for energy. Energy is released by oxidation of inorganic electron donor. The released energy is utilized to reduce carbon dioxide to form celluar organics. Thus, a portion of the energy provided by the electron donor is used to maintain life requirements and the other portion is used for cell synthesis. Half reactions in the cell synthesis, electron acceptor, and electron donor are available (Sawyer and McCarty, 1978). The stoichometic coefficients of the half reaction are written based on one unit of electron transfer. In the combination of half reactions to form the overall reaction two coefficients are used. One is f_s, which is the proportion of energy used for cell synthesis. The other is f_e, which is the proportion of energy used to maintain life requirement. The sum of f_s and f_e is one. A system of the half equations is provided in Appendix B.

2.3 Conventional Methods of Nitrate Removal from Drinking Water Supplies

Ion exchange, biological denitrification, and reverse osmosis have been applied in full-scale facility for nitrate removal. Ion exchange is currently the most common method of nitrate removal from drinking water in the United States (Clifford and Liu, 1993). Kapoor and Viraraghavan (1997) indicated that ion exchange provides an attractive alternative for small and average-size facilities. It was considered suitable for groundwater that was relatively free of dissolved organic matter.

In Europe, where larger scale nitrate removal must be accomplished, heterotrophic biological denitrification is utilized by adding methanol, ethanol, or acetic acid, as the carbon source. Phosphorus and other nutrients may be added (Gayle et al., 1989). Reverse osmosis, distillation, and electrodialysis processes also remove dissolved solids but do not selectively target nitrate. These processes are generally considered too costly for routine nitrate removal from drinking water. Biological denitrification is preferred over physicochemical processes because of the selective removal capability and the formation of harmless end products. Autotrophic denitrification is preferred over heterotrophic denitrification because it does not need organic carbon source.

2.4 Overview of Biological Denitrification

Many researchers have extensively studied denitrification under different environmental conditions. In a review of natural and artificial denitrifiation of groundwater, Hiscock et al. (1991) indicated that natural denitrification does occur in many aquifers, however, natural process cannot remove large quantities of nitrate that may often be present in many aquifers. After comparing various ground and underground techniques for denitrification, these investigators concluded that artificial denitrification applied in above ground installations offered the most desirable nitrate removal and process control capabilities.

2.4.1 Denitrification in Surface Water

In lakes during summer stratification, nitrate and nitrite concentrations are typically highest at the mid-depth due to denitrification in the bottom waters and algae assimilation in the surface waters. A comprehensive review on the fate of nitrate in natural surface waters has been presented by Brezonik (1977). Given anoxic condition and the presence of nitrate, the primary factor affecting the rate of denitrification is the concentration of electron donor.

2.4.2 Denitrification in Groundwater

In-situ, the denitrification of groundwater is usually electron donor limited (Smith et al., 1994). Most common electron donor in groundwater is dissolved organic carbon (DOC). Thurman (1985) reported that in a survey of one hundred groundwaters in the United States, the median DOC was 0.7 mg/L. Barcelona (1984) measured an average total organic carbon (TOC) level of 2.95 mg/L in shallow sand and gravel aquifer in northern Illinolis. It is now well established that denitrification rate in the natural aquifers was increased by adding suitable electron donors (Smith et al., 1994, Dahab, 1991).

Champ et al. (1979) reported that oxidation-reduction potential (ORP) can be used as a verification of denitrification occurrence. The ORP would decline from a highly oxidized state to a reduced state. However, Howard (1985) reported that sometimes apparent lowering in nitrate concentration from above 10 to less than 2 mg/L in the direction of decreasing ORP was not attributable to denitrification; rather, it was primarily due to mixing of waters of different origins.

Currently, in-situ denitrification in a groundwater system has been proved feasible, but the reaction rates and prediction techniques are not fully known. To encourage in-situ biological degradation of wastewater, Korom (1992) reported adding NO_3^- in the groundwater that was contaminated with hazardous organic compounds. In a study of in-situ re-mediation, Reddy (1982) found that the rate of denitrification was independent of the soil type, and was proportional to the concentration of NO_3^- and available organic carbon. Smith and Duff (1988) indicated that available carbon would not be a limiting factor to denitrify small amounts of NO_3^- in an aquifer. Enhanced nitrate removal was found by adding formate or hydrogen as the electron donor, to a slurried core sample obtained from an active zone of denitrification.

2.4.3 Denitrification in Above Ground Reactor

2.4.3.1 Heterotrophic Denitrification

Richard et al. (1980) conducted a study of denitrification of groundwater in France. The study used a pilot-scale fluidized bed reactor with ethanol as the substrate. The reactor was 6 m high, 250 mm in diameter, and operated without effluent recycle. The results showed that the maximum specific nitrate utilization rate was 250 mg NO_3^- per gram of volatile suspended solids per hour. Denitrification rates were greater at lower flow velocities. The optimum up-flow velocity was 20 m/hr for bed expansion and denitrification. The specific denitrification rate at this flow rate was 83 mg NO_3^- per hour per gram of volatile suspended solids in the reactor.

Frick and Richard (1985) reported that a 50 m^3/hr denitrification facility using acetic acid as the carbon source and phosphate as the nutrient was able to remove nitrate concentration from 80 mg/L as NO_3^- in the influent to 30 mg/L as NO_3^- in the effluent. The process applied an up-flow fixed-bed reactor, flocculation, carbon filtration and chlorination. The nitrite concentration in the finished water was less than 0.1 mg/L.

Frank and Dott (1985) reported the performance of a pilot-scale bio-reactor packed with polystyrene beads, and using methanol or ethanol as an energy source. The results showed that the reactor was able to reduce nitrate concentration from 55 mg/L as NO_3^- in the influent to 3 mg/L as NO_3^- in the effluent. Most of the bacteria were verified as genus *Pseudomonas*.

Hoek and Klapwijk (1988b) conducted a hybrid ion-exchange/biological-denitrification study. This process used ion exchange to remove nitrate from groundwater. The concentrated brine consisted of 700 mg/L of $NO_3^- - N$, which was then removed by a biological denitrification unit. The concentrated brine, after denitrification, was recycled and used as a regenerant. However, the brine needed to be replaced every six days due to the sulfate accumulation. This process achieved 80 percent reduction of brine-disposal compared to conventional once through regeneration system.

2.4.3.2 Autotrophic Denitrification

In a study of the physiology and kinetics of autotrophic denitrification by *Thiobacillus denitrifican* using thiosulfate as the electron donor, Claus and Kutzner (1985b) reported that the optimum growth condition was 30 ^oC and pH 7.5 - 8.0. maximum specific growth rate (μ_{max}) = 0.11/h, half-velocity constant (K_s) = 0.2 mg NO_3^-/L, maximum substrate utilization rate (k) = 0.78 g-NO_3^-/g-VSS.h, yield coefficients ($Y_{NO_3^-}$) = 0.129 g-VSS/g-NO_3^-, ($Y_{S_2O_3^{2-}}$) = 0.085 g-VSS/g-$S_2O_3^{2-}$. The denitrification gas was almost pure N_2. The concentrations of N_2O and NO produced in the reactor were below 1 ppm.

Kurt et al. (1987) studied the biodegradation kinetics of autotrophic hydrogen-oxidizing denitrifying bacteria using a bench-scale fluidized bed reactor. The results showed that a residence time of 4.5 hours was required for complete denitrification of 25 mg/L $NO_3^- - N$. The optimum pH was approximately 7.5. The pH increased as a result of denitrification reaction. If the pH was allowed to approach 9.0, nitrite accumulated and the process failed.

In Germany, the performance of a commercial-scale biological-denitrification plant using hydrogen as an electron donor was evaluated (Gross and Treuter, 1986). The reactor was operated at a temperature of 10.5 ^oC. The organisms growth rate varied from 0.1 to 0.3 per day. The sludge production on a dry-weight basis was approximately 0.2 kg per kg nitrate nitrogen removed. A residence time of one to two hours was required to remove 11.3 mg/L $NO_3^- - N$. The denitrification rate varied with the mass and activity of the biomass.

In a slurry batch reactor test, Batchelor (1978b), reported that the observed yield coefficients were 0.089 mg organic-N per mg of $NO_3^- - N$ removed, 2.35 mg $SO_4^{2-} - S$ per mg of $NO_3^- - N$ removed, and 0.113 meq alkalinity destroyed per mg of $NO_3^- - N$ removed. Sikora and Keeney (1976), successfully used elemental sulfur *and Thiobacillus denitrifican* to denitrify septic tank effluent. Bisogni and Driscoll (1977) conducted a research for autotrophic denitrification with *Thiobacillus denitrifican* using sulfide and thiosulfate as electron donors. The yield coefficient was reported as 0.704 mg of VSS per mg of nitrate utilized when sulfide was used as the electron donor. When thiosulfate was used as the electron donor, the yield coefficient was 0.703 mg of VSS per mg of nitrate utilized.

Claus and Kutzner (1985b) successfully isolated *Thiobacillus denitrificans* using thiosulfate as an energy source and nitrate as an electron acceptor and nitrogen source. Later on, an upflow packed-bed reactor with lava-stone media was employed to treat wastewater with high nitrate concentration. In this investigation, thiosulfate was used as an electron donor. The results showed that 95 percent denitrification was achieved at the nitrate loading of 25 kg/m^3.d.

Batchelor and Lawrence (1978b), and Davidson and Ridgeway (1995) conducted research on autotrophic denitrification using powdered slurry of sulfur as an electron donor for *Thiobacillus denitrifican*. The results were promising. Lampe and Zhang (1997), and Flere(1997) studied the autotrophic denitrification of groundwater re-mediation using elemental sulfur and limestone granules. The results showed high nitrate removal efficiency but biofouling of the media caused decreased removal deficiency. It was suggested that backwashing of the bed at 40 to 60 days duration would reduce the biofouling.

Frumai et al. (1996) operated two laboratory-scale biological filters using thiosulfate as the electron donor. The objective was to investigate the effects of alkalinity and pH on the removal of nitrate and nitrite. The reactor was filled with granular anthracite media and was inoculated with activated sludge from a municipal sewage treatment plant. The flow velocity was controlled at 4 m/day, which was in the range of typical slow sand filtration rate. The results showed high nitrite accumulation with below pH of 7.4. The alkalinity level in excess of 150 mg/L as $CaCO_3$ was used to maintain acceptable removal efficiency. Overath et al. (1986) conducted a study on autotrophic denitrification using packed tower filled with elemental sulfur and activated carbon. The reactor was 3-m long and 100 mm in diameter. The reactor was operated with a flow rate of 30 L/hr. The reactor was able to remove nitrate from 35 mg/L in the influent to almost 0 mg/L in the effluent.

Blecon et al. (1983) conducted studies on denitrification in a packed tower filled with elemental sulfur and various ratios of limestone. The results showed that the efficiency increased as the particle size of sulfur decreased. The lowest ratio of limestone to granular sulfur gave the best results for water with a high-alkalinity (305 mg/L HCO_3^-). Lewandowski et al. (1987) conducted denitrification studies using calcium alginate beads suspended in a completely mixed batch reactor. Nitrogen gas was used to provide reactor mixing. Nitrate was reduced from 27 mg/L to 6 mg/L in seven hours. The nitrogen removal rate was 1.6 mg N/L.hr and increased to 4.8 mg N/L.hr. Nitrite accumulated initially but later was reduced to below 2 mg/L as $NO_2^- - N$.

Hashimoto et al. (1989) conducted a study of enrichment of sulfur denitrifying bacteria, and its acclimation to elemental sulfur on a batch reactor. The enrichment of denitrifiers was established through various sources such as activated sludge and bottom mud of polluted river. $NaHCO_3$ was used to provide carbon source and pH adjustment.

$S_2O_3^{2-}$ and S^0 were used as the electron donor. A pure single isolate was obtained from repeated plating cultures and was identified as *T. denitrificans*. The isolated strain showed the following characteristics: (1) short-rod shape, (2) motile, (3) gram-negative, (4) strict autotrophic, (5) facultative anaerobe, and (6) oxidized elemental sulfur, but its oxidation rate was slower than that of $S_2O_3^{2-}$. The denitrifier acclimated to $S_2O_3^{2-}$ was successfully acclimated to elemental sulfur by the gradual and stepwise replacement of $S_2O_3^{2-}$ to elemental sulfur. The ratio of alkalinity destruction to nitrate nitrogen removal was 2.49 when thiosulfate was used as an electron donor, and 4.39 when elemental sulfur was used as an electron donor. The ratio of TOC increase to nitrate nitrogen removal was 0.371 when thiosulfate was used as a substrate, and 0.361 when elemental sulfur was used as a substrate. The denitrification rate followed the zero order kinetics. The specific denitrification rates of enriched microorganism were 9.4 mg $NO_3^- - N$/mg-TOC.d in case of $S_2O_3^{2-}$ and 2.5 mg $NO_3^- - N$/mg TOC.d in case of S^o respectively.

Flere (1997) evaluated the performance of sulfur and limestone autotrophic denitrification (SLAD) in a packed-bed up-flow system. The size of sulfur media was between 2.38 mm and 4.76 mm. Limestone was mixed with sulfur at a ratio of one sulfur to three limestone by volume. The porosity of biofilm reactor determined by tracer studies was 36 percent. The results showed that an optimum denitrification rate of 384 g $NO_3^- - N/m^3$.d was found. The maximum removal efficiency of 95 percent was found at a loading rate of 200 g $NO_3^- - N$/m^3.day. Nitrite concentration in the effluent rose rapidly when the loading rate increased above 600 g $NO_3^- - N$/m^3.day. Biofouling occurred after 6 months of operation with synthetic groundwater, and after 1.3 to 2 months of operation with real groundwater. In both cases the influent nitrate concentrations was 30 mg/L as $NO_3^- - N$. It was suggested by the investigators that backwashing every one to two months was a necessity for a lime stone autotrophic denitrification column system.

Hoek et al. (1992a) conducted a study on the optimization of the sulfur-limestone filtration process for nitrate removal from groundwater. The experiments consisted of four unit operations and processes in series: (1) vacuum deaeration, (2) biological autotrophic nitrate removal, (3) aeration, and (4) passage through soil as post-treatment. The nitrate loading was 960 g $NO_3^- - N$/m^3.d. The sulfur to limestone ratios of 1:2, 1:1, and 2:1 essentially gave the same results with respect to nitrate removal capacities. Based on the theoretical sulfur to limestone consumption rates, he suggested a ratio of 1: 2 to simplify the maintenance of the reactor. The accumulated biomass was responsible for the increased effluent nitrate concentration. Backwashing was a necessity to remove the accumulated biomass. In an evaluation of the performance of a 35 m^3/hr demonstration plant, the plant was operated at a volumetric nitrate loading rate of 552 g NO_3^-/m^3.d. Sulfide production was reported at higher column heights. Maximum sulfide concentrations was 22 mg/L as S^{2-} (Hoek et al., 1992b).

CHAPTER 3

MATERIAL AND METHODS

The experimental program on autotrophic denitrification was carried out in a batch reactor, and in a continuous up-flow reactor. The batch reactor assembly consisted of an anaerobic reaction vessel, gas collection cylinder and leveling reservoir. The results of the batch reactor study were utilized to develop the basic relationships that were utilized to design and operate the continuous flow reactor. The continuous up-flow reactor had three components: influent storage tank, dissolved oxygen (DO) removal trap that was removed later, and the up-flow reactor. The reactors' description is presented below.

3.1 Description of Batch and Continuous Flow Reactor

3.1.1 Batch Reactor

The batch reactor assembly consisted of a 6-liter distillation flask with a flat bottom. It was installed over a magnetic-stirrer hot plate. The stopper on the top of the flask had four holes to provide for the following fittings: (a) a thermometer to measure the liquid temperature; (b) a 6-mm (one-quarter-inch) glass tube connected with a rubber tube for feeding the reactor; (c) a 6-mm glass tube connected with a rubber tube for withdrawal of the reactor fluid; and (d) a 6-mm glass tube connected with a rubber tube for withdrawal of the generated gas.

The gas collection system consisted of a graduated gas collection tube 6 cm in diameter and 68.6 cm long. It was connected with a leveling reservoir for maintaining the desired liquid level in the gas collection tube, and for facilitating withdrawal and feeding of the reactor.

The gas collection tube had a two-holed stopper on the top. A tube was connected to allow free flow of gas from the reactor into the collection tube. The other hole contained a tube to vent the gas as needed. The lower end of the gas collection tube was conical and was connected to the leveling reservoir. The gas collection tube was half filled with a saturated sodium chloride solution containing 5 percent sulfuric acid and methyl orange for color. This solution prevented the CO_2 gas from dissolving into the liquid. A complete batch reactor assembly is shown in Figures 3.1 and 3.2.

3.1.2 Continuous Up-Flow Reactor

The continuous up-flow reactor assembly consisted of one main reactor. This reactor was made of a cylindrical PVC pipe, 10.16 cm (4 inch) in diameter and 228.6 cm (7.5 feet) in length. For a watertight seal, the top and bottom of the column were sealed with PVC caps. At the base of the reactor, a 15.24-cm (6-inch) gravel layer was placed. The gravel size was 0.635 cm (1/4 inch) in diameter. Six sampling ports were provided at a spacing of 30.48 cm (one foot) center-to-center. The details of the reactor are shown in Figures 3.3 and 3.4.

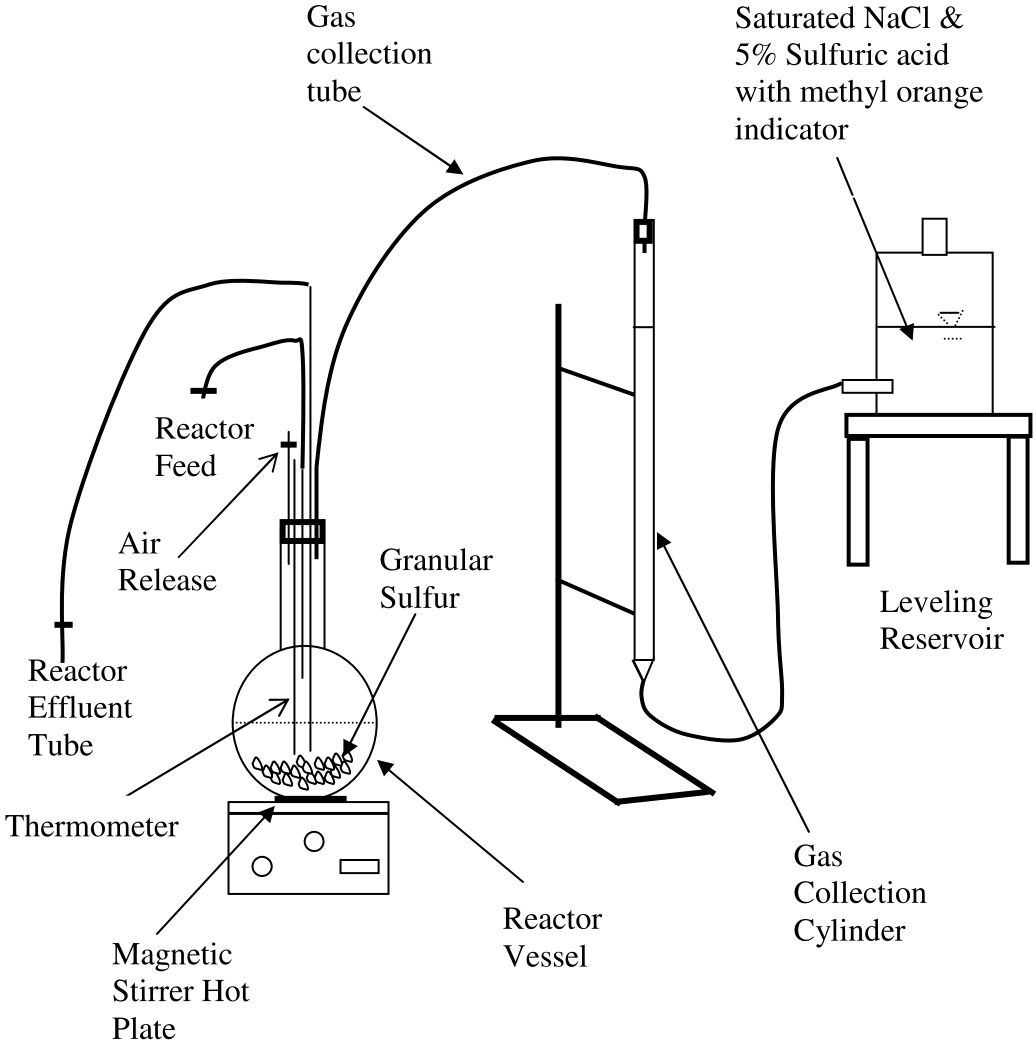


Figure 3.1 Details of Batch Reactor Assembly and Gas Collection System

Figure 3.2 Photograph of Batch Reactor Assembly and Gas Collection System

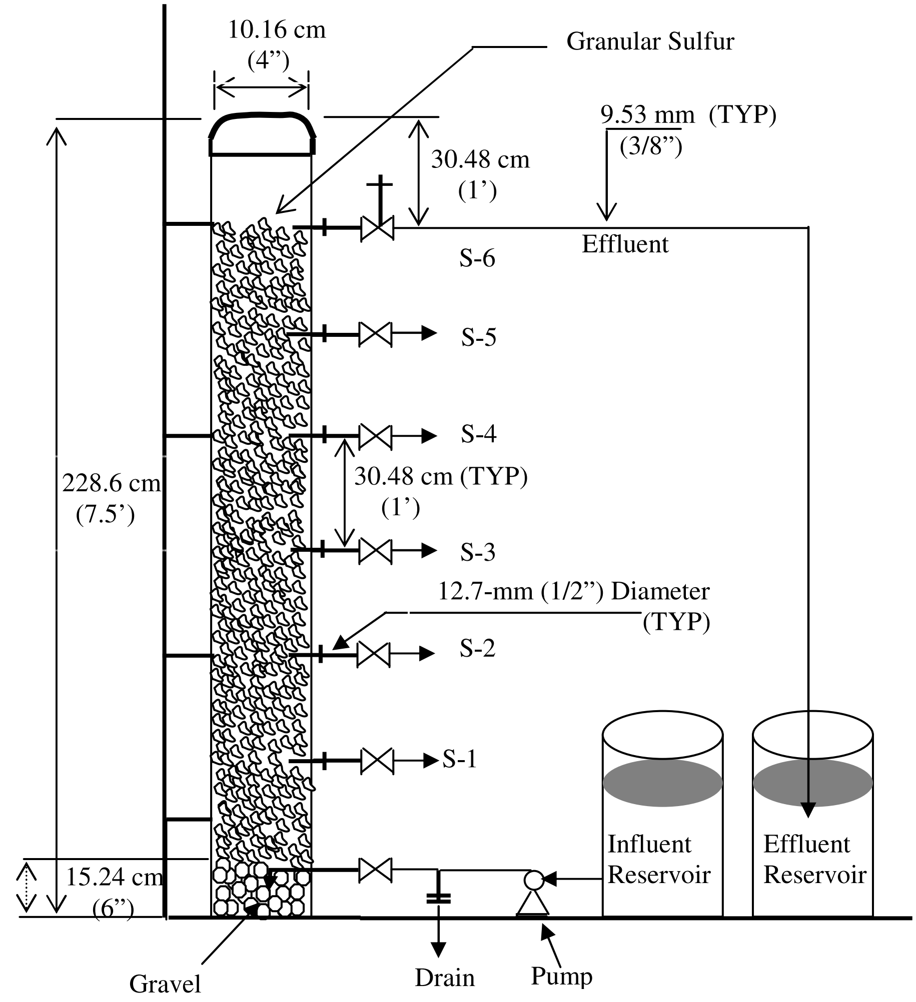


Figure 3.3 Assembly of Sulfur-Packed Continuous Up-Flow Reactor

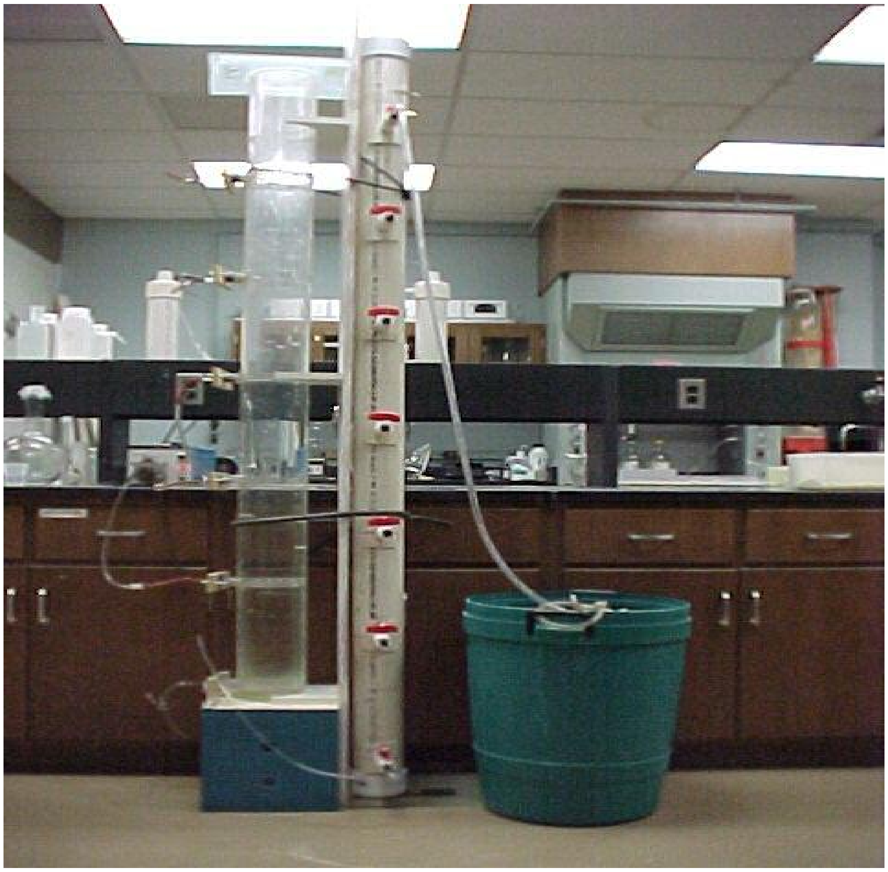


Figure 3.4 Photograph of Sulfur-Packed Continuous Up-Flow Reactor

3.2 Sulfur Media

The media in the packed-bed reactor was elemental sulfur. The elemental sulfur was obtained from the International Sulfur, Inc., Mount Pleasant, Texas. The media size was between 2.38 mm and 4.76 mm in diameter (U.S. Standard Sieve Size #8 and #4). The physical characteristics of the media was determined in the laboratory using the standard procedures. These procedures are provided in Appendix C. The density, void ratio, and specific surface area of the media are provided in Table 3.1.

Table 3.1 The Density, Void Ratio, and Specific Surface Area of the Media

Media Physical Characteristics	Values
Density	1.997 g/cm^3
Void Ratio	0.4
Specific Surface Area	1.45 x 10^3 m^2/m^3

3.3 Reactor Operation

3.3.1 Seed Cultivation in the Batch Reactor

The digested sludge from the Village Creek Wastewater Treatment Plant in Fort Worth, Texas was brought for seed. The bacteria culture was developed by adding 100-mL digested sludge, 300 cm^3 granular sulfur, and 10 mg/L $NO_3^- - N$ in total liquid volume of 4 L in the reaction vessel of the batch reactor. The content of the batch reactor was mixed by the magnetic stirrer. Gas generation started after 7 days of continuous mixing. The normal operation of the batch reactor started by sample withdrawal, reactor feeding, and gas volume measurement.

3.3.2 Operation of Batch Reactor

The batch reactor operation was on a fill and draw basis. Each day approximately 600 mL of reactor content was removed for analyses and equal volume of fresh feed was added. The sample analyses included measurement of pH, concentrations of total alkalinity, $NO_3^- - N$, and SO_4^{2-} of the feed and the withdrawn liquid. Additionally, temperature and gas production each day were recorded. The volume of gas after measurement each day was released, and the level in the gas vessel was reset.

Addition of sodium bicarbonate ($NaHCO_3$) was necessary in the feed to maintain proper alkalinity and pH in the system. The alkalinity addition was 5.0 mg/L alkalinity as $CaCO_3$ per mg/L of nitrate nitrogen added in the reactor. The samples withdrawn were filtered through a 0.45 μm glass fiber paper prior to laboratory analysis. The laboratory data was used to calculate total gas production, sulfate production, and alkalinity destruction for each mg/L of $NO_3^- - N$ removed from the feed.

3.3.3 Operation of Continuous Up-Flow Reactor

The continuous flow reactor was started using a standard procedure. After the denitrification was established in the reactor, the operation mode began. The start up and operational mode are presented below.

3.3.3.1 Start-up Phase

The start up phase of the continuous flow reactor included reactor seeding. Approximately 600 mL of seed from the batch reactor was added into the packed-bed reactor. The remaining volume of the column was filled with feed solution consisting of 20 mg/L of nitrate nitrogen. After one day of media soaking, the internal re-circulation was started. The alkalinity and nitrate concentration in the reactor content was checked daily. After several days of recirculation the nitrate concentration in the recirculating liquid started to drop. At that time the continuous feeding was started. A peristaltic pump (Cole-Parmer Instrument Co.) was used to continuously pump the feed from the bottom of the reactor. Initially the nitrate removal was inconsistent. Tap water was used to prepare the feed. It was speculated that the chlorine residuals in the tap water may have caused the interference. Therefore, the tap water was aerated for three days for dechlorination before preparing the feed. Also sodium bicarbonate and micronutrients were added in the feed. The composition of the micronutrient solution is provided in Table 3.2. After these improvements were made, the nitrate removal stabilized and remained such until the end of this research.

3.3.3.2 Reactor Operation

The continuous flow reactor was operated at three hydraulic loadings. At each hydraulic loading, four different $NO_3^- - N$ concentrations in the feed were tested. These concentrations were 20, 40, 60, and 70 mg/L $NO_3^- - N$ and hydraulic loadings were 0.06, 0.08, and 0.10 m^3/m^2.hr. The reactor was operated for at least 7 days at each nitrate nitrogen concentration in the feed. The reactor operation matrix is provided in Table 3.3.

Table 3.2 Composition of Micro Nutrients for Continuous Up-Flow Reactor

Constituent	Concentration in the feed
$NaHCO_3$	Based on alkalinity ratio of 5 mg/L as $CaCO_3$ per mg/L of $NO_3^- - N$, $NaHCO_3$ was added in the feed
K_2HPO_4	0.2 mg/L as P
NH_4Cl	1 mg/L as N
$MgCl_2.6H_2O$	1 mg/L as $MgCl_2.6H_2O$
$FeCl_3.6H_2O$	1 mg/L as $FeCl_3.6H_2O$
$MnSO_4.H_2O$	1 mg/L as $MnSO_4.H_2O$
pH	8.3~8.6

Note: This composition is a modification of the formula provided by Batchelor (1978)

Table 3.3 Hydraulic Loadings and $NO_3^- - N$ Concentrations in the Feed During Operation Phase

Hydraulic Loading, $m^3/m^2.hr$	$NO_3^- - N$ Concentration in the Feed, mg/L				
0.06	20	50	70	90	-
0.08	20	40	50	60	70
0.10	20	40	60	70	-

3.3.4 Sampling, and Sample Preparation

The influent sample was collected from the influent feed tank. The effluent samples were collected from six effluent ports. The routine measurements and analyses included temperature, pH, total alkalinity, $NO_3^- - N$, $NO_2^- - N$, and SO_4^{2-}. The influent DO was checked on routine basis. The temperature, pH and total alkalinity measurements were made immediately. The samples were filtered through 0.45 μm membrane filter. Effluents were made to complete all analytical measurements on the day of sampling. The samples were stored in the environmental chamber at 5°C to complete the analysis at a later time. The maximum storage time was two days. All stored samples were allowed to warm up to the room temperature before conducting the analytical tests. The details of the analytical procedures are provided in Appendix D.

3.3.5 Applied Hydraulic and Substrate Loadings

The continuous up-flow reactor was operated under variable $NO_3^- - N$ as well as hydraulic loadings. As a result, each sample withdrawn from different ports had different detention time and $NO_3^- - N$ loading. The applied hydraulic loadings and corresponding true hydraulic retention time (THRT) at each sampling port are summarized in Table 3.4. The $NO_3^- - N$ loading at different sampling ports were calculated from the hydraulic loading and the hydraulic retention time. The calculated $NO_3^- - N$ loadings at each port resulting from different $NO_3^- - N$ concentration in the feed and hydraulic loadings are summarized in Tables 3.5-3.10.

Table 3.4 Summary of THRT at Each Sampling Port at Three Hydraulic Loadings

	Sampling Port					
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	True Hydraulic Retention Time (THRT), hrs					
0.06 $m^3/m^2 \cdot hr$	2.0[a]	4.1	6.1	8.1	10.2	12.2
0.08 $m^3/m^2 \cdot hr$	1.5	3.1	4.6	6.1	7.6	9.1
0.10 $m^3/m^2 \cdot hr$	1.2	2.4	3.7	4.9	6.1	7.3

[a] $\dfrac{0.3048 \text{ m} \times 0.4}{0.06 \text{ m}^3/\text{m}^2.\text{hr}} = 2.03 \text{ hr}$

Note :

Throughout this research, whenever the hydraulic retention time (HRT) is mentioned, it refers to the "true" hydraulic retention time (THRT).

$$\text{THRT} = \frac{\text{Volume} \times \text{Void Ratio}}{\text{Flow Rate}} = \frac{\text{Area} \times \text{Column Height} \times \text{Void Ratio}}{\text{Flow Rate}}$$

$$= \frac{A \times h \times e}{Q} = \frac{h \times e}{(Q/A)} = \frac{h \times e}{Q_v}$$

Table 3.5 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 20 mg/L)

	Sampling Port					
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, $g/m^3.d$					
0.06 $m^3/m^2 \cdot hr$	236[a]	118	79	59	47	39
0.08 $m^3/m^2 \cdot hr$	315	157	105	79	63	52
0.10 $m^3/m^2 \cdot hr$	394	197	131	98	79	66

[a] $NO_3^- \text{ - N Loading } = \dfrac{Q \times C}{V} = \dfrac{Q \times C}{A \times h \times e} = \dfrac{Q/A \times C}{h \times e} = \dfrac{Q_v \times C}{h \times e}$

$(0.06 \text{ m}^3/\text{m}^2.\text{hr} \times 20 \text{ g/m}^3)/(0.3048 \text{ m} \times 0.4) \times (24 \text{ hr/d}) = 236.2 \text{ g/m}^3.\text{d}$

Table 3.6 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 40 mg/L)

	Sampling Port					
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, g/m^3.d					
0.06 m^3/m$^2\cdot$hr	472 [a]	236	157	118	94	79
0.08 m^3/m$^2\cdot$hr	630	315	210	157	126	105
0.10 m^3/m$^2\cdot$hr	787	394	262	197	157	131

[a] (0.06 m^3/m^2.hr x 40 g/m^3)/(0.3048 m x 0.4) x (24 hr/d) = 472.4 g/m^3.d

Table 3.7 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 50 mg/L)

	Sampling Port					
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, g/m^3.d					
0.06 m^3/m$^2\cdot$hr	591 [a]	295	197	148	118	98
0.08 m^3/m$^2\cdot$hr	787	394	262	197	157	131
0.10 m^3/m$^2\cdot$hr	984	492	328	246	197	164

[a] (0.06 m^3/m^2.hr x 50 g/m^3)/(0.3048 m x 0.4) x (24 hr/d) = 590.6 g/m^3.d

Table 3.8 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 60 mg/L)

Sampling Port						
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, g/m^3.d					
0.06 m^3/m^2·hr	709[a]	354	236	177	142	118
0.08 m^3/m^2·hr	945	472	315	236	189	157
0.10 m^3/m^2·hr	1181	591	394	295	236	197

[a] (0.06 m^3/m^2.hr x 60 g/m^3)/(0.3048 m x 0.4) x (24 hr/d) = 708.7 g/m^3.d

Table 3.9 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 70 mg/L)

Sampling Port						
Hydraulic Surface loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, g/m^3.d					
0.06 m^3/m^2·hr	827[a]	413	276	207	165	138
0.08 m^3/m^2·hr	1102	551	367	276	220	184
0.10 m^3/m^2·hr	1378	689	459	344	276	230

[a] (0.06 m^3/m^2.hr x 70 g/m^3)/(0.3048 m x 0.4) x (24 hr/d) = 826.8 g/m^3.d

Table 3.10 Summary of $NO_3^- - N$ Loadings at Each Sampling Port at Three Hydraulic Loadings (Influent $NO_3^- - N$ = 90 mg/L)

	Sampling Port					
Hydraulic Surface Loading, Q_v	1	2	3	4	5	6
	$NO_3^- - N$ Loading, g/m^3.d					
0.06 m^3/m^2·hr	1063[a]	531	354	266	213	177
0.08 m^3/m^2·hr	1417	709	472	354	283	236
0.10 m^3/m^2·hr	1772	886	519	443	354	295

[a] (0.06 m^3/m^2.hr x 90 g/m^3)/(0.3048 m x 0.4) x (24 hr/d) = 1,063 g/m^3.d

CHAPTER 4

RESULTS

The experimental program was conducted with two reactors: (1) an anaerobic batch reactor, and (b) a continuous up-flow reactor. Results of each reactor study are presented below.

4.1 Batch Reactor Study

The purpose of the batch reactor study was to (1) develop microbial seed to be used in the continuous flow reactor, and (2) develop relationships between gas volume generated, alkalinity destruction, and sulfate production with respect to $NO_3^- - N$ destruction. The batch reactor study was conducted under anaerobic condition. The reactor contained granular sulfur and the reactor contents were continuously mixed. The effluent and influent samples were withdrawn and fed daily. The graduated gas collection vessel was connected to the reactor, and volume of gas generated daily was recorded. During the initial adjustment phase of reactor operation, the active microbial population was established. This was indicated by consistent gas production. It took approximately two weeks to reach the nearly consistent gas generation, and the data recording began.

On a daily basis, approximately 600 mL of the reactor content was removed and equal volume of nitrate containing substrate was added into the reactor. The concentrations of alkalinity, nitrate-N, and sulfate in the influent and effluent were measured and recorded. Additionally, pH, temperature, and gas volume produced were measured for each day of operation. The volume of gas produced each day was recorded before releasing the gas. Daily pH values, concentrations of $NO_3^- - N$ and SO_4^{2-}, total alkalinity in the feed, and daily volume of feed introduced into the batch reactor are summarized in Tables 4.1. Additionally, the daily pH values, and concentrations of $NO_3^- - N$, SO_4^{2-}, and total alkalinity in the effluent, volume of gas produced, and volume of liquid withdrawn from the reactor are provided in Table 4.2. The data presented in Tables 4.1 and 4.2 are from the day the data recording began. The initial data of approximately two weeks of operation are not included in these tables.

4.2 Continuous Up-Flow Reactor Study

The continuous flow reactor consisted of a column packed with granular sulfur. The purpose of the continuous flow reactor study was to: (1) operate the reactor at different hydraulic and nitrate loadings; (2) develop relationships of alkalinity destruction per unit nitrate nitrogen removal, and sulfate generation per unit nitrate nitrogen removal; and (3) utilize measured data to model the effluent concentrations of $NO_3^- - N$ and $NO_2^- - N$ with respect to THRT.

Table 4.1 Characteristics of Batch Reactor Feed

Consecutive Sampling Days	pH	Nitrate Nitrogen, mg/L as $NO_3^- - N$	Sulfate, mg/L as SO_4^{2-}	Total Alkalinity, mg/L as $CaCO_3$	Daily Feed Volume, mL
0	7.9	100	37.5	460	625
1	8.0	100	39.2	460	550
2	8.3	100	39.2	460	580
3	8.3	100	39.2	460	710
3[a]	ND	0	39.2	18850	50
4	8.3	100	39.2	460	610
5	8.6	100	39.2	460	530
6[b]	ND	ND	ND	ND	ND
7	8.6	100	37.5	578	480
8	8.6	100	37.5	578	640
9	8.6	100	37.5	578	650
10	8.6	100	37.5	578	590
11	8.6	100	37.5	578	660
12	8.1	100	37.5	580	590
13	8.0	100	37.5	580	710
14	8.4	100	37.5	580	545
15	8.4	100	37.5	580	650
16	ND	ND	ND	ND	ND
17	8.4	100	37.5	580	550
18	8.7	100	37.5	580	565
19	8.0	100	37.5	572	650
20	8.3	100	37.5	572	610
21	8.3	100	37.5	572	605

[a] The pH of the reactor content decreased suddenly on the third day. Therefore, 50 mL of prepared solution with high concentration of $NaHCO_3$ was added to raise the alkalinity. The $NO_3^- - N$ concentration in this solution was zero, and SO_4^{2-} concentration was 39.2 mg/L, and alkalinity was 18,850 mg/L as $CaCO_3$.

[b] No data. The effluent sample was not withdrawn and reactor was not fed.

Note: The first data entry in this table is from the day the data collection began. Approximately two weeks of initial unstable data are not included.

Table 4.2 Batch Reactor Daily Effluent Quality Data and Gas Generation

Consecutive Sampling Days	Effluent Sample Contents					
	pH	Nitrate Nitrogen, mg/L as $NO_3^- - N$	Total Alkalinity, mg/L as $CaCO_3$	Sulfate, mg/L as SO_4^{2-}	Sample Volume, mL	Total Gas Volume, mL
0	6.4	52	160	264	570	25
1	6.3	44.1	114	375	580	65
2	6.0	24.2	54	475	585	80
3	5.6	17.2	40	536	663	50
3[a]	ND	31.4	111.9	451.1	0	0
4	6.5	17.2	275	525	722	43
5	6.5	16.8	208	660	573	80
6[b]	ND	ND	ND	ND	ND	ND
7	6.1	4.7	115	842	586	90
8	5.8	1.9	96	824	563	50
9	5.7	0.7	96	860	571	24
10	5.8	0.5	100	853	580	10
11	5.9	0	106	846	570	34
12	6.2	4.6	134	790	619	34
13	6.1	2.2	140	806	739	38
14	6.1	6.3	150	781	570	48
15	6.2	3.3	136	808	570	40
16	ND	ND	ND	ND	ND	ND
17	6.2	0.8	136	771	566	61
18	6.2	0.2	140	774	579	62
19	6.0	0	140	772	573	49
20	6.3	0	156	755	573	57
21	6.1	0	162	775	579	48

[a] See foot note “a” in Table 4.1

[b] See foot note “b” in Table 4.1

Note: The first data entry in this table is from the day the data collection began. Approximately two weeks of initial unstable data are not included.

4.2.1 Start-up Phase

During the start-up phase, the alkalinity and $NO_3^- - N$ destructions were monitored. The influent feed contained: $NO_3^- - N$ = 20 mg/L, and Alkalinity = 184 mg/L as $CaCO_3$. The hydraulic loading was 0.06 m^3/m^2.hr. During the start-up phase, the reactor did not achieve high nitrate nitrogen removal efficiency. The following modifications were made during startup phase: (1) the residual chlorine was removed by aerating the tap water for three days before it was used to prepare the raw feed solution, (2) micronutrients as specified in Table 3.2 were added into the raw feed, and (3) a small granular sulfur-packed PVC pipe was provided in the front of the reactor column to deoxygenate the feed. This trap effectively removed DO in the feed but also removed a portion of $NO_3^- - N$ in the feed. As a result, the DO trap was removed, while aeration for chlorine removal and micronutrients addition were continued during the entire operational phase.

4.2.2 Operation Phase

After all the modifications were made during the start-up phase, the operation phase was carried out for approximately three months at three different hydraulic loadings, and four to five influent $NO_3^- - N$ concentrations. The measured parameters for the continuous flow reactor were total alkalinity, nitrate nitrogen, nitrite nitrogen, sulfate, pH, and temperature. These parameters were measured in the feed as well as in the samples collected from each port of the column.

4.2.2.1 Results at Hydraulic Loading of 0.06 m^3/m^2.hr

The hydraulic loading of 0.06 m^3/m^2.hr was maintained at a feed rate of 8.1 mL per minute (0.000486 m^3/hr) over the column area of 0.0081 m^2. The column at this hydraulic loading was operated from January 30th to March 6th, and the target $NO_3^- - N$ concentrations in the feed were changed in the ascending order of 20, 50, 70, and 90 mg/L. The actual $NO_3^- - N$ concentrations in the feed however, were slightly different from the target concentrations. Only 6 to 11 days of operation data at each influent $NO_3^- - N$ concentration were averaged, while approximately 1 to 2 days of transition data immediately after changing the $NO_3^- - N$ concentration in the feed were ignored. The data are reported in Table 4.3 and illustrated in Figure 4.1.

Table 4.3 Average Concentrations of Measured Parameters in the Influent and Effluent from Each Port at Hydraulic Loading of 0.06 m^3/m^2.hr and Influent Target $NO_3^- - N$ Concentrations of 20, 50, 70, and 90 mg/L

$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
20 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			236[a]	118	79	59	47	39
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	19.0	0	0	0	0	0	0
	$NO_2^- - N$, mg/L	0	0	0	0	0	0	0
	Alkalinity, mg/L as $CaCO_3$	182	106	106	106	106	106	98
	Sulfate, mg/L as SO_4^{2-}	47	198	195	196	195	200	203
	pH	7.0	6.1	6.0	6.0	6.0	6.1	6.0
	Temperature °C	22	ND[b]	ND	ND	ND	ND	ND
50 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			591	295	197	148	118	98
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	50.5	11.4	1.8	0.8	0.7	0.5	0.5
	$NO_2^- - N$, mg/L	1.8	4.9	2.8	1.1	0.7	0.7	0.5
	Alkalinity, mg/L as $CaCO_3$	335	197	165	161	159	158	153
	Sulfate, mg/L as SO_4^{2-}	38	318	400	421	425	429	436
	pH	8.4	6.6	6.4	6.4	6.4	6.4	6.5
	Temperature °C	19.1	20.9	21.0	21.1	21.2	21.4	20.7

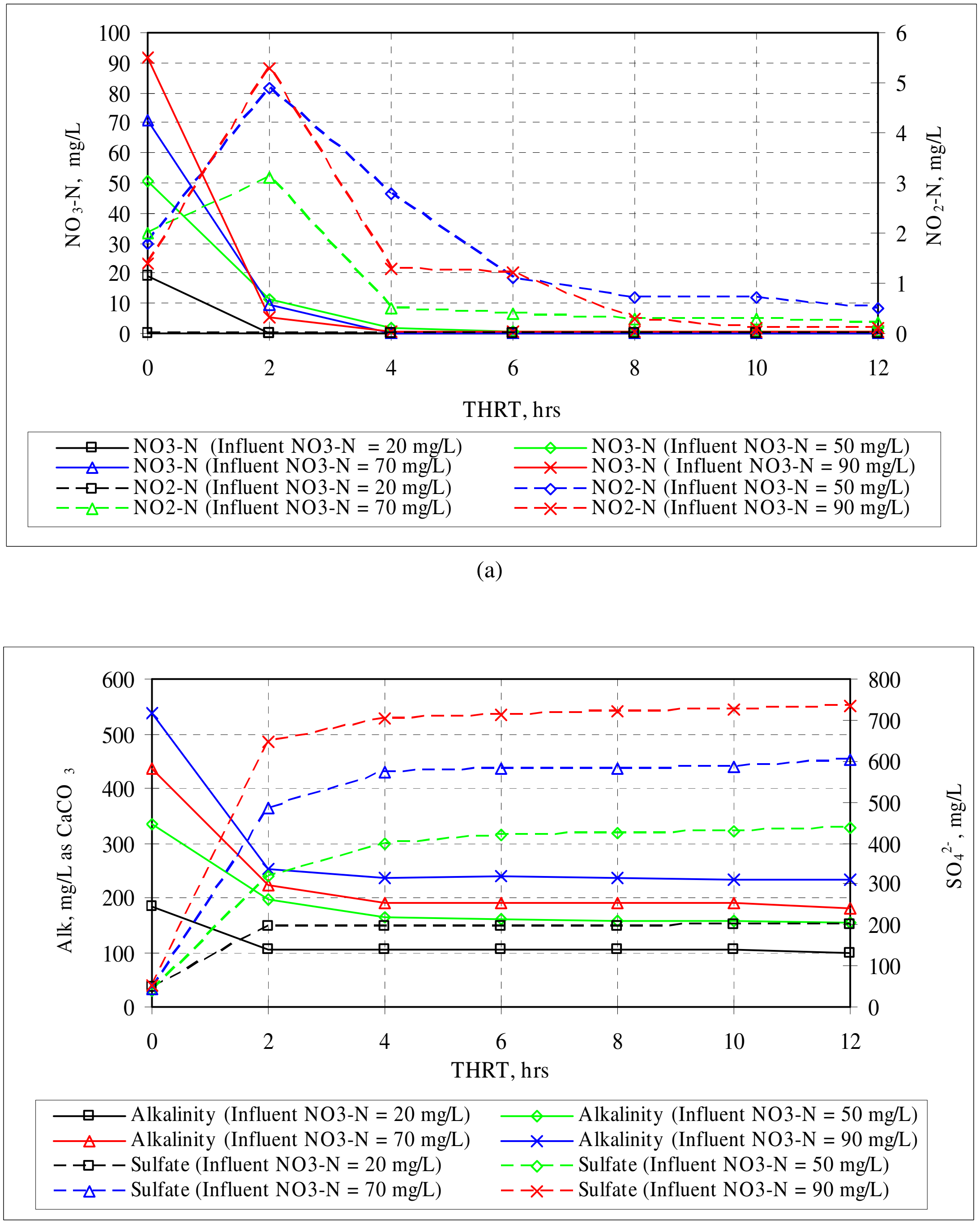
Table 4.3 – Continued

$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
70 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			827	413	276	207	165	138
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	70.9	9.5	0.2	0.1	0.1	0.1	0.2
	$NO_2^- - N$, mg/L	2.0	3.1	0.5	0.4	0.3	0.3	0.2
	Alkalinity, mg/L as $CaCO_3$	437	224	189	190	190	190	179
	Sulfate, mg/L as SO_4^{2-}	44	486	571	581	581	584	603
	pH	8.4	6.5	6.3	6.3	6.3	6.3	6.4
	Temperature ^oC	19.9	21.3	21.4	21.4	21.5	21.6	21.0
90 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			1,063	531	354	266	213	177
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	91.8	5.1	0.7	0.6	0.3	0.3	0.3
	$NO_2^- - N$, mg/L	1.4	5.3	1.3	0.2	0.3	0.1	0.1
	Alkalinity, mg/L as $CaCO_3$	539	252	236	238	236	234	224
	Sulfate, mg/L as SO_4^{2-}	51	646	706	711	723	725	735
	pH	8.6	6.4	6.4	6.4	6.4	6.4	6.4
	Temperature ^oC	19.9	21.5	21.5	21.5	21.6	21.7	21.0

[a] $NO_3^- - N$ loading = C_o x $Q/(Vx0.4)$ = C_o x Hydraulic Loading/(0.4 x Height of port from base)

$$= 20 \frac{g}{m^3} \times \frac{0.06}{0.4} \frac{m^3}{m^2\ hr} \times \frac{24\ hr}{d \times 0.3048\ m} = 236 \frac{g}{m^3\ d}$$

[b] ND = No Data



(a)

(b)

Figure 4.1 Graphical Presentation of Effluent Concentrations (a) $NO_3^- - N$, and $NO_2^- - N$ Concentrations with Respect to THRT, and (b) Alkalinity and Sulfate Concentrations with Respect to THRT at Hydraulic Loading of $0.06\ m^3/m^2.hr$

4.2.2.2 Results at Hydraulic Loading of 0.08 m^3/m^2.hr

The hydraulic loading of 0.08 m^3/m^2.hr was maintained at a feed rate of 10.8 mL per minute (0.000649 m^3/hr) over the column area of 0.0081 m^2.

The column at this hydraulic loading was operated from March 19th to April 18th, and the target $NO_3^- - N$ concentrations in the feed were changed in the descending order of 70, 60, 50, 40, and 20 mg/L. The actual $NO_3^- - N$ concentrations in the feed however, were slightly different from the target concentrations. Only 4 to 6 days of the operation data at each influent $NO_3^- - N$ concentration were averaged, while approximately 1 to 2 days of transition data immediately after changing the $NO_3^- - N$ concentration in the feed were ignored. The data are reported in Table 4.4 and illustrated in Figure 4.2.

Table 4.4 Average Concentrations of Measured Parameters in the Influent and Effluent from Each Port at Hydraulic Loading of 0.08 m^3/m^2.hr and Influent Target $NO_3^- - N$ Concentrations of 20, 40, 50, 60, and 70 mg/L

$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
20 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			315[a]	157	105	79	63	52
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	19.9	0.1	0.1	0.0	0.0	0.0	0.0
	$NO_2^- - N$, mg/L	2.0	0.3	0.1	0.1	0.2	0.2	0.3
	Alkalinity, mg/L as $CaCO_3$	193	117	117	117	117	117	106
	Sulfate, mg/L as SO_4^{2-}	63	229	236	231	237	240	244
	PH	8.3	6.5	6.5	6.5	6.5	6.5	6.5
	Temperature °C	21.0	21.8	21.8	21.9	22.0	22.0	22.0
40 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			630	315	210	157	126	105
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	37.9	1.1	0.7	0.6	0.6	0.6	0.6
	$NO_2^- - N$, mg/L	2.4	6.9	0.3	0.5	0.2	0.2	0.2
	Alkalinity, mg/L as $CaCO_3$	293	165	156	155	152	149	134
	Sulfate, mg/L as SO_4^{2-}	59.3	345	365	378	380	375	390
	pH	8.3	6.5	6.5	6.5	6.5	6.4	6.4
	Temperature °C	21.0	21.9	21.9	21.9	22.0	22.0	21.7

Table 4.4 – Continued

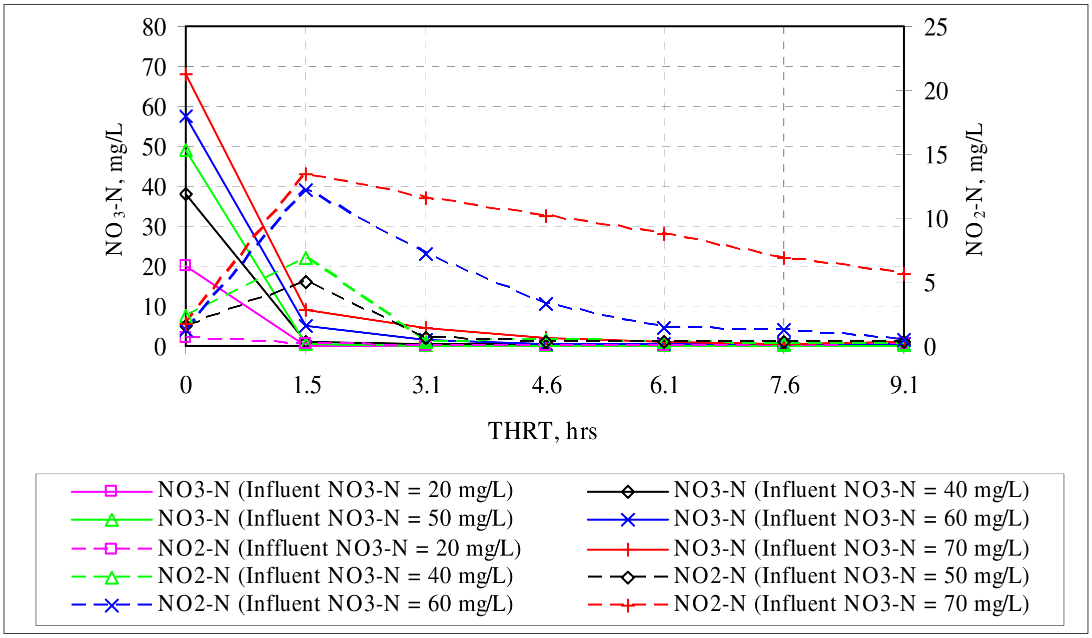
$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
50 mg/L			$NO_3^- - N$ Loading at Each Port, $g/m^3.d$					
			787	394	262	197	157	131
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	48.8	0.4	0.2	0.2	0.2	0.2	0.2
	$NO_2^- - N$, mg/L	1.5	4.9	0.6	0.3	0.4	0.3	0.3
	Alkalinity, mg/L as $CaCO_3$	342	181	174	175	175	174	166
	Sulfate, mg/L as SO_4^{2-}	58	403	419	411	421	428	430
	pH	8.4	6.5	6.4	6.4	6.4	6.4	6.5
	Temperature °C	20.4	21.3	21.3	21.3	21.4	21.5	21.1
60 mg/L			$NO_3^- - N$ Loading at Each Port, $g/m^3.d$					
			945	472	315	236	189	157
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	57.6	5.0	1.5	0.6	0.5	0.4	0.4
	$NO_2^- - N$, mg/L	1.3	12.2	7.2	3.3	1.4	1.2	0.5
	Alkalinity, mg/L as $CaCO_3$	394	214	200	193	192	191	186
	Sulfate, mg/L as SO_4^{2-}	56	419	470	497	501	509	511
	pH	8.5	6.5	6.5	6.4	6.4	6.4	6.5
	Temperature °C	19.4	21.2	21.2	21.2	21.4	21.5	21.0

Table 4.4 – Continued

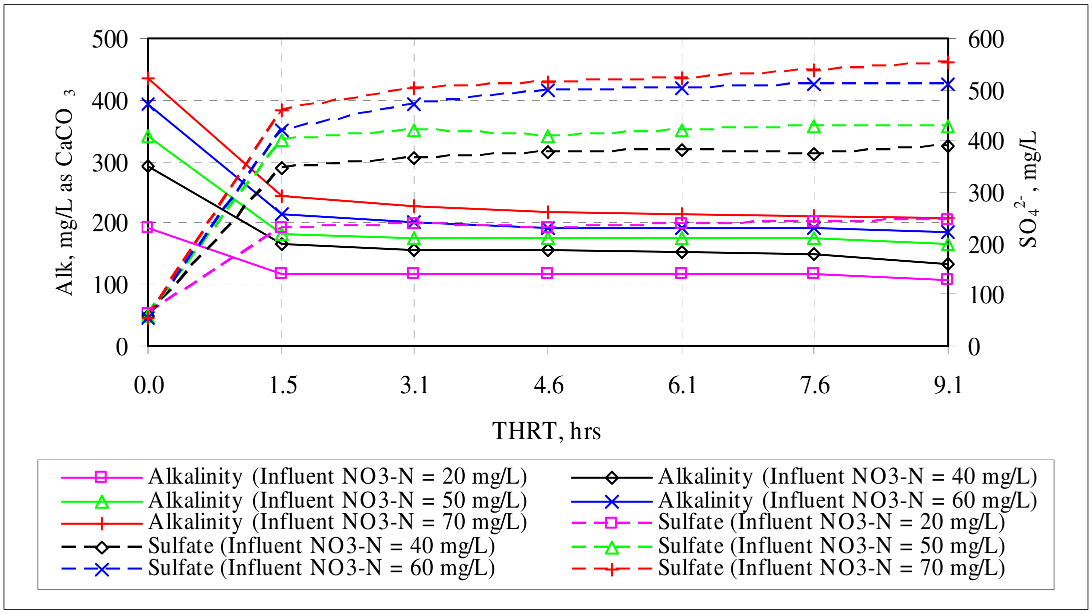
$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
70 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			1,102	551	367	276	220	184
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	68.0	9.1	4.7	2.1	1.1	0.7	0.8
	$NO_2^- - N$, mg/L	1.8	13.5	11.5	10.1	8.8	6.8	5.6
	Alkalinity, mg/L as $CaCO_3$	435	242	228	219	215	212	209
	Sulfate, mg/L as SO_4^{2-}	55	460	501	515	523	539	553
	pH	8.5	6.5	6.5	6.4	6.4	6.4	6.5
	Temperature °C	19.8	21.3	21.4	21.4	21.6	21.7	21.2

[a] $NO_3^- - N$ Loading = C_o x $Q/(V \text{ x } 0.4)$ = C_o x Hydraulic Loading/(0.4 x Height of port from base)

$$20\,\frac{\text{g}}{\text{m}^3} \times \frac{0.08\,\frac{\text{m}^3}{\text{m}^2\ \text{hr}}}{0.4 \times 0.3048\ \text{m}} \times \frac{24\ \text{hr}}{\text{d}} = 315\,\frac{\text{g}}{\text{m}^3\ \text{d}}$$



(a)



(b)

Figure 4.2 Graphical Presentation of Effluent Concentrations (a) $NO_3^- - N$, and $NO_2^- - N$ Concentrations with Respect to THRT, and (b) Alkalinity and Sulfate Concentrations with Respect to THRT at Hydraulic Loading of 0.08 m^3/m^2.hr.

4.2.2.3 Results at Hydraulic Loading of 0.10 m^3/m^2.hr

The hydraulic loading of 0.10 m^3/m^2.hr was maintained at a feed rate of 13.5 mL per minute (0.000811 m^3/hr) over the column area of 0.0081 m^2.

The column at his hydraulic loading was operated from April 19th to May 11th, and the target $NO_3^- - N$ concentrations in the feed were changed in the ascending order of 20, 40, 60, and 70 mg/L. The actual $NO_3^- - N$ concentrations in the feed however, were slightly different from the target concentrations. Only 4 to 6 days of operation data at each influent $NO_3^- - N$ concentration were averaged, while approximately 1 day of transition data immediately after changing the $NO_3^- - N$ concentration in the feed were ignored. The data are reported in Table 4.5 and illustrated in Figures 4.3.

Table 4.5 Average Concentrations of Measured Parameters in the Influent and Effluent from Each Port at Hydraulic Loading of 0.10 m^3/m^2.hr and Influent Target $NO_3^- - N$ Concentrations of 20, 40, 60, and 70 mg/L

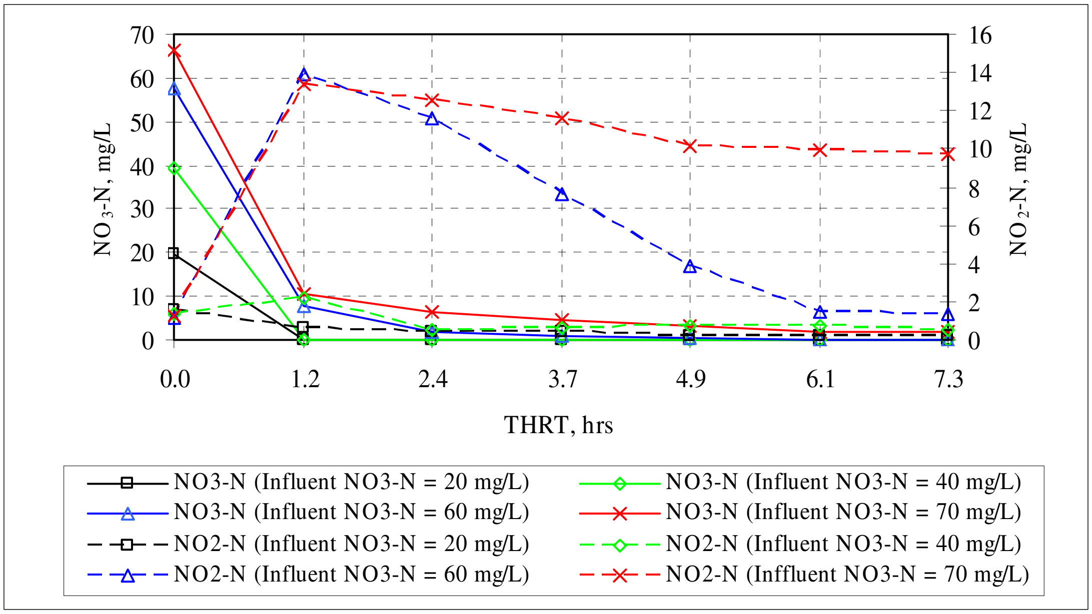
$NO_3^- - N$ Concentration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Sampling Ports					
			1	2	3	4	5	6
20 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			394[a]	197	131	98	79	66
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	19.9	0.0	0.0	0.0	0.0	0.0	0.0
	$NO_2^- - N$, mg/L	1.6	0.6	0.4	0.4	0.2	0.2	0.2
	Alkalinity, mg/L As $CaCO_3$	201	116	117	115	116	115	107
	Sulfate, mg/L as SO_4^{2-}	65	237	237	237	235	236	247
	PH	8.3	6.6	6.6	6.6	6.6	6.6	6.5
	Temperature °C	20.2	21.3	21.3	21.4	21.4	21.5	21.1
40 mg/L			$NO_3^- - N$ Loading at Each Port, g/m^3.d					
			787	394	262	197	157	131
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	39.2	0.2	0.1	0.0	0.0	0.0	0.0
	$NO_2^- - N$, mg/L	1.4	2.2	0.5	0.6	0.7	0.7	0.5
	Alkalinity, mg/L As $CaCO_3$	298	155	153	153	150	149	139
	Sulfate, mg/L as SO_4^{2-}	66	374	380	378	377	372	388
	pH	8.4	6.5	6.5	6.5	6.5	6.5	6.5
	Temperature °C	20.5	21.4	21.4	21.4	21.5	21.5	20.9

Table 4.5 – Continued

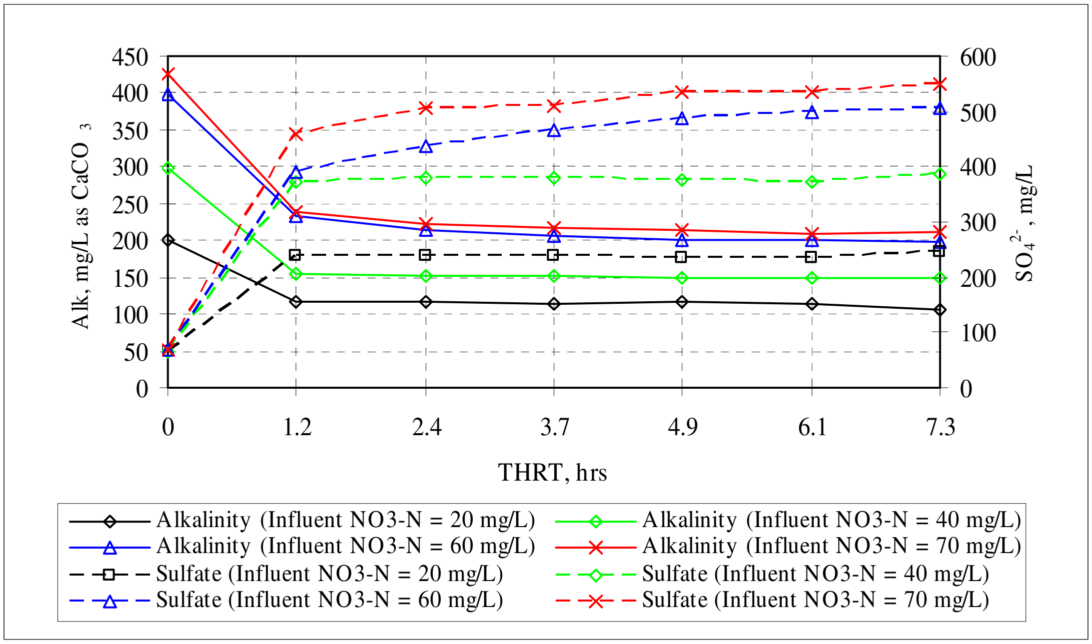
$NO_3^- - N$ Concent-ration in Prepared Feed	Measured Parameter	Measured Concentration in the Feed	Effluent Concentrations					
			1	2	3	4	5	6
60 mg/L			$NO_3^- - N$ Loading at Each Port, $g/m^3.d$					
			1,181	591	394	295	236	197
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	57.6	7.9	2.0	0.9	0.4	0.2	0.2
	$NO_2^- - N$, mg/L	1.1	13.9	11.6	7.6	3.9	1.5	1.4
	Alkalinity, mg/L as $CaCO_3$	398	232	214	205	201	200	197
	Sulfate, mg/L as SO_4^{2-}	67	391	439	465	487	499	507
	pH	8.5	6.6	6.5	6.5	6.5	6.5	6.5
	Temperature ^oC	20.5	21.2	21.2	21.3	21.4	21.5	20.9
70 mg/L			$NO_3^- - N$ Loading at Each Port, $g/m^3.d$					
			1,378	689	459	344	276	230
			Effluent Concentrations					
	$NO_3^- - N$, mg/L	66.5	10.5	6.5	4.6	3.2	1.8	1.9
	$NO_2^- - N$, mg/L	1.3	13.4	12.5	11.6	10.1	9.9	9.7
	Alkalinity, mg/L as $CaCO_3$	426	239	223	217	214	210	211
	Sulfate, mg/L as SO_4^{2-}	68	458	505	508	536	534	550
	pH	8.5	6.6	6.5	6.5	6.5	6.5	6.5
	Temperature ^oC	20.4	21.2	21.1	21.1	21.1	21.2	20.5

[a] $NO_3^- - N$ Loading = C_o x $Q/(V \text{ x } 0.4)$ = C_o x Hydraulic Loading/(0.4 x Height of port from base)

$$20 \frac{g}{m^3} \times \frac{0.10 \frac{m^3}{m^2.hr}}{0.4 \times 0.3048 \ m} \times \frac{24 \ hr}{d} = 394 \frac{g}{m^3.d}$$



(a)



(b)

Figure 4.3 Graphical Presentation of Effluent Concentrations (a) $NO_3^- - N$, and $NO_2^- - N$ Concentrations with Respect to THRT, and (b) Alkalinity and Sulfate Concentrations with Respect to THRT at Hydraulic Loading of 0.10 m^3/m^2.hr.

CHAPTER 5

DISCUSSION

5.1 Batch Reactor Study

The batch reactor was operated to develop microbiological culture for use in the continuous up-flow reactor. Also the batch reactor data was used to develop three important relationships: ratios of alkalinity destruction, sulfate production, and gas generation with respect to nitrate nitrogen destruction.

5.1.1 Reactor Base Line Data

Initially, the reactor operation was unstable. The gas generation, nitrate and alkalinity destruction and sulfate production were inconsistent. It took approximately two weeks to reach a stable condition, and data recording began. At that time, the initial volume of the reactor contents, and concentrations of $NO_3^- - N$, SO_4^{2-}, and alkalinity were respectively: 4,080 mL, 52.0 mg/L as $NO_3^- - N$, 264.4 mg/L as SO_4^{2-}, and 160.0 mg/L as $CaCO_3$. The initial values of pH, temperature, and gas generation were 6.4, 22 °C, and 25 mL/day.

5.1.2 Alkalinity Destruction, SO_4^{2-} Production, and Gas Generation

A material mass balance analysis was conducted to calculate the concentration of various parameters from day-to-day operation. The concentrations of different constituents in the feed and in the effluent were used in these calculations. The constituents were total alkalinity, $NO_3^- - N$, and SO_4^{2-}. Equation (5.1) was used for mass balance calculations.

$$C = \frac{C_1 \times (V_o - V_1) + C_2 \times V_2}{V_3} \quad \text{...} \quad (5.1)$$

Where:

V_0 = volume of reactor content before sample was withdrawn, mL

V_1 = volume of sample withdrawn, mL

V_2 = volume of raw feed added, mL

V_3 = net volume of total content remaining in the reactor ($V_o - V_1 + V_2$), mL

C = calculated concentration of specific components (alkalinity, nitrate nitrogen, and sulfate) in the reactor after the feed was added, mg/L

C_1 = measured concentration of specific components (alkalinity, nitrate, and sulfate) in the sample withdrawn from the reactor obtained from Table 4.2, mg/L

C_2 = measured concentration of specific component (alkalinity, nitrate, and sulfate) added in the feed obtained from Table 4.1, mg/L

The calculated reactor volume, and the concentrations of $NO_3^- - N$, SO_4^{2-}, and alkalinity in the reactor content after the raw feed was added are summarized in Table 5.1. The calculated alkalinity destruction, sulfate production, $NO_3^- - N$ consumption, and total gas generation are presented in Table 5.2.

Table 5.1 Calculated Volume and Concentrations in the Reactor after Addition of Raw Feed

Consecutive Sampling days	Calculated Content in the Reactor After Addition of Raw Feed, mL	Calculated $NO_3^- - N$ in the Reactor After Addition of Raw Feed, mg/L as $NO_3^- - N$	Calculated SO_4^{2-} in the Reactor After Addition of Raw Feed, mg/L as SO_4^{2-}	Calculated Total Alkalinity in the Reactor After Addition of Raw Feed, mg/L as $CaCO_3$
0	4,080	52.0	264.4	160.0
1	4,135 [a]	59.3 [b]	230.1	205.3
2	4,105	51.6	330.3	160.4
3	4,100	34.9	413.5	111.4
3[c]	4,147	31.4	451.1	111.9
4	4,197	31.0	446.2	335.1
5	4,085	29.6	452.3	302.6
6 [d]	ND	ND	ND	ND
7	4,042	27.7	578.3	241.0
8	3,936	16.3	743.6	171.5
9	4,013	17.5	698.8	172.9
10	4,092	16.5	729.3	172.6
11	4,102	14.8	735.7	168.8
12	4,192	15.7	718.6	180.3
13	4,163	18.1	683	197.2
14	4,134	19.0	673.8	215.6
15	4,109	18.7	682	207.0
16	ND	ND	ND	ND
17	4,189	18.3	688	204.9
18	4,173	13.9	674.3	194.5
19	4,159	13.8	673.9	199.8
20	4,236	15.3	659	206.3
21	4,273	14.3	652.2	215.4

[a] Volume in the reactor = 4,080 mL – 570 mL + 625 mL = 4,135 mL

[b] $$NO_3^- - N, \frac{mg}{L} = \frac{52.0\,\frac{mg}{L} \times (4080\text{ mL} - 570\text{ mL}) + 100\,\frac{mg}{L} \times 625\text{mL}}{4135\text{ mL}} = 59.3\,\frac{mg}{L}$$

[c] The pH of the reactor content decreased suddenly on the third day. Therefore, 50 mL of prepared solution with high concentration of $NaHCO_3$ was added to raise the alkalinity. The $NO_3^- - N$ concentration in the prepared solution was zero, SO_4^{2-} concentration was 39.2 mg/L, and alkalinity was 18,850 mg/L as $CaCO_3$.

[d] No Data. The effluent sample was not withdrawn and reactor was not fed on that day.

Table 5.2 Calculated Alkalinity Destruction, Sulfate Production, $NO_3^- - N$ Consumption, and Gas Generation

Consecutive Sampling Days	$NO_3^- - N$ Reduction, mg/L as $NO_3^- - N$	Total Gas Production, mL	Sulfate Production, mg/L as SO_4^{2-}	Alkalinity Destruction, mg/L as $CaCO_3$
1	15.2 [a]	65	145.2 [b]	91.3 [c]
2	27.4	80	144.9	106.4
3	17.7	50	122.7	71.4
3[d]	ND	ND	ND	ND
4	13.8	43	78.8	60.1
5	12.8	80	135.2	94.6
6[e]	ND	ND	ND	ND
7	23.0	90	263.4	126
8	14.4	50	80.7	75.5
9	16.8	24	161.2	76.9
10	16.0	10	123.7	72.6
11	14.8	34	110.2	62.8
12	11.1	34	71	46
13	15.9	38	122.8	57.2
14	12.7	48	106.8	65.6
15	15.4	40	125.5	71.0
16	ND	ND	ND	ND
17	17.5	61	83.0	68.9
18	13.7	62	99.7	54.5
19	13.8	49	97.8	59.8
20	15.3	57	95.6	50.3
21	14.3	48	122.7	53.4

[a] 59.3 mg/L (Table 5.1) – 44.1 mg/L (Table 4.2) = 15.2 mg/L
[b] 375.3 mg/L (Table 4.2) – 230.1 mg/L (Table 5.1) =145.2 mg/L
[c] 205.3 mg/L (Table 5.1) – 114 mg/L (Table 4.2) = 91.3 mg/L
[d] See foot note “a “ in Table 4.1
[e] See foot note “b” in Table 4.1

5.1.3 Statistical Analyses of Alkalinity Destruction, SO_4^{2-} Production, and Gas Generation with Respect to $NO_3^- - N$ Consumption

The relationships of alkalinity destruction, sulfate production, and gas generation with respect to $\mathrm{NO_3^-} - \mathrm{N}$ removal are analyzed by using simple linear regression. The linear relationship is expressed by Equation (5.2).

$$Y = \beta_o + \beta_1 X + \varepsilon \quad (5.2)$$

Where:

Y = a response variable (such as alkalinity destruction, sulfate production and gas generation)

β_o = the intercept of regression line at the *Y* axis

β_1 = the slope of the regression line (the ratio of responsible variable to independent variable)

X = an independent variable (such as nitrate nitrogen reduction)

ε = the vertical deviation of *Y* value from the regression line

The slope, intercept, standard deviation, variance, R^2, and values of upper and lower 95 percent confidence ratios are provided in Table 5.3. The parameter, R^2, is the square of sample correlation coefficient. It is a unit less measure of the strength of the linear relationship between *X* and *Y* values. The value of R^2 interpret the correlation of two variables as follows:

(1) $R^2 = 1$ indicates a perfect linear correlation.

(2) $R^2 = 0$ indicates that there is no relationship between two variables.

Table 5.3 Summary of Statistical Output for Linear Regression of Alkalinity Destruction, SO_4^{2-} Production, and Gas Generation with Respect to $NO_3^- - N$ Consumption (Batch Reactor)

Statistical Parameters	Linear Regression of Alkalinity Destruction and $NO_3^- - N$ Consumption	Linear Regression of SO_4^{2-} Production and $NO_3^- - N$ Consumption	Linear Regression of Gas Generation and $NO_3^- - N$ Consumption
Slope	3.86	6.94	0.61
Intercept	10.54	10.39	10.9
Standard Deviation	0.93	2.18	0.28
Variance	0.86	4.75	0.078
R^2	0.50	0.37	0.22
Upper Value of Slope at 95% Confidence	5.82	11.54	1.20
Lower Value of Slope at 95% Confidence	1.90	2.35	0.02
Observations	19	19	19

Several basic relationships on alkalinity destruction, sulfate production, and gas production with respect to nitrate nitrogen removal were developed from the results presented in Table 5.3. These relations are summarized below.

(1) The ratio of alkalinity destruction to nitrate nitrogen reduction is 3.86 mg-$CaCO_3$/mg-$NO_3^- - N$.

(2) The ratio of sulfate production to nitrate nitrogen reduction is 6.94 mg-SO_4^{2-}/mg-$NO_3^- - N$.

(3) The ratio of gas generation to nitrate nitrogen reduction is 0.61 mL-gas/mg-$NO_3^- - N$.

5.1.4 Theoretical Volume of Nitrogen Gas Generation under Experimental Condition

The volume of gas produced was measured at laboratory temperature. The theoretical volume of nitrogen gas generated per mg of $NO_3^- - N$ consumed is calculated at the laboratory temperature from Equation (5.3).

$$V = \frac{nRT}{P} \qquad (5.3)$$

Where:

P = atmospheric pressure, atm

V = volume of nitrogen, mL

n = number of moles of gas

R = universal gas constant, 0.082 L-atm/mole.^{o}K

T = temperature, ^{o}K (The average temperature in the reactor was 22 degree C.)

W = weight of nitrogen gas, g

M = nitrogen gas mole molecular weight, g/mole

The number of moles of nitrogen gas per mg of N_2 gas is calculated as follows:

$$n = \frac{1\,\text{mg - N}_2 \times 10^{-3}\ \text{g/mg}}{28\,\text{g/mole of N}_2} = 3.57 \times 10^{-5}\ \text{mole}$$

The volume of nitrogen generated per mg of $\text{NO}_3^- - \text{N}$ consumed is:

$$V = \frac{nRT}{P} = 3.57 \times 10^{-5}\ \text{mole} \times 0.082 \frac{\text{L - atm}}{\text{mole }^{\text{o}}\text{K}} \times \frac{1{,}000\,\text{mL}}{\text{L}} \times 295\ ^{\text{o}}\text{K/1 atm}$$

$$= 0.86\,\text{mL - N}_2\,/\,\text{mg - NO}_3^- - \text{N}$$

The theoretical volume of nitrogen gas generated under experimental condition is 0.86 mL per mg of $\text{NO}_3^- - \text{N}$ consumed. The measured volume of gas generation at laboratory temperature is 0.61 mL-gas/mg- $\text{NO}_3^- - \text{N}$. From these results, it is estimated that nearly 71% of $\text{NO}_3^- - \text{N}$ was reduced to nitrogen gas.

5.2 Continuous Up-Flow Reactor Study

The results of the continuous flow reactor study are used to develop several important relationships. These relationships are: ratios of alkalinity destruction, and sulfate production with respect to $\text{NO}_3^- - \text{N}$ reduction. The stoichiometry of autotrophic denitrification is also developed based on the biologically mediated half reaction equations. An analytical model based on conjugate reaction kinetics was utilized. The model provides a tool to predict the nitrate and nitrite nitrogen concentrations in the effluent from the up-flow column. The procedure is presented below.

5.2.1 Alkalinity Destruction and Sulfate Production

Nitrate removal in the continuous flow sulfur-packed up-flow reactor was accompanied by alkalinity destruction and sulfate generation. As nitrate is reduced, sulfur is oxidized to sulfate producing sulfuric acid. Mineral acid rapidly destroys alkalinity and pH is lowered.

5.2.1.1 Alkalinity Destruction

It has been well established that pH below 6.0 is inhibitory to the biological activity (Baalsrud, 1954; Gauntlett and Craft, 1979). Therefore, sufficient alkalinity must be present in the feed to maintain the desired pH. As mentioned in paragraph 3.3.2, sodium bicarbonate was added in the feed in the ratio of 5 mg/L alkalinity as $CaCO_3$ to 1 mg/L of $NO_3^- - N$ consumed. A plot of alkalinity destruction versus $NO_3^- - N$ removal in samples withdrawn from all ports at three hydraulic loadings and at different $NO_3^- - N$ concentrations in the feed is shown in Figure 5.1.

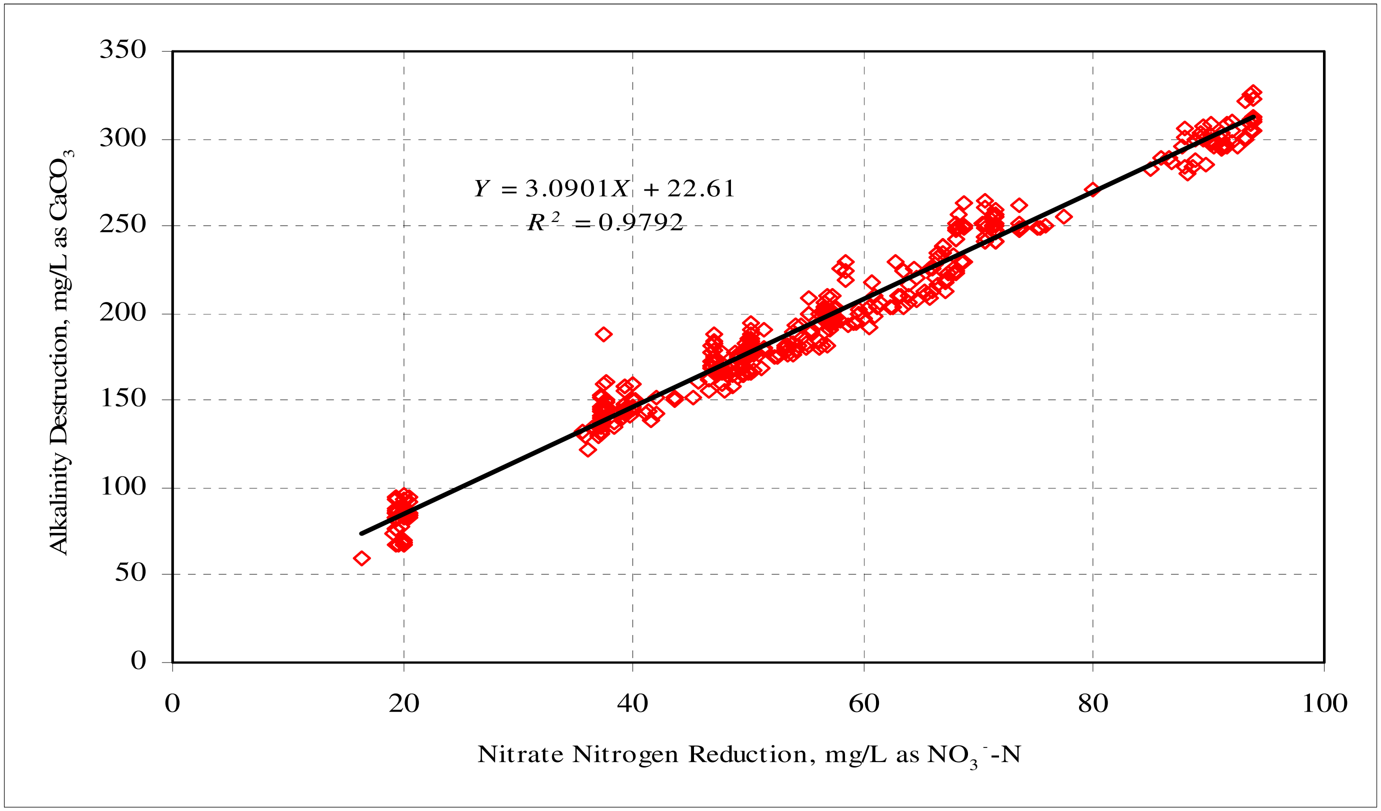


Figure 5.1 Relationship between Alkalinity Destruction and Nitrate Nitrogen Reduction

5.2.1.2 Sulfate Production

The elemental sulfur was packed into the up-flow reactor. The sulfur was used as an electron donor by the autotrophic denitrifiers. As elemental sulfur was oxidized, sulfate ion was produced. A plot of sulfate production versus $NO_3^- - N$ removal in the samples from all ports at three hydraulic loadings and at different $NO_3^- - N$ concentrations in the feed are shown in Figure 5.2.

5.2.2 Statistical Analyses of Alkalinity Destruction, and SO_4^{2-} Production, with Respect to $NO_3^- - N$ Consumption

The relationships of alkalinity destruction, and sulfate production with respect to $NO_3^- - N$ removal are further analyzed by using simple linear regression. The linear relationship is expressed by Equation (5.2). The results are summarized in Table 5.4.

Table 5.4 Summary of Statistical Output for Linear Regression of Alkalinity Destruction, and SO_4^{2-} Production with Respect to $NO_3^- - N$ Consumption (Continuous Flow Reactor)

Statistical Parameters	Linear Regression between Alkalinity Destruction and $NO_3^- - N$ Consumption	Linear Regression between SO_4^{2-} Production and $NO_3^- - N$ Consumption
Slope	3.09	6.91
Intercept	22.61	30.8
Standard Deviation	0.02	0.07
Variance	0.0004	0.0049
R^2	0.98	0.95
Upper Value of Slope at 95% Confidence	3.13	7.05
Lower Value of Slope at 95% Confidence	3.05	6.76
Observations	492	492

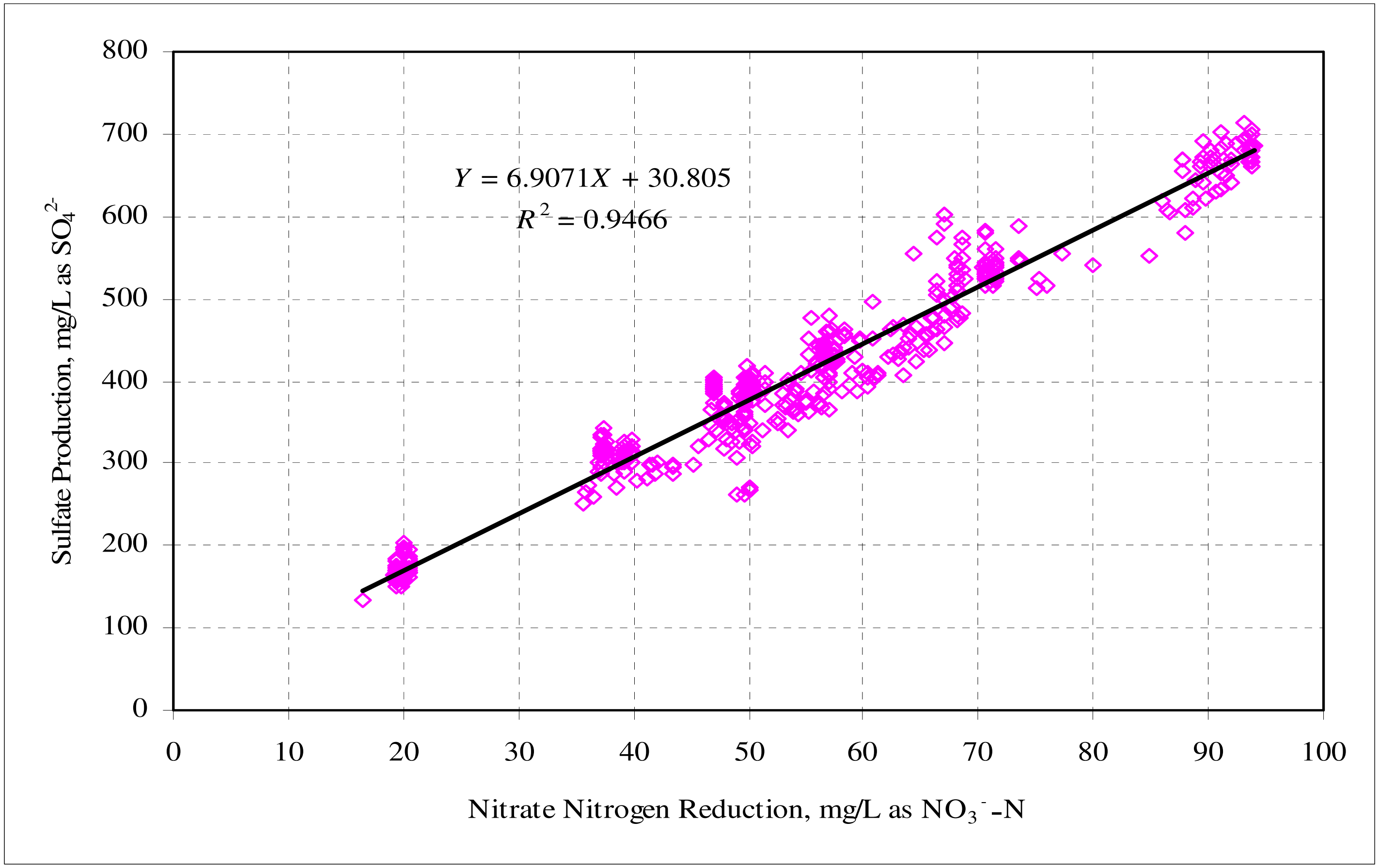


Figure 5.2 Relationship between Sulfate Production and Nitrate Nitrogen Reduction

The relationship between alkalinity destruction and nitrate nitrogen removal showed a linear relationship with an R^2 of 0.98. The R^2 value close to 1 implied a perfect linear relationship. The slope of the line indicates that 3.09 mg/L alkalinity as $CaCO_3$ was destroyed per mg/L of nitrate nitrogen removal. This ratio in the batch reactor study was 3.86. The intercept indicates that there is an initial 22.61 mg/L alkalinity destruction which could be attributed to the DO removal and other unknown factors. This topic is discussed further in Section 5.2.4. The small standard deviation, variance, interval between lower 95% confidence and upper 95% confidence, and high number of observations indicate that the value of slope is reliable and truly representative.

The relationship between sulfate production and nitrate nitrogen removal showed a linear relationship with an R^2 of 0.95. The R^2 value close to one implied a high linear relationship. The slope of the linear line is 6.91 indicating that 6.91 mg/L SO_4^{2-} is generated per mg/L of nitrate nitrogen removed. The sulfate generation ratio in the batch reactor study was 6.94. The intercept indicates that 30.81 mg/L of SO_4^{2-} is initially generated. The initial sulfate generation may be attributed to deoxygenation of raw feed and other unknown factors (see Section 5.2.4 for additional details). The small standard deviations, variance, intervals between lower and upper 95% confidence, and high number of observations indicate that the value of slope is reliable and truly representative.

5.2.3 Stoichiometry of Autotrophic Denitrification

Sawyer and McCarty (1978) reported that most of biologically mediated reactions are composed of three oxidation-reduction half reactions. These reactions are:

(1) an electron donor reaction, R_d, (2) a terminal electron acceptor reaction, R_a, and (3) a cell synthesis reaction, R_c, The combined overall equation is expressed by equation (5.4).

$$R = f_s R_c + f_e R_a - R_d \qquad (5.4)$$

Where:

$$f_e + f_s = 1 \qquad (5.5)$$

R = balanced overall reaction

f_s = proportion of energy used for cell synthesis

f_e = proportion of energy used to maintain life requirement

R_a = half reaction for electron acceptor

R_c = half reaction for cell synthesis

R_d = half reaction for electron donor

5.2.3.1 Half Reaction Equations

A list of useful half reactions for bacterial system has been given by Sawyer and McCarty, 1978. These reactions are provided in Appendix B. In this research, elemental sulfur serves as an electron donor. In the half reactions, it is assumed that the electron acceptor is either oxygen or nitrate, although in reality electrons are also consumed in cell synthesis. Likewise, in the half reactions for cell synthesis, ammonia or nitrate could be the source for nitrogen. Each alternative is derived from half reactions given by Sawyer and McCarty, 1978, and is expressed by equations (5.6) – (5.10).

(1) Oxygen as an electron acceptor

$$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O \qquad (5.6)$$

(2) Nitrate as an electron acceptor

$$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O \qquad (5.7)$$

(3) Sulfur as an electron donor

$$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^- = \frac{1}{6}S + \frac{2}{3}H_2O \qquad (5.8)$$

(4) Cell synthesis with ammonia as a nitrogen source

$$\begin{aligned}&\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- \\ &= \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O\end{aligned} \qquad (5.9)$$

(5) Cell synthesis with nitrate as a nitrogen source

$$\begin{aligned}&\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^- \\ &= \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O\end{aligned} \qquad (5.10)$$

5.2.3.2 Overall Reaction Equations

The overall reactions are developed from equation (5.4) for two cases: (1) electron acceptor being oxygen, and (2) electron acceptor being nitrate. Ammonia or nitrate nitrogen could be a source of nitrogen for cell synthesis in both cases. These relationships are given below.

(1) Oxygen – an electron acceptor

(a) Ammonia – a nitrogen source for cell synthesis

The stoichiometry of autotrophic denitrification is developed by substituting equations (5.5), (5.6), (5.8), and (5.9) into equation (5.4). The stoichiometry is expressed by equation (5.11).

$$\begin{aligned}&\frac{1-f_s}{4}O_2+\frac{1}{6}S+\frac{f_s}{5}CO_2+\frac{f_s}{20}NH_4^+ +\frac{f_s}{20}HCO_3^- +(\frac{10+3f_s}{60})H_2O\\&=\frac{f_s}{20}C_5H_7O_2N+\frac{1}{6}SO_4^{2-}+\frac{1}{3}H^+\end{aligned} \quad \text{...........} \quad (5.11)$$

(b) Nitrate – a nitrogen source for cell synthesis

The stoichiometry of autotrophic denitrification is developed by substituting equations (5.5), (5.6), (5.8), and (5.10) into equation (5.4). The stoichiometry is expressed by equation (5.12).

$$\begin{aligned}&\frac{1-f_s}{4}O_2+\frac{1}{6}S+(\frac{f_s}{28})NO_3^- +\frac{5f_s}{28}CO_2+(\frac{14+9f_s}{84})H_2O\\&=\frac{f_s}{28}C_5H_7O_2N+\frac{1}{6}SO_4^{2-}+(\frac{28-3f_s}{84})H^+\end{aligned} \quad \text{............................} \quad (5.12)$$

(2) Nitrate – an electron acceptor

(a) Ammonia – a nitrogen source for cell synthesis

The stoichiometry of autotrophic denitrification is developed by substituting equations (5.5), and (5.7) – (5.9) into equation (5.4). The stoichiometry is expressed by Equation (5.13).

$$\begin{aligned}&\frac{1-f_s}{5}NO_3^- + \frac{1}{6}S + \frac{f_s}{5}CO_2 + \frac{f_s}{20}NH_4^+ + \frac{f_s}{20}HCO_3^- + (\frac{4+9f_s}{60})H_2O \\ &= \frac{f_s}{20}C_5H_7O_2N + \frac{1-f_s}{10}N_2 + \frac{1}{6}SO_4^{2-} + (\frac{2}{15}+\frac{f_s}{5})H^+\end{aligned} \quad \text{...........} \quad (5.13)$$

(b) Nitrate – a nitrogen source for cell synthesis

The stoichiometry of autotrophic denitrification is developed by substituting equations (5.5), (5.7), (5.8), and (5.10) into equation (5.4). The stoichiometry is expressed by equation (5.14).

$$\begin{aligned}&(\frac{28-23f_s}{140})NO_3^- + \frac{1}{6}S + \frac{5f_s}{28}CO_2 + (\frac{87f_s+28}{420})H_2O \\ &= \frac{f_s}{28}C_5H_7O_2N + \frac{1-f_s}{10}N_2 + \frac{1}{6}SO_4^{2-} + (\frac{69f_s+56}{420})H^+\end{aligned} \quad \text{..............................} \quad (5.14)$$

5.2.3.3 Application of Experimental Data to Determine Overall Stoichiometric Equation Coefficients

The stoichiometric coefficients of overall equation of autotrophic denitrification are developed by comparing the experimental data for the ratio of sulfate production and nitrate nitrogen reduction. In autotrophic denitrification process, nitrate is used as an electron acceptor. The overall equations are developed for two alternatives: ammonia or nitrate being a nitrogen source for cell synthesis. Both alternatives are presented below.

(1) Ammonia – a nitrogen source for cell synthesis

The ratio of sulfate production and nitrate nitrogen destruction is 6.91 (Figure 5.2). By substituting 6.91 equal to the stoichiometric ratio of sulfate production to nitrate destruction in equation (5.13), the value of f_s is calculated.

$$\frac{\frac{1}{6}\mathrm{SO_4^{2-}}}{\frac{1-f_s}{5}\mathrm{NO_3^-}} = 6.91$$

$$\frac{\frac{1}{6}\,\text{mole}\,\mathrm{SO_4^{2-}} \times \frac{96\,\text{g}\,\mathrm{SO_4^{2-}}}{\text{mole}\,\mathrm{SO_4^{2-}}}}{\frac{1\text{-}f_s}{5}\,\text{mole}\,\mathrm{NO_3^-} \times \frac{14\,\text{g}\,\mathrm{NO_3^-}}{\text{mole}\,\mathrm{NO_3^-}}} = 6.91$$

$$f_s = 0.17$$

From Equation (5.5), $f_e = 1 - f_s = 1 - 0.17 = 0.83$

By substituting f_s = 0.17 into equation (5.13), the stoichiometry of autotrophic denitrification using granular sulfur as an electron donor, nitrate as an electron acceptor, and ammonia as the nitrogen source for cell synthesis is obtained. The result is shown by equation (5.15).

$$\begin{aligned} &1.0NO_3^- + 1.0S + 0.2CO_2 + 0.05NH_4^+ + 0.05HCO_3^- + 0.55H_2O \\ &\Rightarrow 0.05C_5H_7O_2N + 0.5N_2 + 1.0SO_4^{2-} + 1.0H^+ \end{aligned} \quad \text{..................} \quad (5.15)$$

(2) Nitrate – a nitrogen source for cell synthesis

The ratio of sulfate production and nitrate nitrogen destruction is 6.91 (Figure 5.2). By substituting 6.91 equal to the stoichiometric ratio of sulfate production to nitrate destruction in equation (5.14), the value of f_s is calculated.

$$\frac{\frac{1}{6}\mathrm{SO_4^{2-}}}{\frac{28-23f_s}{140}\mathrm{NO_3^-}} = 6.91$$

$$\frac{\frac{1}{6}\,\mathrm{mole\,SO_4^{2-}} \times \frac{96\,\mathrm{g\,SO_4^{2-}}}{\mathrm{mole\,SO_4^{2-}}}}{\frac{28-23f_s}{140}\,\mathrm{mole\,NO_3^-} \times \frac{14\,\mathrm{g\,NO_3^-}}{\mathrm{mole\,NO_3^-}}} = 6.91$$

$$f_s = 0.21$$

From Equation (5.5), $f_e = 1 - f_s = 1 - 0.21 = 0.79$

By substituting f_s = 0.21 into equation (5.14), the stoichiometry of autotrophic denitrification using granular sulfur as an electron donor, nitrate as an electron acceptor, and nitrate as the nitrogen source for cell synthesis is obtained. The result is expressed in equation (5.16).

$$\begin{aligned} &1.0NO_3^- + 1.0S + 0.226CO_2 + 0.665H_2O \\ &\Rightarrow 0.045C_5H_7O_2N + 0.477N_2 + 1.0SO_4^{2-} + 1.0H^+ \end{aligned} \quad \text{.....................................} \quad (5.16)$$

5.2.4 Initial Alkalinity Destruction, and Sulfate Production Due to DO Removal

The dissolved oxygen (DO) in the feed was rapidly removed by facultative microorganisms accompanied by alkalinity destruction, and sulfate generation. Based on the half reactions using oxygen as an electron acceptor and elemental sulfur as an electron donor, the theoretical initial alkalinity destruction and sulfate production due to DO removal is calculated. The nitrogen source for cell synthesis may be either ammonia or nitrate nitrogen. Both alternatives are described below.

5.2.4.1 Initial Alkalinity Destruction

(1) Ammonia – a nitrogen source for cell synthesis

The alkalinity destruction is due to the hydrogen ion production which consumes carbonate and bicarbonate. The consumption of one mole/L of HCO_3^- is equivalent to the reduction of one equivalent/L of alkalinity. The calculation procedure is shown below.

Equation (5.11) shows that 1/3 mole of $[H^+]$ is produced, and $f_s/20$ mole of HCO_3^- is consumed. Sawyer and McCarty, 1978, provided a list of $f_{s(\max)}$ which is given in Table B1 of Appendix B. Assuming $f_{s(\max)} = 0.21$, the theoretical initial alkalinity destruction due to deoxygenation is calculated below.

$$\frac{\text{Alkalinity Destruction}}{\text{DO Removal}} = \frac{\frac{1}{3}\text{H}^+ + \frac{f_s}{20}\text{HCO}_3^-}{(\frac{1-f_s}{4})\,\text{O}_2}$$

$$= \frac{(\frac{1}{3} \times \frac{\text{eq. H}^+}{\text{mole H}^+} + \frac{0.21}{20} \times \frac{1\,\text{eq. HCO}_3^-}{\text{mole HCO}_3^-}) \times 50 \frac{\text{g CaCO}_3}{\text{eq CaCO}_3}}{\frac{1-0.21}{4} \times \frac{32\,\text{g O}_2}{\text{mole O}_2}}$$

$$= 2.72 \frac{\text{g CaCO}_3}{\text{g O}_2}$$

$$= 2.72 \frac{\text{mg as CaCO}_3}{\text{mg as O}_2}$$

The influent DO in the feed averaged around 8.5 mg/L. The theoretical alkalinity consumption therefore is 2.72 x 8.5 mg/L = 23.12 mg/L as $CaCO_3$. The intercept of the linear line shown in Figure 5.1 is 22.61 mg/L as $CaCO_3$. The intercept of the linear line expressed by the alkalinity consumed versus nitrate nitrogen reduced is equivalent to the alkalinity consumed due to oxygen consumed in the feed.

(2) Nitrate – a nitrogen source for cell synthesis

Equation (5.12) shows that $(28 - 3f_s)/84$ mole of $[H^+]$ is produced for deoxygenation of feed. Sawyer and McCarty, 1978, provided a list of $f_{s(\max)}$ (Table B1 of Appendix B). Assuming $f_{s(\max)} = 0.21$, the theoretical initial alkalinity destruction due to deoxygenation is calculated below.

$$\frac{\text{Alkalinity Destruction}}{\text{DO Removal}} = \frac{\frac{(28-3f_s)}{84} \text{H}^+}{(\frac{1-f_s}{4}) \text{O}_2}$$

$$= \frac{\left(\frac{(28 - 3\times 0.21)}{84} \times \frac{\text{eq. H}^+}{\text{mole H}^+}\right) \times 50 \frac{\text{g CaCO}_3}{\text{eq CaCO}_3}}{\frac{1 - 0.21}{4} \times \frac{32\text{ g O}_2}{\text{mole O}_2}}$$

$$= 2.58 \frac{\text{g CaCO}_3}{\text{g O}_2}$$

$$= 2.58 \frac{\text{mg CaCO}_3}{\text{mg O}_2}$$

The influent DO in the feed averaged around 8.5 mg/L. The theoretical alkalinity consumption therefore is 2.58 x 8.5 mg/L = 21.93 mg/L as $CaCO_3$. This value is close to 22.61 mg/L alkalinity as $CaCO_3$ indicated by the intercept of linear line shown in Figure 5.1.

5.2.4.2 Initial Sulfate Production

Both equations (5.11) & (5.12) show that 1/6 mole of sulfate is produced, and (1 -f_s)/4 mole of O_2 is consumed. Assuming $f_{s(\max)}$ = 0.21 (Table B1 of Appendix B), the theoretical initial sulfate production due to deoxygenation is calculated below.

$$\frac{\text{Sulfate Production}}{\text{DO Removal}} = \frac{\frac{1}{6}\text{SO}_4^{2-}}{\frac{1 - f_s}{4}\text{O}_2} = \frac{\frac{1}{6}\text{mole SO}_4^{2-} \times \frac{96\text{ g SO}_4^{2-}}{\text{mole SO}_4^{2-}}}{\frac{1 - 0.21}{4}\text{mole O}_2 \times \frac{32\text{ g O}_2}{\text{mole O}_2}} = 2.53 \frac{\text{mg as SO}_4^{2-}}{\text{mg as O}_2}$$

The influent DO in the feed averaged around 8.5 mg/L. The theoretical sulfate consumption therefore is 2.53 x 8.5 mg/L = 21.5 mg/L as SO_4^{2-}. This value is close to 30.81 mg/L as SO_4^{2-} indicated by the intercept of linear line shown in Figure 5.2.

5.2.5 Data Modeling of Sulfur-Packed Continuous Up-Flow Reactor

The data developed by the continuous up-flow reactor are used to develop and calibrate the nitrate-nitrite-nitrogen reduction equations. The reactions involving autotrophic nitrate reduction in a sulfur-packed up-flow column is truly a consecutive reaction. In consecutive reactions, the product of one reaction step becomes the reactant of the subsequent reaction steps. The reduction of nitrate to nitrite and then to nitrogen gas is expressed by Equation (5.17). The pathway from nitrite to nitrogen gas proceeds via two intermediate forms of nitrogen: nitric oxide (NO) and nitrous oxide (N_2O).

$$NO_3^- \xrightarrow[k_1]{} NO_2^- \xrightarrow[k_2]{} N_2 \quad \text{......} \quad (5.17)$$

In this reaction, reactant nitrate is reduced to product NO_2^- at a rate determined by reaction rate constant k_1. Product nitrite (NO_2^-) becomes the reactant for the second step, and is finally reduced to nitrogen gas at an overall reaction rate constant k_2. The intermediate products are nitric oxide gas (NO) that is further reduced to nitrous oxide gas (N_2O). Because nitric oxide and nitrous oxide are present in gaseous forms and the gas components were not analyzed in the continuous flow study, the intermediate gas products of nitric oxide and nitrous oxide are ignored, and nitrogen gas is assumed to be the final end product. If the rates of consecutive reactions from nitrate to nitrite and then nitrogen are assumed first-order, then the differential equations that describe the reduction of $\mathrm{NO_3^- - N}$ to $\mathrm{NO_2^- - N}$ and formation of N_2 are expressed by Equations (5.18) to (5.20).

$$\frac{-dC_{NO_3^- - N}}{dt} = k_1 C_{NO_3^- - N} \quad (5.18)$$

$$\frac{dC_{NO_2^- - N}}{dt} = k_1 C_{NO_3^- - N} - k_2 C_{NO_2^- - N} \quad (5.19)$$

$$\frac{dC_{N_2}}{dt} = k_2 C_{NO_2^- - N} \quad (5.20)$$

The solutions of these differential equations provide the concentrations of constituents at any time *t* as expressed by Equations (5.21) to (5.23).

$$C_{NO_3^- - N} = C^o_{NO_3^- - N} e^{-k_1 t} \quad (5.21)$$

$$C_{NO_2^- - N} = \frac{k_1 C^o_{NO_3^- - N}}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + C^o_{NO_2^- - N} e^{-k_2 t} \quad (5.22)$$

$$C_{N_2} = \frac{C^o_{NO_3^- - N}(1 - k_2 e^{-k_1 t} - k_1 e^{-k_2 t})}{k_2 - k_1} + C^o_{NO_2^- - N}(1 - e^{-k_2 t}) + C^o_{N_2} \quad (5.23)$$

Where:

$C_{NO_3^- - N}$ = concentrations of $NO_3^- - N$ at any time t, mg/L

$C_{NO_2^- - N}$ = concentrations of $NO_2^- - N$ at any time t, mg/L

C_{N_2} = concentrations of N_2 at any time t, mg/L

$C^o_{NO_3^- - N}$ = concentrations of $NO_3^- - N$ at $t = 0$, mg/L

$C^o_{NO_2^- - N}$ = concentrations of $NO_2^- - N$ at $t = 0$, mg/L

$C^o_{N_2}$ = concentrations of N_2 at $t = 0$, mg/L

k_1 = reaction rate constant for $NO_3^- - N$ reduction, h^{-1}

k_2 = reaction rate constant for $NO_2^- - N$ reduction, h^{-1}

t = true hydraulic retention time, h

The results of the sulfur-packed continuous up-flow reactor are used to calibrate the nitrate reduction expressed by equations (5.21) to (5.23). The reaction rate constants k_1 and k_2 are estimated to best fit the experimental data.

The experimental data representing the ratios of effluent and influent $NO_3^- - N$ concentrations (C/C_o) at six sampling ports are plotted against the true hydraulic retention time (THRT). As described in Chapter 3, THRT is the total void volume divided by the influent flow rate. The THRT at three hydraulic loadings and at each sampling ports are provided in Table 3.4. The results of C/C_o versus THRT for

$NO_3^- - N$ at three hydraulic loadings and different initial $NO_3^- - N$ concentrations are shown in Figures 5.3 to 5.5. Finally, the data points of three figures are combined into a single line by averaging all data points corresponding to the plotting THRT. This curve is shown in Figure 5.6. The plot of $NO_3^- - N$ reduction clearly follows the classical first order reaction rate.

5.2.5.1 Determination of Nitrate Reaction Rate Constant k_1

The reaction rate constant k_1 is determined using the classical methods that have been applied for the first order BOD stabilization. These methods are Thomas, Least Square, Fujimoto and others (Metcalf and Eddy, 2003). In this study, Fujimoto method is applied to determine the reaction rate constants k_1 and $(1 - C/C_o)$ from the experimental data. The procedure is presented in Appendix G (Table G1 and Figure G1). The average value of k_1 and the ultimate value of $(1 - C/C_o)$ are 1.41/hr and 97.6 percent.

5.2.5.2 Determination of Nitrite Reaction Rate Constant k_2

$NO_2^- - N$ was measured at many sampling ports. Presence of $NO_2^- - N$ is an indication of incomplete reduction of nitrate. At lower sampling ports and at higher $NO_3^- - N$ loadings, the $NO_2^- - N$ concentration was quite high. Figure 5.6 indicates that the average $NO_2^- - N$ production reached 11 percent of the initial $NO_3^- - N$ concentration in the feed at THRT of 1.2 hr. The $NO_2^- - N$ concentration decreases gradually at higher THRT.

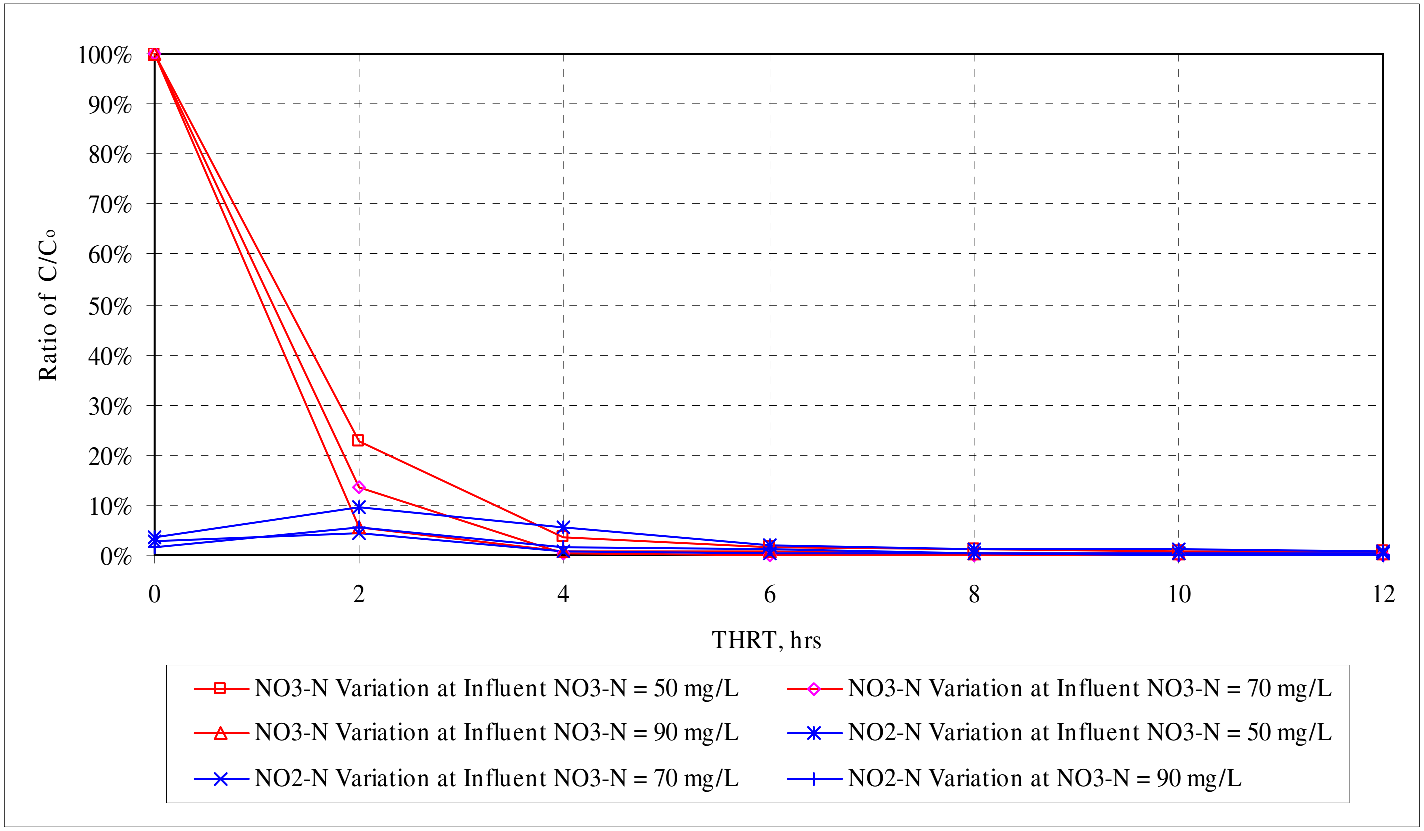


Figure 5.3 Relationship between C/C$_o$ and THRT at Hydraulic Loading of 0.06 m^3/m^2.hr

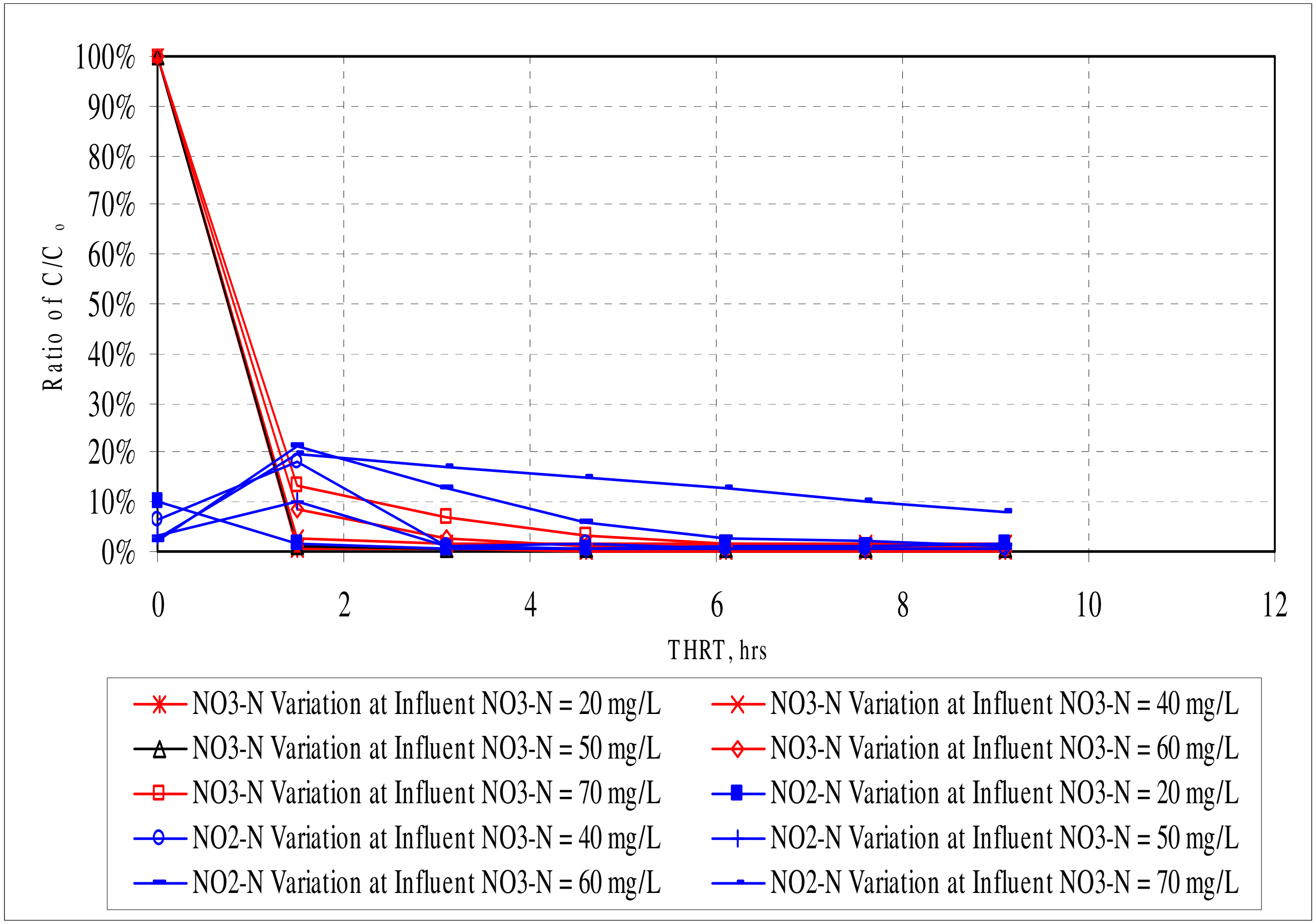


Figure 5.4 Relationship between C/C_o and THRT at Hydraulic Loading of 0.08 m^3/m^2.hr

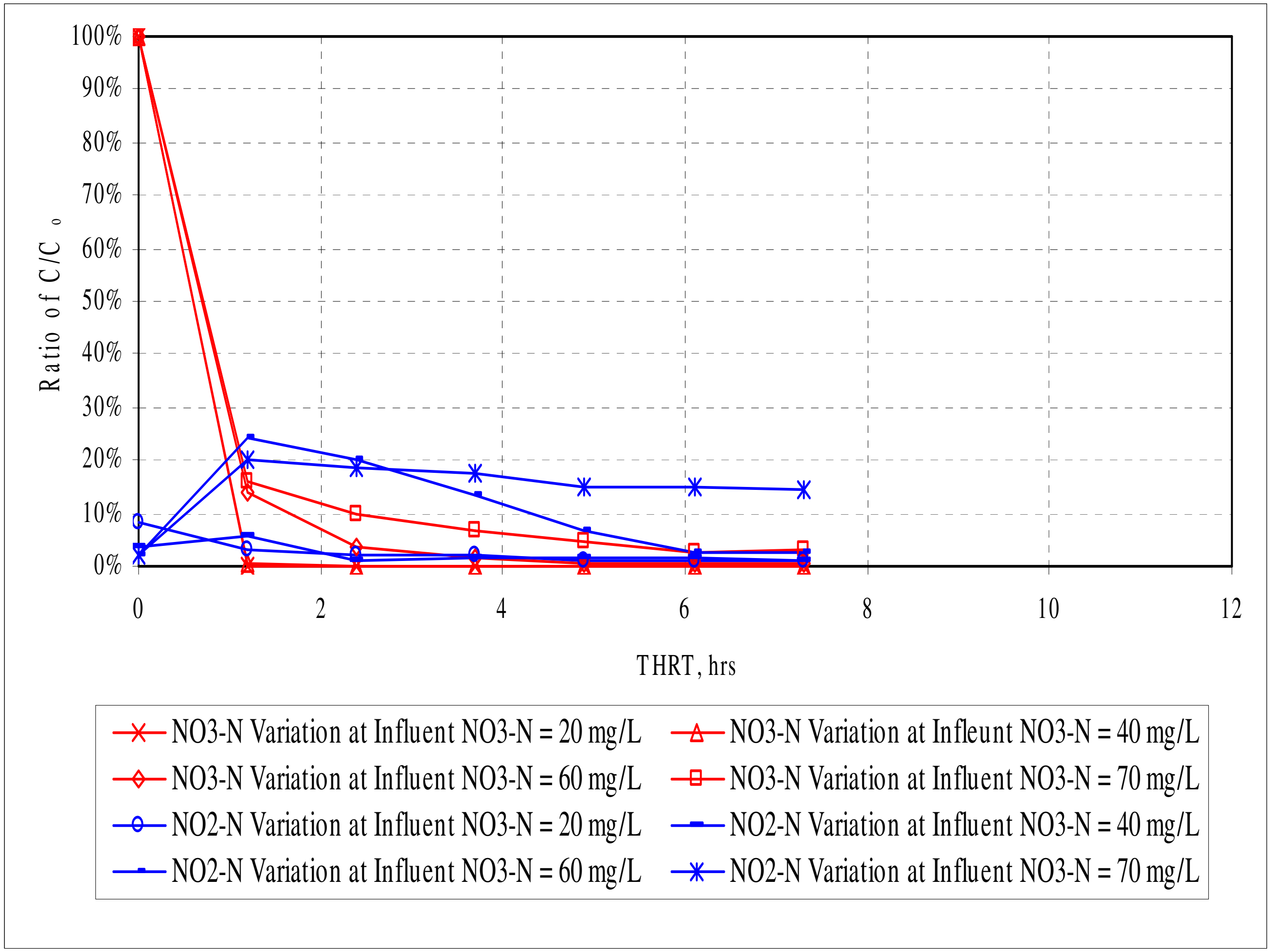


Figure 5.5 Relationship between C/C_o and THRT at Hydraulic Loading of 0.10 m^3/m^2.hr

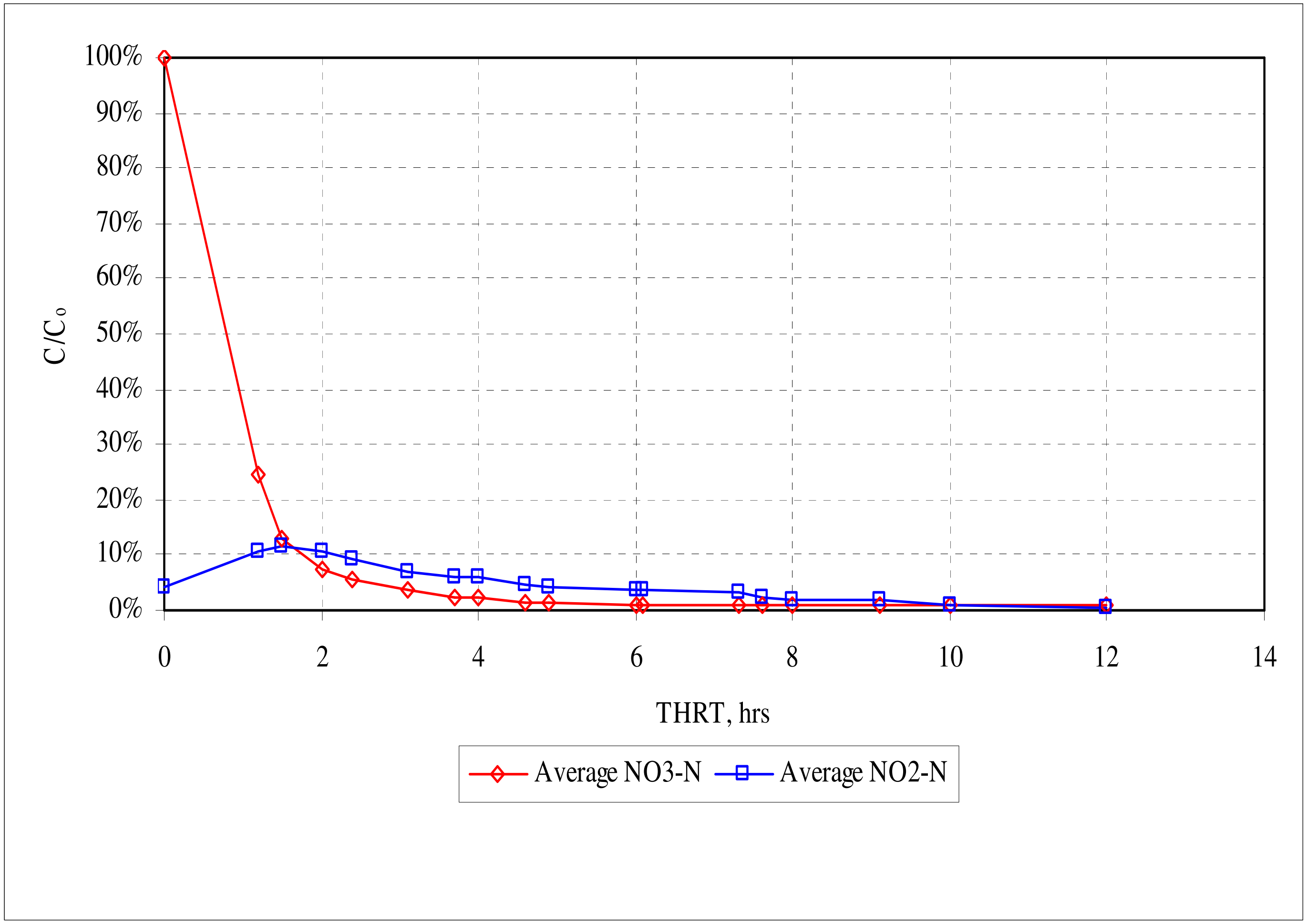


Figure 5.6 Relationship between Average C/C$_o$ and THRT at Three Combined Hydraulic Loadings

The value of reaction rate constant k_2 is obtained by a trial and error procedure. Different values of k_2 are substituted in equation (5.22) to obtain C/C_o ratios closest to the experimental curve shown in Figure 5.6. A value of k_2 equal to 3.15/hr provided the best data fit. In Figure 5.7, the $NO_3^- - N$ and $NO_2^- - N$ ratios from experimental data points, and smooth curves from equations (5.18) and (5.19) using derived k_1 and k_2 are compared. The results of model prediction are close to the experimental data. Because nitrogen production was not measured in the continuous flow reactor study, the generation rate is predicted from Equation (5.23).

5.2.5.3 Sensitivity Analysis of the Model

The sensitivity of the model is dependent upon the reaction rate constants k_1 and k_2. The $NO_3^- - N$ and $NO_2^- - N$ profiles are prepared by assuming different k_1 and k_2 values and then comparing them with the best fit profiles shown in Figure 5.7 (k_1 = 1.41/hr and k_2 = 3.15/hr). In this investigation the sensitivity analysis was performed by varying the values of one parameter 50% higher and 50% lower than the optimum value while keeping the other parameter constant at optimum value. The optimum values of the constants are those that gave the best experimental data fit (Figure 5.7). The steps involved in the sensitivity analysis are shown below.

Constant k_1 = 1.41/hr k_2 changed from 3.15/hr	k_2 = 4.73/hr, 50% higher
	k_2 = 1.58/hr, 50% lower
Constant k_2 = 3.15/hr k_1 changed from 1.41/hr	k_1 = 2.11/hr, 50% higher
	k_1 = 0.71/hr, 50% lower

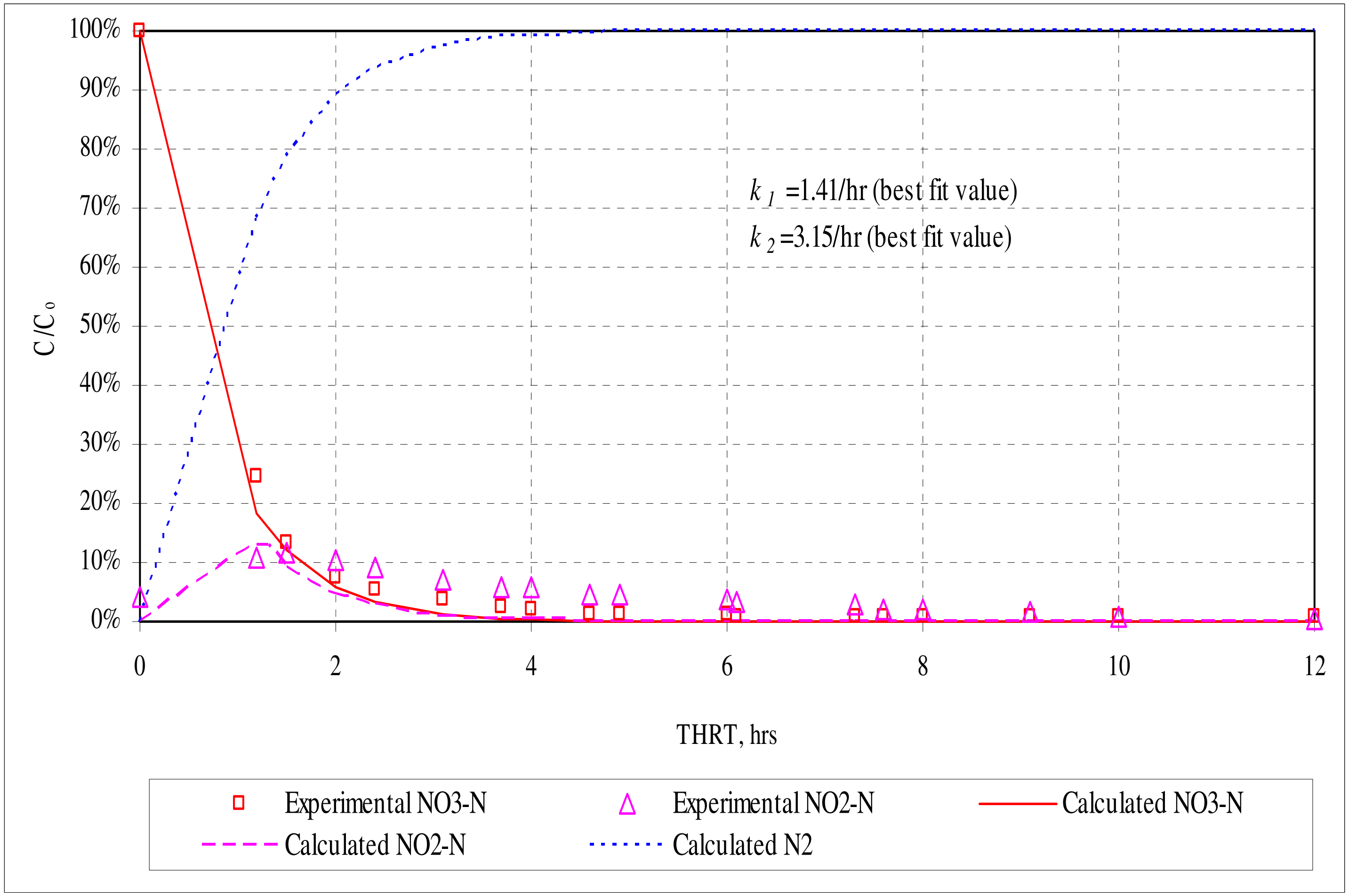


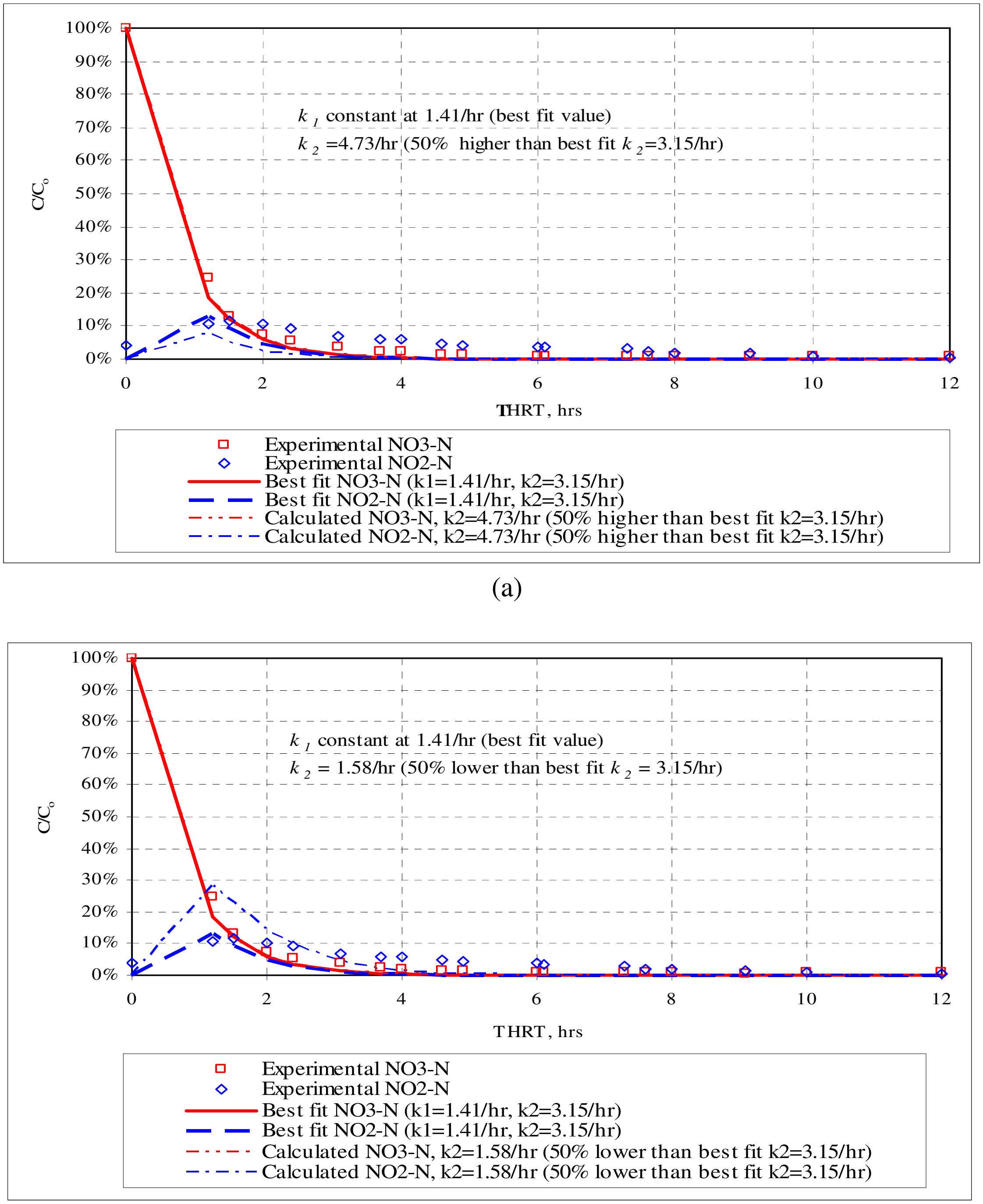
Figure 5.7 Comparison of Average C/C$_o$ from Experimental Result and Calculated C/C$_o$ from Derived k_1 and k_2

The results of sensitivity analysis are illustrated in Figures 5.8 and 5.9. The key results are summarized below.

(1) The $NO_2^- - N$ concentrations are sensitive to change in reaction rate constant k_2. As k_2 value is increased over the optimum k_2, the model predicts lower $NO_2^- - N$ concentration (Figure 5.8(a)). Alternatively, as k_2 is decreased from the optimum k_2, the model predicts significantly higher $NO_2^- - N$ concentration (Figure 5.8(b)). The largest change however, occurs at THRT of around 1.2 hr.

(2) The $NO_3^- - N$ concentrations are sensitive to the change in reaction rate constant k_1. As k_1 value is increased over the optimum k_1, the model predicts lower $NO_2^- - N$ concentration (Figure 5.9(a)). The $NO_3^- - N$ concentrations are very sensitive to the decrease in reaction rate constant k_1. It shows a significant rise at THRT of around 1.2 hr (Figure 5.9(b)). The change of $NO_3^- - N$ concentrations is much less with the increase in k_1.

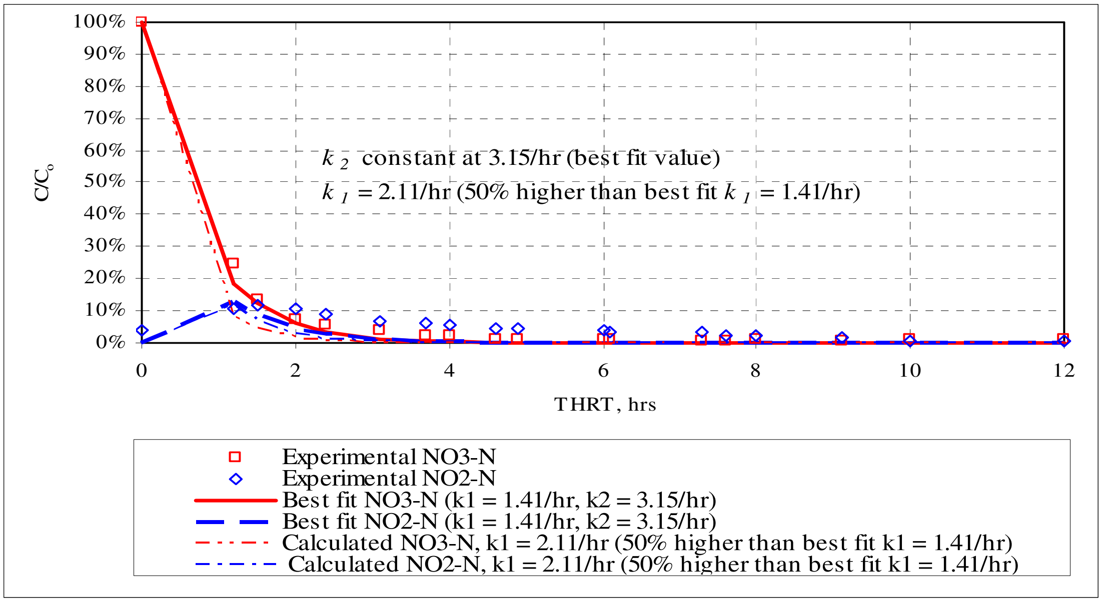
(3) The concentration of both $NO_3^- - N$ and $NO_2^- - N$ are very sensitive to the change in k_1 and k_2 at THRT of around 1.2 hr. As the THRT is increased, the results become less sensitive to the change in reaction rate constants k_1 and k_2. At THRT of 4.0 hr, concentrations of $NO_3^- - N$ and $NO_2^- - N$ approach zero.



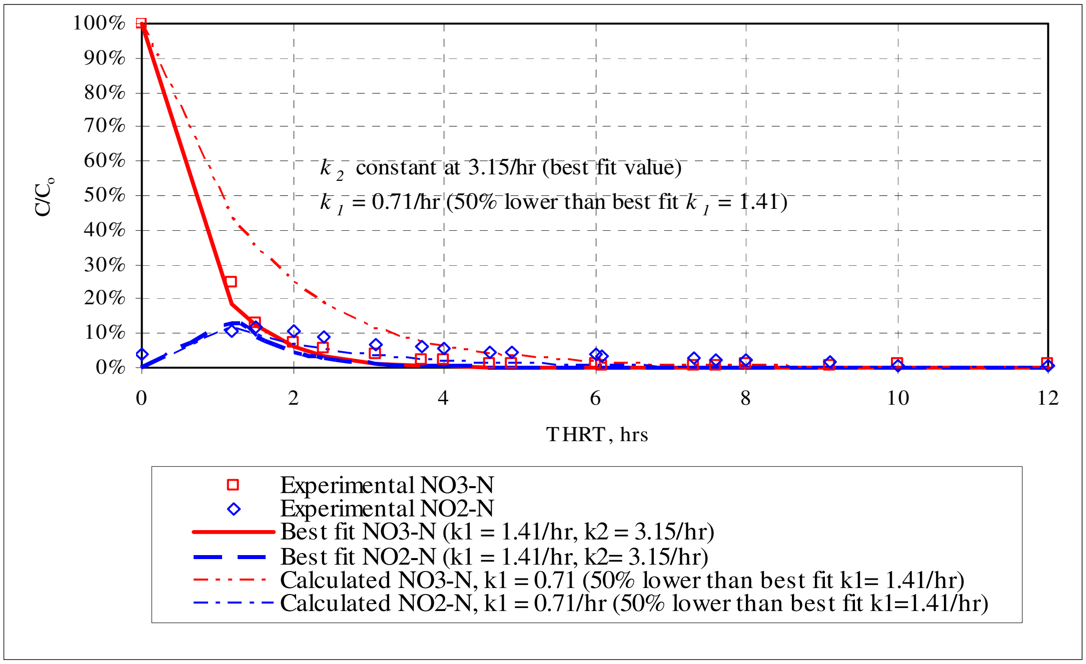
(a)

(b)

Figure 5.8 Sensitivity Analysis and Concentration Profiles (a) k_1 Constant at 1.41/hr (best fit value) and k_2 = 4.73/hr (50% higher than the best fit k_2 = 3.15/hr), and (b) k_1 Constant at 1.41/hr (best fit value) and k_2 = 1.58/hr (50% lower than the best fit k_2 = 3.15/hr)



(a)



(b)

Figure 5.9 Sensitivity Analysis and Concentration Profiles (a) k_2 Constant at 3.15/hr (best fit value) and k_1 = 2.11/hr (50% higher than the best fit k_1 = 1.41/hr), and (b) k_2 Constant at 3.15/hr (best fit value) and k_1 = 0.71/hr (50% lower than the best fit k_1 = 1.41/hr)

5.2.6 Design Example

A design example is presented in this section to illustrate the procedure for designing an up-flow sulfur packed column to remove nitrate from a groundwater supply source. The average nitrate nitrogen concentration is 50 mg/L, and average flow is 0.5 mgd. The finished water quality must meet the Safe Drinking Water Quality Standards; the MCLs for $NO_3^- - N$ and $NO_2^- - N$ below 10 mg/L and 1 mg/L respectively. Determine the dimension of the sulfur-packed up-flow reactor.

(A) Design Criteria Used

The design criteria for the sulfur-packed up-flow column.

Design flow =	0.5 mgd = 1,893 m^3/d
Reactor type =	up-flow granular sulfur-packed column
Raw water $NO_3^- - N$ concentration =	50 mg/L
Raw water $NO_2^- - N$ concentration =	0 mg/L
Finished water $NO_3^- - N$ concentration ≤	10 mg/L
Finished water $NO_2^- - N$ concentration ≤	1 mg/L

(B) Solution

1. Determine the true hydraulic detention time (THRT)

THRT is obtained from equation (5.21)

$$\frac{C_{NO_3^--N}}{C^o_{NO_3^--N}} = e^{-k_1 t}$$

$$\frac{C}{C^o} = \frac{\mathrm{NO_3^-} - \text{N concentration in the finished water}}{\mathrm{NO_3^-} - \text{N concentration in raw water}} = \frac{10\ \text{mg/L}}{50\ \text{mg/L}} = 0.2$$

Use the reaction rate constants k_1 = 1.41/hr and k_2 = 3.15/hr to achieve optimum solution

$$0.2 = \mathrm{e}^{(\frac{-1.41}{\mathrm{hr}}) \times t}$$

t = 1.14 hr

2. Check the nitrite nitrogen concentration in treated water

Ratio of nitrite nitrogen concentration is obtained from equation (5.22)

$$C_{NO_2^--N} = \frac{k_1 C^o_{NO_3^--N}}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + C^o_{NO_2^--N} e^{-k_2 t}$$

$$\begin{aligned} C_{NO_2^--N} &= \frac{1.41 \times 100\%}{3.15 - 1.41}(e^{-1.41/\mathrm{hr} \times 1.14\mathrm{hr}} - e^{-3.15/\mathrm{hr} \times 1.14\mathrm{hr}}) + 0\% \times e^{-3.15/\mathrm{hr}\, x\, 1.14\mathrm{hr}} \\ &= 0.14 \times 100\% \\ &= 14\% \end{aligned}$$

$$\begin{aligned} \text{Concentration of nitrite nitrogen} &= 14\% \times C^o_{NO_3^--N} \\ &= 14\% \times 50 \frac{\mathrm{mg}}{\mathrm{L}} \\ &= 7.0 \frac{\mathrm{mg}}{\mathrm{L}} > 1 \frac{\mathrm{mg}}{\mathrm{L}} \end{aligned}$$

Reiterate the above calculations until the nitrite nitrogen concentration in the finished water $\leq$ 1 mg/L. The results of iterative solution are summarized below.

$NO_3^- - N$, mg/L	THRT, hrs	$NO_2^- - N$, mg/L
10	1.14	7.0
5	1.63	3.8
2.5	2.12	1.99
1.5	2.49	1.19
1.25	2.62	1.00

3. Determine the dimensions of the up-flow reactor

Assume void ratio, *e*, of the sulfur-packed reactor = 0.4

Required reactor volume $= (1{,}893\frac{\text{m}^3}{\text{d}} \times \frac{\text{d}}{24\,\text{hrs.}} \times 2.62\,\text{hrs.})/0.4$

$= 517\ \text{m}^3$

Select the reactor height = 3.5 m

Required reactor surface area = 517 m^3/3.5 m

=148 m^2

Check hydraulic loading $= 1{,}893\frac{\text{m}^3}{\text{d}} \times \frac{\text{d}}{24\,\text{hrs.}} \times \frac{1}{148\,\text{m}^2}$

$= 0.53\frac{\text{m}^3}{\text{m}^2.\text{hr}}$

Select the reactor diameter of 7 m

Surface area of each reactor $= \frac{\pi}{4} \times (7\ \text{m})^2$

$= 38.5\ \text{m}^2$

Required number of reactors = 148 m^2/38.5 m^2

= 3.8, use 4 reactors

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results of an extensive literature search, and detailed experimental programs with a batch reactor and a continuous up-flow sulfur-packed column, it is concluded that the autotrophic denitrification is a viable process for nitrate removal from groundwater supply sources. This process offers a distinct advantage over the heterotrophic denitrification as no external carbon source is necessary. As a result, the question regarding precursors and THMs formation potential in the finished water supply does not arise. Under this research program many specific conclusions have been drawn. These conclusions are summarized below.

(1) The elemental sulfur is oxidized and sulfate is produced linearly at a rate of 6.91 mg/L as SO_4^{2-} per mg/L $NO_3^- - N$ reduced. In this process however alkalinity is destroyed linearly at a rate of 3.09 mg/L as $CaCO_3$ per mg/L $NO_3^- - N$ reduced. As a result, alkalinity must be added to maintain the pH in the physiological range. pH below 6 is detrimental to the established microbiological process. In this investigation pH was successfully maintained by addition of sodium bicarbonate in the ratio of 5/1 in the feed as a source of

alkalinity (5 mg/L sodium bicarbonate expressed as $CaCO_3$ per mg/L $NO_3^- - N$ destroyed). Other investigators have used limestone packed columns with limited success.

(2) The nitrate in the raw feed was reduced to nitrite and then to nitrogen. The concentration of $NO_2^- - N$ increased initially in the effluent due to limiting reaction rate from $NO_3^- - N$ to N_2. As a result, there was significant build up of $NO_2^- - N$ at higher nitrate loadings.

(3) The concentrations of nitrate and nitrite nitrogen in the effluent were successfully modeled by conjugate reaction kinetics. The reaction rate constants k_1 = 1.41/hr and k_2 = 3.15/hr predicted the $NO_3^- - N$ and $NO_2^- - N$ concentrations close to the experimental data.

(4) Nitrogen gas generation was not measured in the continuous flow reactor. It was however, accurately modeled by conjugate reaction kinetics. The N_2 gas generation ratio C/C_o approached 1.0 at around 4.0 hours of THRT. In the batch reactor the gas generation was measured, and the ratio of gas generation was 0.61 mL N_2 per mg of $NO_3^- - N$ reduced.

(5) The dissolved oxygen in the feed was clearly a source of interference in the anoxic denitrification of nitrate. Although the dissolved oxygen in the groundwater sources is normally low, the DO level in the feed was in the range of 6.0 – 8.5 mg/L. Dissolved oxygen, being an electron acceptor, was

consumed. Therefore, initial sulfate production and alkalinity destruction may be due to deoxygeneration reactions.

(6) The autotrophic denitrification reactions based on biologically medicated half reaction equations are developed. These equations are based on (a) ammonia as a nitrogen source for cell synthesis, and (b) nitrate as a nitrogen source for cell synthesis. The cell mass in both equations is expressed by $C_5H_7O_2N$.

(7) The analytical model based on conjugate reaction kinetics developed in this research is a valuable tool to predict nitrate and nitrite concentration in the effluent from the up-flow column. This model has potential for use in designing a continuous up-flow column for removal of nitrate from the groundwater.

(8) A design example has been presented to develop the design procedure for an up-flow sulfur packed column based on a given design flow, nitrate nitrogen concentration in the feed, and limiting concentrations of nitrate and nitrite nitrogen in the finished water. The limiting nitrite concentration in the finished water governed the reactor size.

6.2 Recommendations

Many factors have been identified in this research that directly or indirectly affect the performance of an up-flow sulfur packed autotropohic denitrification column. These factors are temperature, dissolved oxygen in the feed, sulfur grain-size distribution, granular sulfur, porosity ratio, hydraulic and nitrate loading, and true hydraulic detention time. Many useful relationships have been developed in this research in terms of these factors.

It is an established fact that the autotrophic denitrification is very sensitive to lower temperature. Below 10 ^{o}C the performance of the process may drop significantly. Additionally, the granular sulfur is constantly lost in the effluent as sulfate ions causing a reduction in grain size. New material must be added in the column to maintain the required sulfur mass in the column. As a result, the porosity ratios of sulfur in the packed column will constantly change affecting the contact surface area of the sulfur media, and true hydraulic detention time.

It is recommended that a full-scale or pilot plant field study be initiated. This study will provide an opportunity to evaluate the effects of many key factors upon the performance of the autotrophic denitrification process. The research strategy for field investigation may include:

(1) The effect of temperature variation upon the nitrate removal, and nitrite build up in the finished water will be established from the seasonal temperature variation data. The results will be used to include the temperature effects upon the

reaction rate constants k_1 and k_2, and thus expand the capabilities of the conjugate reaction model to include the temperature effects.

(2) The research performance on autotrophic denitrification was conducted using the nitrate-spiked tap water. Micronutrients were added and the feed was stored for several days for dechlorination. All these steps were taken to simulate the groundwater. The DO in the feed however, remained high. The groundwater is normally low in DO. Therefore, the field investigations performed on groundwater will be a true representation of the real situation, and the effects of DO and micronutrients will be confirmed.

(3) Claus and Kutzner (1985) conducted autotrophic denitrification using granular sulfur as the electron donor and reported that there was no need of ammonium for the nitrogen source. However, it is still a controversial issue. It is therefore recommended to include in the future study the need for NH_4^+ as a nitrogen source for cell synthesis.

(4) The NO_2^- buildup in the effluent at high $NO_3^- - N$ loading is a cause of concern. It is recommended the effluent recycle through the reactor be investigated. Effluent recycle will increase the $NO_2^- - N$ *reductase* concentrations in the feed thus may accelerate the limiting reaction rate from $NO_2^- - N$ to N_2.

APPENDIX A

STOICHIOMETRIC RELATIONSHIP OF BIOLOGICAL DENITRIFICATION

The stoichiometric relationships for biological denitrification are obtained from various published sources. These relationships are summarized below.

Heterotrophic Denitrification

McCarty (1969)

$$NO_3^- + 1.08\ CH_3OH + H^+ \longrightarrow 0.065\ C_5H_7O_2N + 0.47\ N_2 + 0.76\ CO_2 + 2.44\ H_2O$$

Metcalf and Eddy (1972)

$$6\ NO_3^- + 5\ CH_3OH \longrightarrow 5\ CO_2 + 3\ N_2 + 7\ H_2O + 6\ OH^-$$

Richard et al., (1980)

$$5\ C_2H_5OH + 12\ NO_3^- \longrightarrow 6\ N_2 + 10\ CO_2 + 9\ H_2O + 12\ OH^-$$

Frick and Richard, (1985)

$$5\ CH_3COOH + 8\ NO_3^- \longrightarrow 4\ N_2 + 10\ CO_2 + 6\ H_2O + 8\ OH^-$$

Barrenstein et al., (1986)

$$5\ CH_4 + 8\ NO_3^- + 8H^+ \longrightarrow 5\ CO_2 + 4\ N_2 + 14\ H_2O$$

Frunzke and Meyer, (1990)

$$5\ CO + 2\ NO_3^- + 2H^+ \longrightarrow 5\ CO_2 + N_2 + H_2O$$

Autotrophic Denitrification

Batchelor, Bill (1978)

$$1.0\ NO_3^- + 1.10\ S + 0.40\ CO_2 + 0.76\ H_2O + 0.08\ NH_4^+$$
$$\longrightarrow 0.08\ C_5H_7O_2N + 0.5\ N_2 + 1.10\ SO_4^{2-} + 1.28\ H^+$$

Kelly, (1982)

$$2\ S + 2\ H_2O + 3\ O_2 \longrightarrow 2\ H_2SO_4$$

(Claus and Kutzner, 1985)

$$5S_2O_3^{2-} + 8\,NO_3^- + H_2O \longrightarrow 4\ N_2 + 10\ SO_4^{2-} + 2\ H^+$$

(Barrenstein et al. 1986)

$$5\ S^{2-} + 8\ NO_3^- + 8H^+ \longrightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O$$

(Kurt et al, 1987)

$$2\ NO_3^- + 5H_2 \longrightarrow N_2 + 4\ H_2O + 2\ OH^-$$

APPENDIX B

USEFUL HALF-REACTIONS INVOLVED IN BIOLOGICAL SYSTEMS

Many useful half-reactions involved in biological system are summarized below. The typical values of $f_{s(\max)}$ for autotrophic bacterial reactions are provided in Table B1 (Sawyer and McCarty, 1978).

Reaction Number **Half Reaction**

Reactions for bacterial cell synthesis (R_c)

Ammonia as nitrogen source

1 $\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$

Nitrate as nitrogen source

2 $\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$

Reactions for electron acceptors (R_a)

Oxygen

3 $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$

Nitrate

4 $\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O$

Sulfate

5 $\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^- = \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$

Carbon dioxide (methane fermentation)

6 $\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$

Reactions for electron donors (R_d)

Organic donors (heterotrophic reactions)

Domestic wastewater

7 $$\frac{9}{50}CO_2+\frac{1}{50}HCO_3^-+\frac{1}{50}NH_4^++H^++e^-=\frac{1}{50}C_{10}H_{19}O_3N+\frac{9}{25}H_2O$$

Protein (amino acids, proteins, nitrogenous organics)

8 $$\frac{8}{33}CO_2+\frac{2}{33}NH_4^++\frac{31}{33}H^++e^-=\frac{1}{66}C_{16}H_{24}O_5N_4+\frac{27}{66}H_2O$$

Carbohydrates (cellulose, starch, sugars)

9 $$\frac{1}{4}CO_2+H^++e^-=\frac{1}{4}CH_2O+\frac{1}{4}H_2O$$

Grease (fats and oils)

10 $$\frac{4}{23}CO_2+H^++e^-=\frac{1}{46}C_8H_{10}O+\frac{15}{46}H_2O$$

Acetate

11 $$\frac{1}{8}CO_2+\frac{1}{8}HCO_3^-+H^++e^-=\frac{1}{8}CH_3COO^-+\frac{3}{8}H_2O$$

Propionate

12 $$\frac{1}{7}CO_2+\frac{1}{14}HCO_3^-+H^++e^-=\frac{1}{14}CH_3CH_2COO^-+\frac{5}{14}H_2O$$

Benzoate

13 $$\frac{1}{5}CO_2+\frac{1}{30}HCO_3^-+H^++e^-=\frac{1}{30}C_6H_5COO^-+\frac{13}{20}H_2O$$

Ethanol

14 $$\frac{1}{6}CO_2+H^++e^-=\frac{1}{12}CH_3CH_2OH+\frac{1}{4}H_2O$$

Lactate

15 $$\frac{1}{6}CO_2+\frac{1}{12}HCO_3^-+H^++e^-=\frac{1}{12}CH_3CHOHCOO^-+\frac{1}{3}H_2O$$

Pyruvate

16 $$\frac{1}{5}CO_2+\frac{1}{10}HCO_3^-+H^++e^-=\frac{1}{10}CH_3COCOO^-+\frac{2}{5}H_2O$$

Methanol

17 $$\frac{1}{6}CO_2+H^++e^-=\frac{1}{6}CH_3OH+\frac{1}{6}H_2O$$

Inorganic donors (autotrophic reactions)

18 $$Fe^{3+}+e^-=Fe^{2+}$$

19 $$\frac{1}{2}NO_3^-+H^++e^-=\frac{1}{2}NO_2^-+\frac{1}{2}H_2O$$

20 $$\frac{1}{8}NO_3^-+\frac{5}{4}H^++e^-=\frac{1}{8}NH_4^++\frac{3}{8}H_2O$$

21 $$\frac{1}{6}NO_2^-+\frac{4}{3}H^++e^-=\frac{1}{6}NH_4^++\frac{1}{3}H_2O$$

22 $$\frac{1}{6}SO_4^{2-}+\frac{4}{3}H^++e^-=\frac{1}{6}S+\frac{2}{3}H_2O$$

23 $$\frac{1}{8}SO_4^{2-}+\frac{19}{16}H^++e^-=\frac{1}{16}H_2S+\frac{1}{16}HS^-+\frac{1}{2}H_2O$$

24 $$\frac{1}{4}SO_4^{2-}+\frac{5}{4}H^++e^-=\frac{1}{8}S_2O_3^{2-}+\frac{5}{8}H_2O$$

25 $$H^++e^-=\frac{1}{2}H_2$$

26 $$\frac{1}{2}SO_4^{2-} + H^+ + e^- = \frac{1}{2}SO_3^{2-} + \frac{1}{2}H_2O$$

Table B1 - Typical Values of $f_{s(\max)}$ for Autotrophic Bacterial Reactions

Electron donor	Electron acceptor	$f_{s(\max)}$
S	O_2	0.21
$S_2O_3^{2-}$	O_2	0.21
$S_2O_3^{2-}$	NO_3^-	0.20
NH_4^+	O_2	0.10
H_2	O_2	0.24
H_2	CO_2	0.04
Fe_2^+	O_2	0.07

Note: Adopted from Sawyer & McCarty (1978), P. 245

APPENDIX C

PHYSICAL CHARACTERISTICS OF SULFUR MEDIA USED IN THIS RESEARCH PROGRAM

The physical characteristics of sulfur media include density, void ratio and specific surface area. These physical parameters were determined in the laboratory. Procedures are given below.

Media Density

The media density was measured by the following procedures: (1) weigh a 250-mL clean beaker, (2) take certain volume of granular sulfur into the beaker, (3) weigh the beaker and sulfur, (4) measure equal volume of tap water in a 250-mL graduated cylinder, (5) gradually transfer the sulfur from the beaker into the cylinder containing the water, (6) measure the final water level after sulfur has been added, (7) calculate the water volume replaced by sulfur, (8) determine the weight of sulfur, (9) calculate the density of granular sulfur.

Void Ratio

The void ratio of the sulfur media was calculated by the following procedures: (1) fill the granular sulfur into a 250-mL graduated cylinder to a certain volume, (2) fill a second 250-mL graduated cylinder with tap water, (3) gradually pour the tap water from the second graduated cylinder into the first graduated cylinder until the water level reached to the top layer of the sulfur media, (4) determine the water volume filling the voids, (5) calculate the void ratio.

Specific Surface Area

The specific surface area is defined as the ratio of the total surface area of the media and the total volume of the media.

Assume average equivalent diameter of the sulfur media = d

Surface area = $\phi \times \pi \times d^2$

Where:

ϕ = shape factor. The shape factor is defined as the ratio of the surface area of an equal volume of sphere to the surface area of the grain.

The media volume = $\frac{4}{3} \times \pi \times (\frac{d}{2})^3$

The media diameter is the average grain size retained between the US Standard Sieve #8 (2.38 mm) and #4 (4.76 mm).

$$d = \frac{2.38 \text{ mm} + 4.76 \text{ mm}}{2} = 3.57 \text{ mm}$$

$$\text{Specific surface area} = \frac{\phi \times \pi \times d^2}{(\frac{4}{3}) \times \pi \times (\frac{d}{2})^3} = \frac{\phi \times 6}{d} = \frac{0.86 \times 6}{3.57 \text{ mm}} = 1.45 \times 10^3 \text{ m}^2/\text{m}^3$$

APPENDIX D

ANALYTICAL METHODS

The analytical procedures used in this investigation are the standard procedures as listed in Table D1. Only the procedures of $NO_3^- - N$ and $NO_2^- - N$ are adapted from the literature. These procedures are briefly presented.

Table D1- Analytical Procedures

Determinations	**Analytical Methods**
pH	Orion Scientific pH meter (Mode 920$^+$) with temperature compensation
Alkalinity	Standard Methods, 2320B, 18th Edition, 1992
Sulfate	Standard Methods, 4500E, 18th Edition, 1992
Nitrate-N	Salicyclic Sulfuric Acid Method (Cataldo, et al., 1975)
Nitrite-N	HACH Water Analysis Handbook, 1992

<u>Nitrate Determination by Salicylic Sulfuric Acid Method</u> (Cataldo, et al. 1975)

<u>Reagents:</u>

1. 5% Salicylic-Sulfuric Acid Reagent
2. 4 N Sodium Hydroxide
3. Standard Nitrate (100 mg/L N)

<u>Procedures:</u>

1. Pipet 0.4 mL sample solution into a reaction tube
2. Pipet 1.6 mL 5% Salicyclic-Sulfuric acid reagent into tube and swirl.
3. After 30 minutes, add 18.0 mL of 4N NaOH solution and swirl.

4. Allow contents to cool and read transmittance at a wavelength of 410 nm in the Spectronic-20D (Model 333183).
5. Read the nitrate concentration from the standard calibration curve prepared on the same machine.

Nitrite Measurement (HACH Water Analysis Handbook, 1992)

Reagent

1. NitriVer 2 Nitrite Reagent Power Pillows (HACH)
2. Sodium Nitrite, ACS

Procedure

1. Filter through a 0.45-µm membrane filter to remove suspended solids.
2. Set wavelength at 585 nm in Spectronic-20D (Model 333183).
3. Fill a sample cell with 10 mL of sample.
4. Add the contents of one NitriVer 2 Nitrite Reagent Power Pillow, shake to dissolve. A greenish-brown color will develop.
5. Read the transmittance after 10 min in the Spectronic-20D (Model 333183).
6. Read the nitrite concentration from the standard calibration curve prepared on the same machine.

APPENDIX E

RAW EXPERIMENTAL DATA OF BATCH REACTOR STUDY

The experimental data on volume of batch reactor contents, $NO_3^- - N$, SO_4^{2-}, and alkalinity of reactor contents and feed, and pH of feed are summarized in Table E1. The concentrations of $NO_3^- - N$, SO_4^{2-}, and alkalinity, pH of the batch reactor effluent, and gas generation data are provided in Table E2.

Table E1 – The Experimental Data on Batch Reactor Contents and Feed Quality

Date	Reactor Content Data				Influent Data			
	Volume, mL	$NO_3^- - N$, mg/L	SO_4^{2-}, mg/L	Alkalinity, mg/L as $CaCO_3$	$NO_3^- - N$, mg/L	SO_4^{2-}, mg/L	Alkalinity, mg/L as $CaCO_3$	pH
9/5/00	4000	10	50	ND	10	37.5	100	7.92
9/6/00	4000	ND	ND	ND	10	37.5	100	7.92
9//7/00	4000	ND	ND	ND	10	37.5	100	7.92
9/8/00	4000	ND	ND	ND	100	37.5	460	7.92
9/9/00	4000	ND	ND	ND	100	37.5	460	7.92
9/10/00	4070	ND	ND	ND	100	37.5	460	7.92
9/11/00	4070	ND	ND	ND	100	37.5	460	7.92
9/12/00	4070	ND	ND	ND	100	37.5	460	7.92
9/12/00	4070	ND	ND	ND	100	37.5	460	7.92
9/12/00	4070	ND	ND	ND	100	37.5	460	7.92
9/13/00	4070	ND	ND	ND	100	37.5	460	7.92
9/13/00	4100	ND	ND	ND	100	37.5	460	7.92
9/14/00	4035	ND	ND	ND	100	37.5	460	7.92
9/15/00	4080	52.0	264.4	160.0	100	37.5	460	7.92
9/16/00	4135	59.3	230.1	205.3	100	39.2	460	7.97
9/17/00	4105	51.6	330.3	160.4	100	39.2	460	8.26
9/18/00	4147	31.4	451.1	111.9	0	39.2	18850	ND
9/18/00	4197	33.7	451.1	335.1	100	39.2	460	8.26
9/19/00	4085	29.6	452.3	302.6	100	39.2	460	8.55
9/20/00	4042	27.7	578.3	241.0	ND	ND	ND	ND
9/21/00	4042	25.6	578.3	241.0	100	37.5	578	8.56
9/22/00	3936	16.3	743.6	171.5	100	37.5	578	8.56
9/23/00	4013	17.5	698.8	172.9	100	37.5	578	8.56
9/24/00	4092	16.5	729.3	172.6	100	37.5	578	8.56
9/25/00	4102	14.8	735.7	168.8	100	37.5	578	8.56
9/26/00	4192	15.7	718.6	180.3	100	37.5	580	8.14
9/26/00	4163	18.1	683.0	197.2	100	37.5	580	8.03
9/27/00	4134	19.0	673.8	215.6	100	37.5	580	8.35
9/28/00	4109	18.7	682.0	207.0	100	37.5	580	8.35
9/29/00	ND	ND	ND	ND	ND	ND	ND	ND

Table E1 – Continued

Date	Reactor Content Data				Influent Data			
	Volume, mL	$NO_3^- - N$, Mg/L	SO_4^{2-}, mg/L	Alkalinity, mg/L as $CaCO_3$	$NO_3^- - N$, mg/L	SO_4^{2-}, mg/L	Alkalinity, mg/L as $CaCO_3$	pH
9/30/00	4189	18.3	688.0	204.9	100	37.5	580	8.35
10/1/00	4173	13.9	674.3	194.5	100	37.5	580	8.65
10/2/00	4159	13.8	673.9	199.8	100	37.5	572	8.03
10/3/00	4236	15.3	659.0	206.3	100	37.5	572	8.25
10/4/00	4273	14.3	652.2	215.4	100	37.5	572	8.25
10/5/00	4299	14.1	671.1	219.7	100	ND	ND	ND
10/6/00	4147	31.4	451.1	111.9	0	39.2	18850	ND

Note: ND = No data

Table E2 – The Experimental Data on Batch Reactor Effluent Quality and Gas Generation

Date	Effluent				Influent Feed Volume, mL	Sample Volume Withdrawal, mL	Gas Volume Reading, mL	Gas Volume Reset, mL	Gas Volume Generated, mL
	NO_3^-, mg/L	SO_4^{2-}, mg/L	Alkalinity as $CaCO_3$, mg/L	pH					
9/5/00	ND	10	50	7.2	570	570	ND	ND	ND
9/6/00	ND	ND	ND	ND	570	570	ND	ND	ND
9//7/00	ND	ND	ND	ND	570	570	ND	500	ND
9/8/00	ND	ND	ND	ND	570	570	505	500	5
9/9/00	ND	ND	ND	ND	570	500	490	500	ND
9/10/00	ND	ND	ND	ND	570	570	485	485	ND
9/11/00	ND	ND	ND	6.79	570	570	485	498	ND
9/12/00	ND	ND	ND	6.89	570	570	510	495	12
9/12/00	ND	ND	ND	6.57	570	570	495	495	ND
9/12/00	ND	ND	ND	6.78	570	570	495	480	ND
9/13/00	ND	ND	ND	6.57	600	570	480	540	ND
9/13/00	ND	ND	ND	6.61	495	560	540	495	ND
9/14/00	ND	ND	ND	6.48	610	565	515	555	20
9/15/00	52	264	160	6.38	625	570	580	515	25
9/16/00	44.1	375	114	6.34	550	580	580	495	65
9/17/00	24.2	475	54	6.04	580	585	575	495	80
9/18/00	17.2	536	40	5.6	710	663	545	485	50
9/18/00	17.2	451.1	111.9	ND	50	0	485	485	ND
9/19/00	17.2	525	275	6.52	610	722	528	475	43
9/20/00	16.8	660	208	6.45	530	573	555	475	80
9/21/00	ND	ND	ND	ND	ND	ND	ND	ND	ND
9/22/00	4.7	842	115	6.07	480	586	565	480	90
9/23/00	1.9	824	96	5.8	640	563	530	501	50
9/24/00	0.7	860	96	5.7	650	571	525	500	24
9/25/00	0.5	853	100	5.76	590	580	510	476	10
9/26/00	0	846	106	5.9	660	570	510	478	34
9/26/00	4.6	790	134	6.2	590	619	512	477	34
9/27/00	2.2	806	140	6.1	710	739	515	477	38
9/28/00	6.3	781	150	6.1	545	570	525	490	48
9/29/00	3.3	808	136	6.23	650	570	530	500	40
9/30/00	ND	ND	ND	ND	ND	ND	ND	ND	ND
10/1/00	0.8	771	136	6.19	550	566	561	498	61
10/2/00	0.2	774	140	6.2	565	579	560	479	62
10/3/00	0	772	140	6.03	650	573	528	478	49
10/4/00	0	755	156	6.25	610	573	535	479	57
10/5/00	0	775	162	6.13	605	579	527	479	48

Note: ND = No data

APPENDIX F

RAW EXPERIMENTAL DATA OF SULFUR-PACKED CONTINUOUS UP-FLOW REACTOR STUDY

The experimental results on the influent and effluent quality from different sampling ports of sulfur-packed up-flow reactor are summarized in this appendix. The $NO_3^- - N$, $NO_2^- - N$, alkalinity, sulfate, pH, and temperature data are provided separately in Tables F1 through F6 respectively.

Table F1 - Experimental Data of $NO_3^- - N$ Concentration in the Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	$NO_3^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/30/2001	50.1	14.6	9.9	8.2	6.6	6.6	6.6	TD
1/31/2001	50.3	11.8	3.0	2.3	2.4	1.7	1.7	
2/1/2001	50.3	14.4	2.5	1.1	1.1	0.7	0.4	
2/2/2001	50.3	12.0	1.1	0.5	0.2	0.3	0.4	
2/3/2001	ND							
2/4/2001	50.3	13.7	3.6	1.2	0.60	0.52	0.5	
2/5/2001	51.4	10.2	2.1	0.6	< 0.1	< 0.1	< 0.1	
2/6/2001	50.4	9.0	0.4	0.2	< 0.1	< 0.1	0.2	
2/7/2001	50.4	8.3	0.1	0.1	0.1	0.1	0.1	
2/8/2001	68.3	23.0	1.3	0.3	0.3	0.3	0.3	TD
2/9/2001	70.6	20.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
2/10/2001	70.6	16.7	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
2/11/2001	ND							
2/12/2001	68.3	11.8	0.3	0.1	0.1	0.1	0.1	
2/13/2001	71.3	5.3	0.1	0.1	< 0.1	< 0.1	0.1	
2/14/2001	70.9	10.1	0.4	0.4	0.2	0.2	0.2	
2/15/2001	70.9	7.5	0.5	0.2	0.2	0.2	0.2	
2/16/2001	73.7	5.1	0.2	0.2	0.1	0.2	0.2	
2/17/2001	69.0	6.4	0.3	0.4	0.4	0.4	0.4	
2/18/2001	71.6	8.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
2/19/2001	71.6	5.6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
2/20/2001	71.6	7.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
2/21/2001	91.1	16.0	2.3	< 0.1	< 0.1	< 0.1	< 0.1	TD
2/22/2001	93.6	16.3	4.0	1.2	0.4	0.5	0.5	TD
2/23/2001	91.8	16.5	3.2	1.5	0.4	0.5	0.3	TD
2/24/2001	92.1	16.3	4.2	1.5	0.7	0.2	0.2	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	93.9	9.0	0.3	0.2	0.2	0.2	0.3	
2/28/2001	93.9	5.9	0.2	0.3	0.1	0.3	0.2	

Table F1 – Continued

Date	$NO_3^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/1/2001	90.4	3.9	0.2	0.2	0.2	0.2	0.2	
3/2/2001	93.9	2.0	0.3	0.3	0.2	0.2	0.2	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	88.3	8.4	2.4	1.6	0.6	0.5	0.5	
3/6/2001	90.1	1.4	0.6	0.7	0.6	0.6	0.7	
3/7/2001	50.3	0.6	0.1	0.1	0.1	0.1	0.1	TD
3/8/2001	50.3	1.4	0.1	0.1	0.1	0.1	0.1	TD
3/9/2001	50.3	1.1	0.1	0.1	0.1	0.1	0.1	TD
3/10/2001	53.4	4.4	0.8	0.3	0.1	0.1	0.1	TD
3/11/2001	53.4	5.5	2.2	0.9	0.2	0.1	0.3	TD
3/12/2001	48.4	6.8	1.9	0.7	0.9	0.6	0.9	TD
3/13/2001	ND							
3/14/2001	47.0	0.1	0.1	0.1	0.1	0.1	0.1	TD
3/15/2001	ND							
3/16/2001	47.0	0.3	0.1	0.1	0.1	0.1	0.1	TD
3/17/2001	47.0	0.1	0.1	0.1	0.1	0.1	0.1	TD
3/18/2001	ND							
3/19/2001	68.2	5.9	1.8	1.1	0.7	0.7	2.6	
3/20/2001	66.6	6.1	2.6	0.7	0.4	0.2	0.2	
3/21/2001	68.8	8.8	5.6	2.6	0.7	0.7	0.4	
3/22/2001	68.2	11.3	5.7	2.3	1.1	0.5	0.4	
3/23/2001	68.2	13.2	7.8	3.7	2.4	1.1	0.5	
3/24/2001	58.7	4.8	1.6	0.7	0.3	0.3	0.3	TD
3/25/2001	58.0	4.9	1.3	0.6	0.3	0.3	0.3	
3/26/2001	55.7	9.9	4.2	1.1	0.4	0.4	0.3	
3/27/2001	57.2	5.0	1.0	0.4	0.3	0.2	0.2	
3/28/2001	60.2	4.1	1.1	0.6	0.6	0.6	0.6	
3/29/2001	57.1	3.1	0.7	0.5	0.6	0.5	0.6	
3/30/2001	57.1	3.0	0.6	0.6	0.6	0.6	0.6	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	49.7	1.1	0.2	0.2	0.2	0.2	0.2	
4/3/2001	49.7	0.2	0.2	0.2	0.2	0.2	0.2	
4/4/2001	48.0	0.2	0.2	0.2	0.2	0.2	0.2	
4/5/2001	48.0	0.2	0.2	0.2	0.2	0.2	0.2	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	37.9	1.0	0.5	0.5	0.5	0.5	0.5	
4/9/2001	37.9	1.9	0.8	0.6	0.6	0.6	0.6	
4/10/2001	37.9	0.7	0.7	0.7	0.7	0.7	0.7	
4/11/2001	37.9	0.7	0.7	0.7	0.7	0.7	0.7	
4/12/2001	ND							
4/13/2001	20.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	

Table F1 – Continued

Date	$NO_3^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/14/2001	19.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/15/2001	19.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	TD
4/16/2001	21.3	4.9	1.6	2.0	2.1	1.5	1.2	
4/17/2001	ND							
4/18/2001	20.6	0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/19/2001	20.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/20/2001	19.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/21/2001	20.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/22/2001	19.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/23/2001	20.6	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/24/2001	ND							
4/25/2001	39.2	0.4	0.3	< 0.1	< 0.1	< 0.1	< 0.1	
4/26/2001	39.9	0.3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/27/2001	39.2	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/28/2001	37.7	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/29/2001	39.9	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
4/30/2001	39.2	0.4	0.26	< 0.1	< 0.1	< 0.1	< 0.1	
5/1/2001	ND							
5/2/2001	57.6	9.1	3.8	2.0	1.0	0.4	0.3	
5/3/2001	57.6	8.4	2.6	0.6	0.3	<	<	
5/4/2001	57.6	7.2	1.0	0.5	0.3	0.3	0.3	
5/5/2001	57.6	7.6	1.4	0.6	0.2	0.1	0.1	
5/6/2001	57.6	7.4	1.2	0.5	0.2	0.2	0.2	
5/7/2001	56.2	8.0	1.9	0.6	0.1	<	<	TD
5/8/2001	66.5	13.1	7.4	5.2	2.4	1.3	1.0	
5/9/2001	65.5	10.3	6.7	6.1	4.6	1.9	1.7	
5/10/2001	67.1	12.7	9.1	6.7	5.8	4.0	4.9	
5/11/2001	67.1	6.2	2.7	0.6	0.1	0.1	0.1	

Note: ND = No data
TD = Transition data, not used

Table F2 - Experimental Data of $NO_2^- - N$ Concentration in the Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	$NO_2^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/30/2001	2.4	6.9	9.6	8.5	8.0	8.0	9.6	TD
1/31/2001	2.4	6.9	5.9	3.7	3.2	3.2	3.2	
2/1/2001	2.3	4.2	3.7	1.6	0.8	0.4	0.0	
2/2/2001	2.4	3.2	2.4	0.8	0.3	0.3	0.3	
2/3/2001	ND							
2/4/2001	0.7	5.2	3.5	0.5	0.0	0.0	0.0	
2/5/2001	1.2	6.3	3.2	0.9	0.2	0.1	0.0	
2/6/2001	1.7	4.5	1.0	0.0	0.3	0.3	0.0	
2/7/2001	1.9	3.8	0.0	0.3	0.3	0.3	0.0	
2/8/2001	0.5	4.8	0.5	0.0	0.3	0.3	0.0	TD
2/9/2001	1.6	3.2	0.0	0.3	0.5	0.3	0.3	
2/10/2001	2.1	3.2	1.3	0.5	0.0	0.0	0.0	
2/11/2001	ND							
2/12/2001	1.1	1.3	0.8	0.5	0.5	0.3	0.0	
2/13/2001	1.8	2.1	1.3	0.4	0.0	0.2	0.3	
2/14/2001	2.1	4.2	0.5	0.8	0.5	0.5	0.5	
2/15/2001	2.6	3.7	0.0	0.3	0.3	0.3	0.3	
2/16/2001	1.6	3.2	0.2	0.3	0.3	0.3	0.3	
2/17/2001	2.1	3.2	0.2	0.3	0.1	0.1	0.0	
2/18/2001	2.4	3.2	0.5	0.3	0.3	0.3	0.3	
2/19/2001	2.5	2.9	0.3	0.5	0.5	0.4	0.3	
2/20/2001	2.4	3.7	0.3	0.3	0.3	0.3	0.3	
2/21/2001	1.7	5.9	3.7	0.5	0.3	0.5	0.4	TD
2/22/2001	1.9	5.6	5.1	2.6	0.3	0.1	0.1	TD
2/23/2001	2.3	5.6	4.8	2.6	0.6	0.4	0.1	TD
2/24/2001	2.4	6.1	6.7	2.7	2.1	1.6	0.5	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	1.1	8.3	0.8	0.1	0.0	0.0	0.1	
2/28/2001	1.9	7.2	0.3	0.3	0.0	0.0	0.0	
3/1/2001	2.4	4.8	0.1	0.1	0.0	0.0	0.0	
3/2/2001	0.8	1.0	0.2	0.3	0.0	0.0	0.2	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	1.1	7.5	6.1	6.1	1.6	0.0	0.5	
3/6/2001	1.3	3.2	0.3	0.3	0.3	0.3	0.0	
3/7/2001	0.0	1.6	0.1	0.0	0.0	0.0	0.0	TD
3/8/2001	0.1	4.3	0.3	0.0	0.0	0.0	0.0	TD
3/9/2001	0.3	4.3	1.3	0.3	0.0	0.0	0.0	TD
3/10/2001	0.8	7.7	6.7	3.1	1.6	0.8	0.3	TD
3/11/2001	1.3	8.0	7.5	5.9	3.9	3.5	4.8	TD
3/12/2001	0.5	8.3	8.7	5.6	4.8	4.3	6.9	TD

Table F2 – Continued

Date	$NO_2^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/13/2001	ND							
3/14/2001	1.9	0.3	0.0	0.3	0.5	0.0	0.0	TD
3/15/2001	ND							
3/16/2001	1.9	1.8	0.4	0.3	0.0	0.0	0.0	TD
3/17/2001	1.9	1.9	0.4	0.3	0.1	0.1	0.0	TD
3/18/2001	ND							
3/19/2001	1.5	11.8	9.3	9.1	7.5	8.0	7.5	
3/20/2001	2.1	13.6	11.2	7.2	3.7	1.9	1.6	
3/21/2001	1.0	12.6	9.3	7.2	8.3	5.9	7.2	
3/22/2001	2.0	14.4	13.2	12.6	10.4	5.3	3.2	
3/23/2001	2.1	15.2	14.2	14.3	14.2	12.8	8.7	
3/24/2001	1.5	14.6	10.7	6.7	3.1	2.6	0.7	TD
3/25/2001	ND							
3/26/2001	1.4	15.1	12.0	9.9	5.6	4.6	1.1	
3/27/2001	1.6	14.0	9.3	3.5	0.5	0.5	0.4	
3/28/2001	0.6	12.0	5.3	1.3	0.4	0.2	0.4	
3/29/2001	1.1	11.8	4.8	1.1	0.3	0.3	0.3	
3/30/2001	1.9	8.0	4.5	0.5	0.3	0.5	0.3	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	1.6	6.4	0.8	0.3	0.3	0.3	0.3	
4/3/2001	1.0	3.2	0.3	0.3	0.4	0.3	0.3	
4/4/2001	1.3	4.8	0.5	0.3	0.3	0.3	0.3	
4/5/2001	2.2	5.3	0.6	0.3	0.4	0.3	0.3	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	2.7	8.3	0.0	0.3	0.0	0.0	0.0	
4/9/2001	1.5	9.5	0.8	0.7	0.2	0.3	0.3	
4/10/2001	2.4	5.9	0.5	0.5	0.3	0.3	0.3	
4/11/2001	2.9	4.0	0.0	0.5	0.5	0.3	0.3	
4/12/2001	ND							
4/13/2001	1.6	0.4	0.3	0.3	0.3	0.3	0.3	
4/14/2001	1.9	0.5	0.0	0.0	0.3	0.3	0.3	
4/15/2001	ND							
4/16/2001	1.3	5.9	3.5	3.2	3.2	3.2	2.4	
4/17/2001	ND							
4/18/2001	2.4	0.0	0.0	0.0	0.0	0.0	0.3	
4/19/2001	1.3	0.5	0.5	0.5	0.5	0.5	0.5	
4/20/2001	1.6	0.5	0.5	0.5	0.0	0.0	0.0	
4/21/2001	1.5	0.5	0.3	0.3	0.3	0.3	0.4	
4/22/2001	1.5	0.5	0.1	0.1	0.1	0.0	0.3	
4/23/2001	2.1	0.8	0.3	0.3	0.3	0.3	0.3	

Table F2 – Continued

Date	$NO_2^- - N$ Concentration, mg/L							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/24/2001	ND							
4/25/2001	1.5	1.9	0.5	0.5	0.7	0.5	0.5	
4/26/2001	1.1	2.4	0.3	0.5	0.8	0.5	0.5	
4/27/2001	1.3	2.2	0.4	0.5	0.7	0.6	0.5	
4/28/2001	1.3	2.4	0.5	0.8	0.6	0.6	0.6	
4/29/2001	1.5	2.6	0.8	0.7	0.7	0.7	0.7	
4/30/2001	1.9	1.4	0.6	0.5	0.5	0.5	0.4	
5/1/2001	ND							
5/2/2001	1.0	14.7	12.6	9.1	6.7	1.9	0.4	
5/3/2001	1.1	13.6	12.1	8.1	4.7	1.6	0.8	
5/4/2001	1.1	13.7	10.9	5.3	1.9	2.1	3.1	
5/5/2001	1.2	13.8	11.1	7.2	2.8	1.2	1.7	
5/6/2001	1.3	13.8	11.1	8.1	3.3	0.7	1.0	
5/7/2001	1.3	13.9	11.2	9.1	3.7	0.3	0.3	TD
5/8/2001	0.8	14.0	14.7	14.5	12.4	11.5	11.8	
5/9/2001	1.1	16.2	16.7	15.4	15.6	15.1	15.0	
5/10/2001	2.1	13.9	14.2	14.0	12.3	13.1	12.3	
5/11/2001	1.1	9.5	4.3	2.4	0.0	0.0	0.0	

Note: ND = No data
TD = Transition data, not used

Table F3 - Experimental Data of Alkalinity Concentration in the Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	Alkalinity Concentration, mg/L as $CaCO_3$							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/29/2001	336	204	186	182	190	192	192	TD
1/30/2001	332	200	181	180	180	180	182	TD
1/31/2001	332	194	168	166	162	161	164	
2/1/2001	334	204	163	158	158	157	154	
2/2/2001	336	201	161	160	157	157	150	
2/3/2001	ND							
2/4/2001	336	201	174	162	160	159	153	
2/5/2001	340	198	172	164	161	160	150	
2/6/2001	336	192	161	160	160	158	152	
2/7/2001	334	192	156	156	154	154	151	
2/8/2001	437	285	198	190	194	194	185	TD
2/9/2001	436	269	192	192	192	192	185	
2/10/2001	433	257	192	192	192	192	184	
2/11/2001	ND							
2/12/2001	440	238	191	191	191	191	183	
2/13/2001	437	211	187	187	187	187	181	
2/14/2001	438	220	186	187	187	187	174	
2/15/2001	438	214	186	186	186	186	177	
2/16/2001	437	208	186	190	188	188	175	
2/17/2001	440	210	190	191	190	190	177	
2/18/2001	438	214	188	190	187	190	179	
2/19/2001	438	212	192	192	192	190	182	
2/20/2001	434	214	193	193	193	193	177	
2/21/2001	442	193	154	143	146	148	139	TD
2/22/2001	441	186	156	146	141	140	120	TD
2/23/2001	437	188	153	142	142	140	128	TD
2/24/2001	438	188	154	141	142	140	128	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	540	258	232	232	229	228	215	
2/28/2001	539	259	235	235	234	231	216	
3/1/2001	532	243	230	232	235	234	223	
3/2/2001	541	237	230	233	231	228	214	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	541	270	252	254	245	240	235	
3/6/2001	542	243	235	241	240	242	239	
3/7/2001	342	165	158	159	158	157	151	TD
3/8/2001	344	167	162	160	159	159	150	TD
3/9/2001	341	170	162	162	160	158	153	TD
3/10/2001	340	177	164	159	159	159	159	TD
3/11/2001	340	184	172	165	163	161	160	TD
3/12/2001	337	198	181	171	171	168	171	TD

Table F3 – Continued

Date	Alkalinity Concentration, mg/L as $CaCO_3$							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/13/2001	ND							
3/14/2001	336	168	166	166	167	169	157	TD
3/15/2001	ND							
3/16/2001	337	164	160	156	154	154	149	TD
3/17/2001	329	156	152	156	154	158	145	TD
3/18/2001	ND							
3/19/2001	431	227	215	213	208	208	220	
3/20/2001	434	230	218	209	206	202	199	
3/21/2001	437	240	227	217	214	213	207	
3/22/2001	437	255	234	224	218	214	204	
3/23/2001	438	258	246	231	229	225	213	
3/24/2001	408	219	199	183	189	184	178	TD
3/25/2001	399	217	198	188	191	187	179	
3/26/2001	395	234	215	202	196	196	186	
3/27/2001	390	215	196	193	193	190	180	
3/28/2001	390	210	196	190	190	190	188	
3/29/2001	394	206	196	193	192	188	190	
3/30/2001	396	203	197	193	192	192	190	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	340	182	175	176	176	174	166	
4/3/2001	344	179	175	174	174	174	167	
4/4/2001	343	181	174	175	174	174	166	
4/5/2001	342	182	173	175	174	174	164	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	292	163	154	152	144	132	104	
4/9/2001	295	173	160	160	159	158	150	
4/10/2001	294	162	154	150	148	153	141	
4/11/2001	292	161	156	156	155	154	140	
4/12/2001	ND							
4/13/2001	189	122	121	120	120	119	106	
4/14/2001	193	117	117	116.5	116.5	116.5	105.5	
4/15/2001	ND							
4/16/2001	190	130	122	122	116	112	105	
4/17/2001	ND							
4/18/2001	197	112	113	113	113	114	105	
4/19/2001	201	111	114	113	113	114	105	
4/20/2001	200	117	118	112	115	115	107	
4/21/2001	200	117	117	116	116	116	107	
4/22/2001	201	118	118	118	118	115	107	
4/23/2001	202	118	118	118	116	116	107	

Table F3 – Continued

Date	Alkalinity Concentration, mg/L as $CaCO_3$							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/24/2001	ND							
4/25/2001	297	155	154	152	149	149	139	
4/26/2001	298	157	153	151	148	148	138	
4/27/2001	297	157	155	152	149	149	139	
4/28/2001	299	155	153	154	150	149	138	
4/29/2001	298	154	152	153	152	148	139	
4/30/2001	296	152	153	153	154	152	140	
5/1/2001	ND							
5/2/2001	397	232	214	207	201	196	187	
5/3/2001	400	232	217	207	202	198	197	
5/4/2001	399	232	210	202	199	204	204	
5/5/2001	397	231	213	204	201	202	200	
5/6/2001	396	231	215	205	201	201	198	
5/7/2001	395	230	216	206	202	200	196	TD
5/8/2001	450	274	255	246	240	237	239	
5/9/2001	449	268	256	255	251	245	243	
5/10/2001	404	222	208	202	198	194	200	
5/11/2001	400	190	174	166	166	162	162	

Note: ND = No data
TD = Transition data, not used

Table F4 - Experimental Data of Sulfate Concentration in the Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	Sulfate Concentration, mg/L as SO_4^{2-}							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/29/2001	33.4	279.6	333.4	309.9	323.9		310.8	TD
1/30/2001	34.3	285.3	314.0	320.4	333.1	329.9	320.4	TD
1/31/2001	36.5	307.6	377.7	384.1	387.2	384.1	384.1	
2/1/2001	37.8	301.6	390.4	415.9	425.4	431.8	441.4	
2/2/2001	37.1	323.6	425.4	435.0	438.2	454.1	454.1	
2/3/2001	ND							
2/4/2001	38.4	298.1	384.1	422.3	422.3	441.4	435.0	
2/5/2001	38.7	320.4	384.1	422.3	428.6	438.2	447.7	
2/6/2001	38.7	336.3	422.3	438.2	444.6	425.4	438.2	
2/7/2001	38.7	339.5	415.9	428.6	431.8	428.6	447.7	
2/8/2001	41.3	339.5	519.7	551.6	551.6	551.6	557.9	TD
2/9/2001	41.6	390.4	557.9	570.7	583.4	564.3	570.7	
2/10/2001	42.2	425.4	570.7	570.7	577.1	583.4	602.5	
2/11/2001	ND							
2/12/2001	43.2	448.4	558.6	580.9	573.9	568.1	584.1	
2/13/2001	43.2	518.5	565.0	573.2	584.7	559.9	586.0	
2/14/2001	42.9	494.3	570.7	574.5	568.1	584.1	622.3	
2/15/2001	44.1	511.1	581.8	584.1	579.6	588.5	627.7	
2/16/2001	45.4	528.0	593.0	593.6	591.1	593.0	633.1	
2/17/2001	47.0	513.4	570.0	596.8	580.9	612.1	620.4	
2/18/2001	46.4	487.3	568.1	579.6	586.0	595.5	586.6	
2/19/2001	46.4	523.6	571.3	588.5	589.8	591.1	594.2	
2/20/2001	44.8	509.5	571.3	582.8	581.5	588.5	605.7	
2/21/2001	47.0	559.9	691.7	678.9	698.1	729.9	749.0	TD
2/22/2001	48.6	603.8	671.3	736.3	742.6	729.9	761.7	TD
2/23/2001	47.6	570.7	658.6	717.2	717.2	717.2	736.3	TD
2/24/2001	48.0	564.3	628.0	678.9	698.1	710.8	717.2	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	51.1	602.5	723.5	736.3	723.5	729.9	749.0	
2/28/2001	51.1	659.8	710.8	729.9	736.3	736.3	755.4	
3/1/2001	51.1	659.8	710.8	710.8	717.2	723.5	729.9	
3/2/2001	50.5	691.7	729.9	717.2	710.8	717.2	749.0	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	49.6	591.7	668.1	654.7	704.4	717.2	717.2	
3/6/2001	50.5	672.6	691.7	717.2	742.6	723.5	710.8	
3/7/2001	50.8	314.1	317.4	317.4	317.4	317.4	317.4	TD
3/8/2001	50.5	312.5	320.9	320.9	320.9	320.9	320.9	TD
3/9/2001	50.2	403.2	447.7	444.6	454.1	438.2	447.7	TD
3/10/2001	52.2	358.6	400.0	438.2	447.7	447.7	454.1	TD
3/11/2001	52.4	371.3	393.6	406.3	419.1	425.4	422.3	TD
3/12/2001	50.6	349.0	380.9	406.3	406.3	419.1	406.3	TD

Table F4 – Continued

Date	Sulfate Concentration, mg/L as SO_4^{2-}							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/13/2001	ND							
3/14/2001	51.5	425.4	438.2	441.4	447.7	450.9	454.1	TD
3/15/2001	ND							
3/16/2001	53.6	419.1	441.4	444.6	447.7	444.6	444.6	TD
3/17/2001	53.9	438.2	454.1	450.9	447.7	454.1	457.3	TD
3/18/2001	ND							
3/19/2001	53.9	517.8	557.9	551.6	542.0	541.4	510.2	
3/20/2001	54.6	464.3	511.5	513.4	532.5	564.3	577.1	
3/21/2001	53.3	466.9	487.9	516.6	527.4	531.8	528.7	
3/22/2001	56.1	421.0	488.5	515.3	522.9	556.7	605.7	
3/23/2001	54.5	431.2	459.2	479.0	492.4	501.3	545.2	
3/24/2001	54.4	415.6	477.4	505.1	508.6	517.8	510.2	TD
3/25/2001	54.3	411.0	474.4	504.1	511.6	525.5	512.1	
3/26/2001	55.3	375.8	424.8	465.0	486.0	505.7	532.5	
3/27/2001	54.3	406.4	471.3	503.2	514.6	533.1	514.0	
3/28/2001	54.6	424.8	483.4	507.0	502.5	502.5	506.4	
3/29/2001	56.4	445.2	477.1	501.3	500.6	487.9	494.3	
3/30/2001	59.0	449.0	486.0	498.7	493.0	499.4	505.7	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	54.6	396.2	424.2	426.1	430.6	433.1	428.0	
4/3/2001	58.4	398.7	416.6	401.3	418.5	421.0	429.3	
4/4/2001	59.3	404.8	417.2	406.4	417.8	426.1	430.9	
4/5/2001	60.2	410.8	417.8	411.5	417.2	431.2	432.5	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	59.0	347.8	359.2	377.7	394.3	383.4	403.2	
4/9/2001	58.9	332.1	358.6	377.7	374.5	374.5	393.6	
4/10/2001	59.3	355.4	374.5	377.7	377.7	374.5	390.4	
4/11/2001	59.9	345.8	368.1	377.7	374.5	368.1	371.3	
4/12/2001	ND							
4/13/2001	62.2	219.4	225.5	220.1	223.9	234.4	226.8	
4/14/2001	63.1	228.8	235.8	231.0	237.4	240.0	243.5	
4/15/2001	ND							TD
4/16/2001	65.1	198.1	215.6	215.9	229.0	231.2	235.3	
4/17/2001	ND							
4/18/2001	64.0	238.2	246.2	242.0	251.0	245.5	260.2	
4/19/2001	64.1	260.8	261.8	255.1	259.2	253.5	266.9	
4/20/2001	64.0	224.8	220.1	226.8	227.7	223.2	232.8	
4/21/2001	64.7	234.4	237.6	234.4	228.0	231.2	237.6	
4/22/2001	64.1	231.2	234.4	237.6	231.2	234.4	247.1	
4/23/2001	66.0	231.8	231.8	231.8	229.0	236.3	250.3	

Table F4 – Continued

Date	Sulfate Concentration, mg/L as SO_4^{2-}							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/24/2001	ND							
4/25/2001	66.0	372.6	383.4	378.8	381.4	378.0	390.9	
4/26/2001	66.6	378.4	388.5	380.3	386.6	386.0	394.3	
4/27/2001	66.0	366.9	378.4	377.4	376.1	370.1	387.6	
4/28/2001	65.3	373.3	383.4	378.8	381.4	378.0	390.9	
4/29/2001	64.7	386.0	375.8	376.7	373.5	366.1	385.9	
4/30/2001	65.5	365.6	368.2	374.5	365.6	354.2	380.9	
5/1/2001	ND							TD
5/2/2001	64.1	389.8	428.0	450.3	467.5	488.5	526.1	
5/3/2001	66.2	393.6	438.9	457.3	489.8	490.4	493.0	
5/4/2001	67.9	388.5	451.6	467.5	496.2	505.7	500.9	
5/5/2001	67.6	391.7	441.4	473.9	491.7	505.4	505.6	
5/6/2001	67.4	393.3	436.3	477.1	489.5	505.2	507.9	
5/7/2001	67.3	394.9	431.2	480.3	487.3	505.1	510.2	TD
5/8/2001	67.9	408.3	477.1	474.5	520.4	504.5	523.6	
5/9/2001	67.3	428.7	463.7	454.8	470.1	474.5	506.4	
5/10/2001	67.9	428.7	454.8	460.5	479.0	495.5	496.8	
5/11/2001	69.8	565.6	624.2	643.9	672.6	661.8	672.6	

Note: ND = No data
TD = Transition data, not used

Table F5 - Experimental Data of pH of Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	pH							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/29/2001	8.41	6.64	6.56	6.54	6.56	6.6	6.81	TD
1/30/2001	8.37	6.61	6.52	6.51	6.51	6.51	6.63	TD
1/31/2001	8.35	6.58	6.45	6.42	6.42	6.42	6.58	
2/1/2001	8.39	6.62	6.42	6.40	6.40	6.38	6.53	
2/2/2001	8.48	6.62	6.42	6.39	6.38	6.39	6.46	
2/3/2001	ND							
2/4/2001	8.38	6.62	6.46	6.40	6.39	6.38	6.48	
2/5/2001	8.52	6.58	6.45	6.41	6.41	6.41	6.42	
2/6/2001	8.37	6.56	6.39	6.39	6.39	6.39	6.39	
2/7/2001	8.40	6.55	6.38	6.38	6.39	6.38	6.40	
2/8/2001	8.42	6.69	6.35	6.32	6.33	6.35	6.49	TD
2/9/2001	8.38	6.63	6.31	6.32	6.31	6.31	6.40	
2/10/2001	8.44	6.59	6.31	6.31	6.32	6.32	6.41	
2/11/2001	ND							
2/12/2001	8.38	6.51	6.33	6.32	6.32	6.32	6.40	
2/13/2001	8.38	6.42	6.31	6.31	6.31	6.31	6.34	
2/14/2001	8.40	6.44	6.31	6.30	6.30	6.29	6.39	
2/15/2001	8.40	6.40	6.32	6.32	6.32	6.33	6.39	
2/16/2001	8.38	6.38	6.30	6.30	6.30	6.30	6.34	
2/17/2001	8.40	6.39	6.31	6.30	6.30	6.33	6.43	
2/18/2001	8.45	6.45	6.33	6.33	6.34	6.35	6.49	
2/19/2001	8.57	6.42	6.34	6.34	6.36	6.34	6.42	
2/20/2001	8.64	6.43	6.34	6.34	6.34	6.34	6.42	
2/21/2001	8.37	6.32	6.15	6.12	6.13	6.13	6.16	TD
2/22/2001	8.39	6.30	6.17	6.12	6.11	6.11	6.14	TD
2/23/2001	8.48	6.31	6.17	6.12	6.11	6.11	6.16	TD
2/24/2001	8.62	6.33	6.20	6.14	6.13	6.14	6.24	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	8.43	6.40	6.32	6.32	6.31	6.32	6.34	
2/28/2001	8.44	6.39	6.33	6.33	6.33	6.32	6.38	
3/1/2001	8.50	6.36	6.33	6.34	6.34	6.34	6.42	
3/2/2001	8.46	6.36	6.32	6.32	6.32	6.33	6.41	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	8.74	6.47	6.42	6.41	6.39	6.39	6.49	
3/6/2001	8.78	6.37	6.36	6.36	6.36	6.37	6.39	
3/7/2001	8.40	6.40	6.37	6.37	6.38	6.38	6.48	TD
3/8/2001	8.43	6.40	6.40	6.39	6.41	6.40	6.39	TD
3/9/2001	8.52	6.44	6.42	6.41	6.41	6.41	6.44	TD
3/10/2001	8.45	6.49	6.42	6.41	6.40	6.40	6.37	TD
3/11/2001	8.51	6.53	6.47	6.44	6.43	6.43	6.48	TD
3/12/2001	8.41	6.54	6.46	6.43	6.43	6.41	6.42	TD

Table F5 – Continued

Date	pH							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/13/2001	ND							
3/14/2001	8.44	6.43	6.41	6.42	6.40	6.40	6.41	TD
3/15/2001	ND							
3/16/2001	8.42	6.43	6.42	6.42	6.41	6.41	6.43	TD
3/17/2001	8.48	6.41	6.39	6.39	6.40	6.40	6.42	TD
3/18/2001	ND							
3/19/2001	8.45	6.45	6.43	6.41	6.42	6.42	6.44	
3/20/2001	8.56	6.50	6.45	6.41	6.41	6.41	6.44	
3/21/2001	8.45	6.51	6.47	6.44	6.43	6.42	6.50	
3/22/2001	8.47	6.58	6.52	6.46	6.45	6.44	6.43	
3/23/2001	8.58	6.60	6.54	6.50	6.47	6.46	6.46	
3/24/2001	8.82	6.52	6.40	6.40	6.42	6.40	6.40	TD
3/25/2001	8.64	6.52	6.43	6.42	6.42	6.41	6.42	
3/26/2001	8.44	6.60	6.51	6.46	6.44	6.42	6.45	
3/27/2001	8.46	6.51	6.45	6.43	6.42	6.42	6.44	
3/28/2001	8.43	6.48	6.42	6.40	6.40	6.40	6.44	
3/29/2001	8.50	6.47	6.43	6.42	6.42	6.43	6.45	
3/30/2001	8.65	6.49	6.45	6.42	6.42	6.42	6.56	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	8.47	6.47	6.45	6.44	6.44	6.42	6.42	
4/3/2001	8.34	6.44	6.44	6.42	6.41	6.42	6.46	
4/4/2001	8.31	6.46	6.44	6.43	6.43	6.43	6.46	
4/5/2001	8.27	6.48	6.44	6.44	6.44	6.44	6.45	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	8.46	6.49	6.46	6.43	6.36	6.33	6.28	
4/9/2001	8.23	6.57	6.52	6.50	6.50	6.49	6.45	
4/10/2001	8.16	6.52	6.49	6.48	6.48	6.48	6.46	
4/11/2001	8.15	6.51	6.48	6.47	6.46	6.46	6.43	
4/12/2001	ND							
4/13/2001	8.32	6.62	6.60	6.58	6.58	6.57	6.53	
4/14/2001	8.34	6.53	6.51	6.50	6.50	6.49	6.46	
4/15/2001	ND							TD
4/16/2001	8.33	6.67	6.60	6.60	6.52	6.44	6.37	
4/17/2001	ND							
4/18/2001	8.35	6.44	6.42	6.41	6.42	6.41	6.38	
4/19/2001	8.34	6.55	6.53	6.53	6.52	6.52	6.50	
4/20/2001	8.26	6.61	6.59	6.58	6.58	6.58	6.58	
4/21/2001	8.30	6.58	6.56	6.56	6.55	6.55	6.54	
4/22/2001	8.28	6.60	6.58	6.57	6.57	6.57	6.56	
4/23/2001	8.24	6.61	6.59	6.58	6.57	6.56	6.53	

Table F5 – Continued

Date	pH							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/24/2001	ND							
4/25/2001	8.35	6.53	6.52	6.49	6.49	6.48	6.46	
4/26/2001	8.33	6.52	6.50	6.48	6.47	6.47	6.45	
4/27/2001	8.37	6.55	6.51	6.50	6.48	6.48	6.45	
4/28/2001	8.32	6.54	6.52	6.51	6.49	6.49	6.45	
4/29/2001	8.39	6.50	6.50	6.49	6.49	6.49	6.46	
4/30/2001	8.44	6.51	6.48	6.49	6.48	6.48	6.48	
5/1/2001	ND							
5/2/2001	8.51	6.62	6.53	6.50	6.49	6.47	6.54	
5/3/2001	8.43	6.60	6.51	6.48	6.48	6.48	6.46	
5/4/2001	8.49	6.60	6.51	6.50	6.49	6.49	6.49	
5/5/2001	8.49	6.60	6.51	6.50	6.49	6.48	6.48	
5/6/2001	8.49	6.60	6.51	6.49	6.49	6.48	6.47	
5/7/2001	8.49	6.60	6.51	6.49	6.49	6.47	6.46	TD
5/8/2001	8.48	6.65	6.56	6.53	6.52	6.50	6.50	
5/9/2001	8.54	6.65	6.60	6.58	6.56	6.55	6.53	
5/10/2001	8.49	6.59	6.52	6.49	6.48	6.48	6.48	
5/11/2001	8.38	6.37	6.30	6.26	6.27	6.27	6.28	

Note: ND = No data
TD = Transition data, not used

Table F6 - Experimental Data of Temperature of Influent and Effluent from Different Ports of the Sulfur-Packed Continuous Up-Flow Reactor

Date	Temperature, °C							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
1/29/2001	19.4	20.6	20.9	21	21.1	21.2	20.3	TD
1/30/2001	19.2	21.1	21.1	21.2	21.2	21.2	20.3	TD
1/31/2001	19	21.4	21.5	21.6	21.7	21.8	21.3	
2/1/2001	18.5	20.9	21.1	21.2	21.2	21.4	20.6	
2/2/2001	18.7	21.4	21.5	21.6	21.7	21.8	21.1	
2/3/2001	ND							
2/4/2001	18.8	19.5	19.6	19.7	19.9	20	19.6	
2/5/2001	19.7	21.1	21.2	21.4	21.5	21.7	20.7	
2/6/2001	19.7	21	21.2	21.3	21.4	21.4	20.7	
2/7/2001	19.4	21	21.1	21.2	21.3	21.4	20.7	
2/8/2001	19.7	20.6	20.8	20.9	21	21.1	20.6	TD
2/9/2001	20.2	21.6	21.8	21.7	21.7	21.8	21.2	
2/10/2001	19.5	20.9	21	21.1	21.3	21.4	20	
2/11/2001	ND							
2/12/2001	20	21.6	21.6	21.7	21.8	21.9	21	
2/13/2001	20.3	21.6	21.6	21.6	21.6	21.6	21.2	
2/14/2001	20.1	21.3	21.2	21.2	21.2	21.2	20.8	
2/15/2001	20.3	21.9	21.9	21.9	22	22.1	21.8	
2/16/2001	20.2	22	21.9	21.9	22	22	21.6	
2/17/2001	19.6	20.8	21	21	21.3	21.7	20.7	
2/18/2001	18.8	20	20.2	20.3	20.6	20.9	20.3	
2/19/2001	19.8	21.7	21.6	21.6	21.7	21.7	21.4	
2/20/2001	20	21.4	21.5	21.5	21.7	21.7	21	
2/21/2001	20.3	21.4	21.4	21.5	21.5	21.6	21	TD
2/22/2001	20.1	21.8	21.7	21.7	21.9	22	21.2	TD
2/23/2001	19.5	21.3	21.3	21.3	21.3	21.3	20.9	TD
2/24/2001	19.5	20.8	20.9	21	21.2	21.3	21	TD
2/25/2001	ND							
2/26/2001	ND							
2/27/2001	20.3	21.4	21.4	21.3	21.4	21.5	20.9	
2/28/2001	21	22.2	22.2	22.2	22.2	22.3	21.4	
3/1/2001	19.8	21.4	21.6	21.8	22	22.1	20.9	
3/2/2001	20.2	21.5	21.4	21.5	21.6	21.7	20.8	
3/3/2001	ND							
3/4/2001	ND							
3/5/2001	18.8	20.7	20.7	20.8	20.9	21	21.1	
3/6/2001	19	21.5	21.4	21.4	21.5	21.5	20.8	
3/7/2001	20.9	22	22.1	22.1	22.3	22.3	22	TD
3/8/2001	21.1	22.2	22.2	22.3	22.5	22.5	22	TD
3/9/2001	20	21.6	21.6	21.7	21.7	21.7	21.1	TD
3/10/2001	19.6	20.3	20.4	20.5	20.6	20.8	20.5	TD
3/11/2001	19.4	20.5	20.6	20.6	20.7	20.8	20.7	TD
3/12/2001	20.2	20.8	20.9	21	21.2	21.3	20.9	TD

Table F6 – Continued

Date	Temperature, °C							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
3/13/2001	ND							
3/14/2001	19.9	21.3	21.4	21.5	21.6	21.7	21	TD
3/15/2001	ND							
3/16/2001	199	21.7	21.8	21.8	21.8	21.9	21	TD
3/17/2001	18.8	20.4	20.4	205	206	20.7	19.4	TD
3/18/2001	ND							
3/19/2001	20.1	21.6	21.7	21.8	21.9	22	21.3	
3/20/2001	19.5	21.5	21.6	21.7	21.9	22	21.3	
3/21/2001	20.2	21.6	21.6	21.5	21.6	21.6	21.4	
3/22/2001	20	21	21.1	21.2	21.4	21.5	21.1	
3/23/2001	19.4	20.8	20.9	21	21.1	21.3	21	
3/24/2001	19.4	20.6	20.8	20.8	21	21.1	20.6	TD
3/25/2001	ND							
3/26/2001	19.5	20.8	20.8	20.9	21	21.3	20.9	
3/27/2001	19.3	20.8	20.8	20.9	21.1	21.2	20.9	
3/28/2001	19.9	21.2	21.2	21.2	21.4	21.6	21.3	
3/29/2001	19.7	21.4	21.5	21.5	21.6	21.7	20.9	
3/30/2001	18.7	21.6	21.7	21.7	21.7	21.8	21.1	
3/31/2001	ND							
4/1/2001	ND							
4/2/2001	20.2	21.5	21.5	21.5	21.6	21.5	21.1	
4/3/2001	20.5	21.1	21.1	21.1	21.2	21.3	20.8	
4/4/2001	20.5	21.3	21.3	21.3	21.4	21.5	21.1	
4/5/2001	20.5	21.4	21.4	21.4	21.5	21.6	21.4	
4/6/2001	ND							
4/7/2001	ND							
4/8/2001	21.6	22.7	22.7	22.8	23	23.2	22.9	
4/9/2001	20.9	21.4	21.3	21.3	21.4	21.4	21	
4/10/2001	20.4	21.5	21.4	21.4	21.3	21.3	21	
4/11/2001	21	22	22	22.1	22.1	22.1	21.7	
4/12/2001	ND							
4/13/2001	21	21.7	21.7	21.8	21.8	21.8	21.7	
4/14/2001	21.0	21.8	21.8	21.9	22.0	22.0	21.6	
4/15/2001	ND							TD
4/16/2001	20.9	21.3	21.2	21.3	21.4	21.4	20.9	
4/17/2001	ND							
4/18/2001	20.9	21.8	21.9	22	22.1	22.1	21.4	
4/19/2001	19.9	21.5	21.5	21.6	21.7	21.7	21.2	
4/20/2001	20.4	21.1	21.1	21.1	21.1	21.2	21	
4/21/2001	20.2	21.3	21.3	21.4	21.4	21.5	21.1	
4/22/2001	20.2	21.3	21.4	21.4	21.4	21.5	21	
4/23/2001	20.3	21.3	21.4	21.4	21.4	21.5	21.1	

Table F6 – Continued

Date	Temperature, °C							
	Influent	Effluent from Sampling Ports						Remarks
		S-1	S-2	S-3	S-4	S-5	S-6	
4/24/2001	ND							
4/25/2001	20.3	21.2	21.3	21.3	21.4	21.4	20	
4/26/2001	20.5	21.3	21.3	21.4	21.4	21.4	21	
4/27/2001	20.4	21.2	21.2	21.2	21.3	21.3	20.9	
4/28/2001	20.5	21.3	21.3	21.3	21.4	21.4	21.0	
4/29/2001	20.4	21.2	21.2	21.3	21.3	21.3	20.9	
4/30/2001	21	21.9	21.9	22	22	22	21.3	
5/1/2001	ND							
5/2/2001	20.4	21.2	21.2	21.3	21.4	21.4	20.9	
5/3/2001	20.6	21.3	21.3	21.3	21.4	21.6	21	
5/4/2001	20.4	21.1	21.1	21.2	21.3	21.4	20.9	
5/5/2001	20.5	21.2	21.2	21.3	21.4	21.5	21.0	
5/6/2001	20.5	21.2	21.2	21.2	21.3	21.5	20.9	
5/7/2001	21.2	21.9	21.9	21.9	21.9	21.9	21.1	TD
5/8/2001	20.5	21.2	21.1	21.2	21.3	21.4	20.8	
5/9/2001	20.3	21.1	21	21	21	21	20.3	
5/10/2001	20.3	20.9	20.8	20.8	20.8	20.8	20.1	
5/11/2001	20.6	21.4	21.4	21.4	21.4	21.4	20.9	

Note: ND = No data
TD = Transition data, not used

APPENDIX G

DETERMINATION OF REACTION RATE CONSTANT k_1 USING FUJIMOTO METHOD

The ratios of $\mathrm{NO_3^- - N}$ concentration at any time *t* to initial $\mathrm{NO_3^- - N}$ concentration, and calculation steps of Fujimoto method are summarized in Table G1. The determination procedures for ultimate value of (1-*C/C$_o$*) are plotted in Figure G1.

Table G1 - Determination of Reaction Rate Constant k_1 Using Fujimoto Method

C/C_o	$(1-C/C_o)_n$	$(1-C/C_o)_{n+1}$	$(1-C/C_o)$ Ultimate	k_1	*t,* hrs
100.00%	0.00%	75.33%	97.6%		0
24.67%	75.33%	86.84%	97.6%	1.23	1.2
13.16%	86.84%	92.59%	97.6%	1.47	1.5
7.41%	92.59%	94.49%	97.6%	1.49	2
5.51%	94.49%	96.34%	97.6%	1.44	2.4
3.66%	96.34%	97.62%	97.6%	1.41	3.1
2.38%	97.62%	97.83%	-	-	3.7
2.17%	97.83%	98.62%	-	-	4
1.38%	98.62%	98.75%	-	-	4.6
1.25%	98.75%	98.92%	-	-	4.9
1.08%	98.92%	99.16%	-	-	6
0.84%	99.16%	99.23%	-	-	6.1
0.77%	99.23%	99.25%	-	-	7.3
0.75%	99.25%	99.10%	-	-	7.6
0.90%	99.10%	99.27%	-	-	8
0.73%	99.27%	99.04%	-	-	9.1
0.96%	99.04%	99.08%	-	-	10
0.92%	99.08%				12
Average				1.41	

Note: $k_1 = \dfrac{\ln(1-(1-C/C_o)_n/(1-C/C_o)_{ultimate})}{t_2 - t_1}$

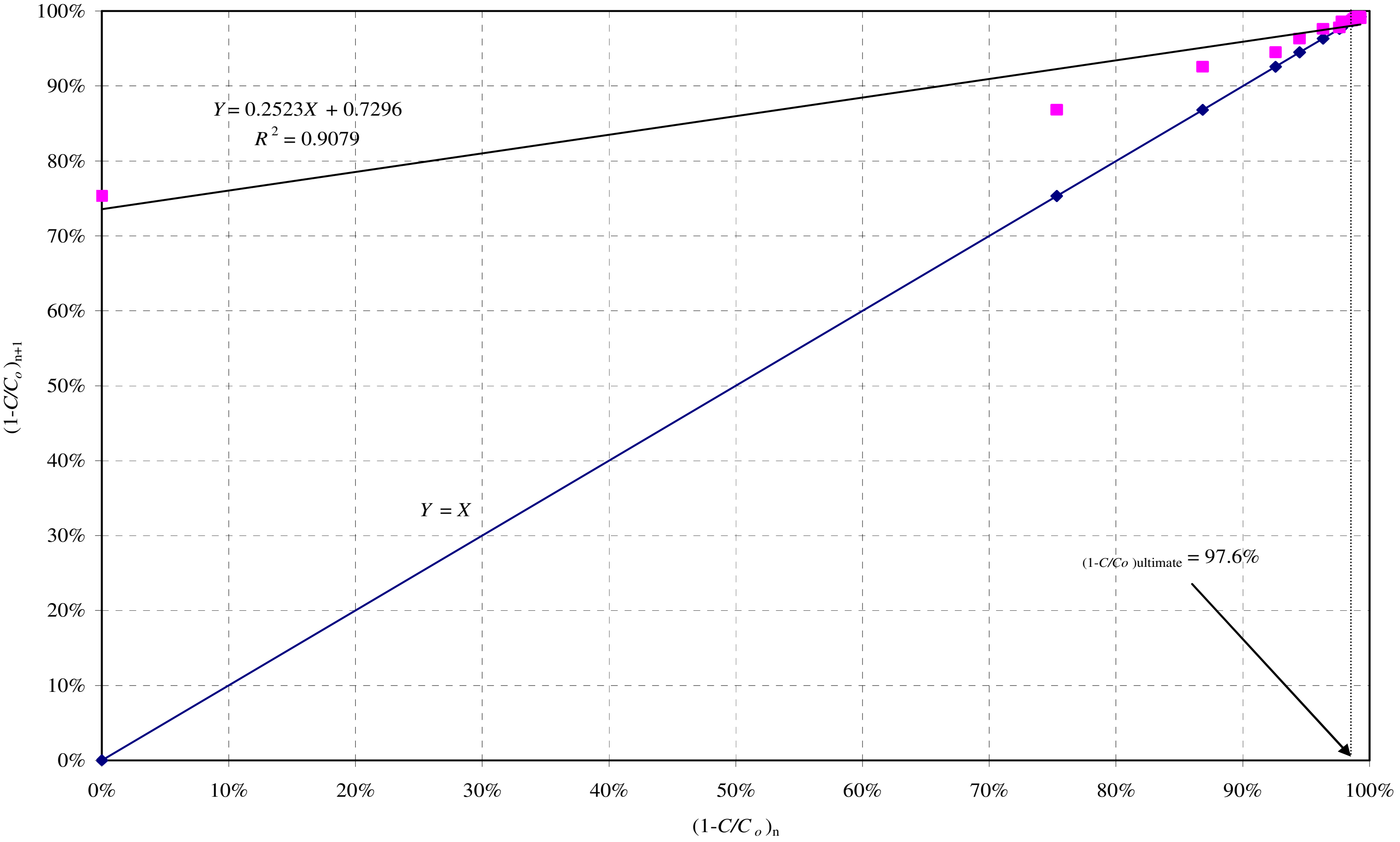


Figure G1 Determination of $(1-C/C_o)_{\text{ultimate}}$ using Fujimoto method

REFERENCES

1 American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), 1992 "*Standard Methods for the Examination of Water and Wastewater,*" 18th Edition, Washington, D.C.

2 American Water Works Association (AWWA), 1995, "Survey Reveal Pesticides, Nitrates in Water from Farming Areas," *Journal of American Water Works Association*, vol. 87, No. 10, pp. 13-14

3 Baalsrud, K. and K. S. Baalsrud, 1954, "Studies on Thiobacillus Denitrificans," *Archives of Microbiology*, vol. 20, No. 1, pp. 34-62

4 Barcelona, M. J., 1984, "TOC Determinations in Groundwater," *Ground Water*, vol. 22, No. 1, pp. 18-24

5 Barrenstein, A., U. Kramer, and P. Obermann, 1986, "Underground Treatment of Nitrate Rich Groundwater by Infiltration with Treated Wastewater or Methane-Rich Natural Gas," DVGW-Schriftenreihe, Wasser, Frankfort, West Germany, vol. 106, pp. 99-116

6 Batchelor, B., and A. W. Lawence, 1978a. "A Kinetic Model for Autotrophic Denitrification Using Elemental Sulfur," *Water Research*, vol. 12, No 12, pp. 1075-1084

7 Batchelor, B., and A. W. Lawence, 1978b, "Autotrophic Denitrification Using Elemental Sulfur," *Journal of Water Pollution Control Federation*, vol. 50, No 8, pp. 1986-2001

8 Batchelor, B., and A. W. Lawence, 1978c. "Stoichiometry of Autotrophic Denitrification Using Elemental Sulfur," In: A.J. Rubin (Ed.), *Chemistry of Wastewater Technology*. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, pp. 421-440.

9 Bergey, D. H., R. E. Buchanan, and N. E. Gibbons, 1974, "Bergey's Manual of Determinative Bacteriology," Eighth Edition, *Williams & Wilkins Company*, Baltimore, MD, pp. 456-461

10 Bergey, D. H., N. R. Krieg, and J. G. Holt, 1986, "Bergey's Manual of Systematic Bacteriology," Volume II, *Williams & Wilkins Company*, Baltimore, MD, pp. 427-438

11 Bisogni, J. J. Jr., and C. T. Driscoll, 1977, "Denitrification Using Thiosulfate and Sulfide," *Journal of Environmental Engineering Division,* American Society of Civil Engineering, vol. 103, No. 4, pp. 593-604

12 Blecon, G., M. Gillet, G. Martin, and J. M. Philipot, 1983, "Autotrophic Biological Denitrification by Thiobacillus Denitrificans on Sulfur-Marl," *Revue Francaise des Science de I'Eau*, vol. 2, No. 3, pp. 267-279

13 Bouchard, D. C., M. K. Williams, and R.Y. Surampalli, 1992, "Nitrate Contamination of Groundwater: Sources and Potential Health Effects," *Journal of American Water Works Association*, vol. 84, No. 9, pp. 85-90

14 Bouwer, E. J., and P. B. Crowe, 1988, "Biological Processes in Drinking Water Treatment," *Journal of American Water Works Association*, vol. 80, No. 9, pp. 82-93

15 Brezonik, P. L., 1977, "Denitrification in Natural Waters," *Progress in Water Technology*, vol. 8, No. 4-5, pp. 373-392

16 Brock, T. D., M. T. Madigan, J. Martinko, and J. Parker, 1994, "Biology of Microorganisms," Prentice Hall International Editions, Seventh Edition, Englewood Cliffs, New Jersey.

17 Challis, B. C., 1973, "Rapid Nitrosation of Phenols and its Implications for Health Hazards from Dietary Nitrites," *Nature*, vol. 244 (5416), p. 466

18 Champ, D. R., J. Gulens, and R.E. Jackson, 1979, "Oxidation-Reduction Sequences in Groundwater Flow Systems," *Canadian Journal of Earth Science,* vol. 16, No. 1, pp. 12-23

19 Cizinska, S., V. Mateju, J. Krejci, T. Janoch and E. Kyslikova, 1992, "Water Denitrification by an Immobilized Biocatalyst," *Journal of Chemical Technology and Biotechnology*, vol. 55, No. 1, pp. 33-38

20 Claus, G., and H. J. Kutzner, 1985a, "Autotrophic Denitrification by *Thiobacillus Denitrificans* in a Packed Bed Reactor," *Applied Microbiology and Biotechnology*, vol. 22, No. 3, pp. 289-296

21 Claus, G., and H. J. Kutzner, 1985b, "Physiology and Kinetics of Autotrophic Denitrification by *Thiobacillus Denitrificans*," *Applied Microbiology and Biotechnology*, vol. 22, No. 2, pp. 283-288

22 Clifford, D., and X. Liu, 1993, "Ion Exchange for Nitrate Removal," *Journal of Ammerican Water Works Association*, vol. 85, No. 4, pp. 135-143

23 Dahab, M. D., 1991, "Nitrate Treatment Methods: an Overview," North Atlantic Treaty Organization Advanced Science Institutes, Series, G 30. *Nitrate Contamination*, Edit by Bogardi, I., and Kuzelka, R.D., Spring-Verlag, Berlin Heidelberg, pp. 349-368

24 Davidson, M. S., and H. F. Ridgeway, 1995, "Biological Denitrification of Groundwater Using Sulfur-Oxidizing Bacteria, : Construction and Evaluation of a 25 gpm Pilot System," Report of A Cooperative Project of Orange County Water District, and the Metropolitan Water District of Southern California

25 Davies, T. R., 1973, " Isolation of Bacteria Capable of Utilizing Methane as a Hydrogen Donor in the Process of Denitrification," *Water Research*, vol. 7, No. 4, April, pp. 575-579

26 Delanghe, B., F. Nakamura, H. Myoga, and Y. Magara, 1994a, "Biological Denitrification with Ethanol in a Membrane Bioreactor," *Environmental Technology*, vol. 15, No. 1, pp. 61-67

27 Delanghe, B., J. Nakamura, H. Myoga, Y. Magara and E. Guibal, 1994b, "Drinking Water Denitrification in a Membrane Bioreactor," *Water Science Technology*, vol. 30, No. 6, pp. 157-160

28 Exner, M. E., and R. F. Spalding, 1990, "Occurrence of Pesticides and Nitrate in Nebraska's Groundwater," *Water Center Publication*, No. 1. Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln.

29 Flere, J. M., 1997, "Remediation of Nitrate-Contaminated Water Using Sulfur and Limestone Autotrophic Denitrification Processes," M.S. Thesis, Department of Civil Engineering, University of Nebraska-Lincoln at Omaha Campus, Omaha, NE

30 Frank, C., and W. Dott, 1985, "Nitrate Removal from Drinking-Water by Biological Denitrification," *Vom Wasser*, Weinheim, Germany, Vol. 65, pp. 287-295.

31 Frick, B. R., and Y. Richard, 1985, “Experience with Biological Denitrification in a Full Scale Drinking Water Treatment Plant,” *Vom Wasser*, vol. 64, pp. 145-154

32 Frunzke, K., and O. Meyer, 1990, “Nitrate Respiration, Denitrification, and Utilization of Nitrogen Sources by Aerobic Carbon Monoxide-Oxidizing Bacteria, *Archives of Microbiology*, vol. 154, No. 2, pp. 168-174

33 Furumai, H., H. Tagui, and K. Fujita, 1996, “Effects of pH and Alkalinity on Sulfur-Denitrification in a Biological Granular Filter,” *Water Science Technology*, Printed in Great Britain, vol. 34, No. 1-2, pp. 355-362

34 Gantzer, C. J., 1995, “ Membrane Dissolution of Hydrogen for Biological Nitrate Removal,” *Proceedings of Water Environment Federation* 8th Annual Conference, Miami Beach, Florida

35 Gaudy, A. F. Jr., and E. T. Gaudy, 1980, “Microbiology for Environmental Scientists and Engineers,” *McGraw-Hill, Inc*., New York, p. 195

36 Gauntlett, R. B., and D. G. Craft, 1979, “Biological Removal of Nitrate from River Water,” *Technical Report TR 98*, Water Research Centre, Medmenham, England

37 Gayle, B. P., G. D. Boardman, J.H. Sherrard, and R.E. Benoit, 1989, “Biological Denitrification of Water,” *Journal of Environmental Engineering Division*, ASCE, vol. 115, No. 5, pp. 930-943

38 Gross, H., and K. Treuter, 1986, “Biological Denitrification Process with Hydrogen-Oxidizing Bacteria for Drinking Water Treatment,” *Aquarium*, vol. 5, No. 5, pp. 288-290

39 Hach Water Analysis Handbook, 1992, 2nd Edition, Hach Company, Loveland, Colorado

40 Hashimoto, S., K. Furikawa, and M. Shiyana, 1989, “Enrichment of Sulfur Denitrifying Bacteria and Its Accumulation to Elemental Sulfur,” *Journal of Water Pollution Research*, vol. 12, No. 7, pp. 431-440

41 Hiscock, K. M., J. W. Lloyd, and D. N. Lerner, 1991, “Review of Natural and Artificial Denitrification of Groundwater,” Printed in Great Britain, *Water Resources,* vol. 25, No. 9, pp. 1099-1111

42 van der Hoek, J. P., W. A. M. Hijnen, C. A. van Bennekom, and B. J. Mijnarends, 1992a, "Optimization of the Sulfur-Limstone Filtration Process for Nitrate Removal from Groundwater," *Journal of Water SRT-AQUA,* vol. 41, No. 4, pp. 209-218

43 van der Hoek, J. P., J. W. N. M. Kappelhof, and W. A. M. Hijnen, 1992b, "Biological Nitrate Removal from Ground Water by Sulphur/Limestone Denitrifcation," *Journal of Chemical Technology and Biotechnology*, vol. 54, No. 2, pp. 197-200

44 van der Hoek, J. P., P. J. M. van der Ven, and A. Klapwijk, 1988b, "Combined Ion Exchange/Biological Denitrification for Nitrate Removal from Groundwater Under Different Process Conditions," *Water Research*, vol. 22, No.6, pp. 679-684

45 Howard, K. W. F., 1985, "Denitrification in a Major Lime Stone Aquifer," *Journal of Hydrology*, vol. 76, pp. 265-280

46 Justin P., and D. P. Kelly, 1978a, "Growth Kinetics of Thiobacillus Denitrificans in Anaerobic and Aerobic Chemostat Culture," *Journal of General Microbiology,* vol. 107, No. 1, pp. 123-130

47 Kapoor, A., and T. Viraraghavan, 1997, "Nitrate Removal From Drinking Water-Review," *Journal of Environmental Engineering Division,* ASCE, vol. 123, No. 4, April, pp. 371-380

48 Kelly, D. P., 1982, "Biochemistry of the Chemolithotrophic Oxidation of Inorganic Sulphur," *Philosphical Transactions*, Royal Society of London, Series B 298, pp. 499-528

49 Kurt, M., I. J. Dunn, and J. R. Bourne, 1987, "Biological Denitrification of Drinking Water Using Autotrophic Organisms with Hydrogen in a Fludized-Bed Biofilm Reactor," *Biotechnology Bioengineering*, vol. 29, No. 4, pp. 493-501

50 Korom, S., 1992, "Natural Denitrification in the Saturated Zone: A Review," *Water Resources Research*, vol. 28 No. 6, pp. 1657-1668

51 Lampe, D. G., and T.C. Zhang, 1997. "Sulfur-based Autotrophic Denitrification for Remediation of Nitrate-contaminated Water," *In Situ and On-Site Bioremediation Symposium*. New Orleans, April 28 – May 1. Battelle Press

52 Lewandowski, I., R. Bakke, and W. G. Characklis, 1987, "Nitrification and Autotrophic Denitrification in Calcium Alginate Beads," *Water Science Technology*, vol. 19, pp. 175-182

53 Lyubchenko, O. A., N. F. Mogilevich, and P. I. Gvozdyak, 1996, “Microbial Nitrification and Sewage Treatment,” *Journal of Water Chemistry and Technology*, vol. 18, No. 1, pp. 98-112

54 McCarty, P. L., “Energetics and Bacterial Growth,” Presented at the July, 1969, 5th Rudolf Research Conference, Rutgers University, New Brunswick, N.J.

55 Metcalf and Eddy, Inc. Revised by Tchobanoglous, G., L. Franklin, H. Burton, and D. Stensel, 2003, “Wastewater Engineering: Treatment and Reuse,” *McGraw Hill Companies Inc*. Fourth Edition, New York, NY.

56 Mirvish, S., 1991, “The significance for Human Health of Nitrate, Nitrite, and Nitroso Compounds,” North Atlantic Treaty Organization, Advanced Science Institutes, Series G30. *Nitrate Contamination*. Edit by Bogardi, I., and R. D. Kuzelka, pp. 253-266, Springer-Verlag Berlin Heidelberg

57 Myers, R. J. K., 1972, “Effect of Sulfide on Nitrate Reduction in Soil,“ *Plant and Soil,* vol. 37, No. 2, pp. 431-433

58 Overath, H., A. Hussmann, and K. Haberer, 1986, “Biological Denitrification by Thiobacillus Denitrificans Using Elemental Sulfur Fixed on Granular Activated Carbon as Electron Donor,” *Vom Wasser*, vol. 66, pp.59-83

59 Pontius, F. W., 1993, “Nitrate and Cancer: Is There a Link,” *Journal of American Water Works Association*, vol. 85, No. 4, pp. 12-14.

60 Power, J. F. and J. S. Schepers, 1989, “Nitrate Contamination of Groundwater in North America,” *Agriculture, Ecosystems and Environment*, vol. 26, No. 3-4, pp. 165-187

61 Reddy, K. R., P. S. C. Rao, and R. E. Jessup, 1982, “The Effect of Carbon Mineralization on Denitrification Kinetics in Mineral and Organic Soils,” *Soil Science Society of America Journal*, vol. 46, No. 1, pp. 62-68

62 Richard, Y., A. Leprince, G. Martin, and C. Leblanc, 1980, “Denitrification of Water for Human Consumption,” *Progress in Water Technology*, Oxford, England, vol. 12, No. 6, pp. 173-191

63 Rittmann, B. E., and P. M. Huck, 1989, “Biological Treatment of Public Water,” *Chemical Rubber Company, Critical Reviews in Environmental Control*, vol. 19, No. 2, pp. 119-184

64 Sawyer, C. N. and P. L. McCarty., 1978 “Chemistry for Environmental Engineering” Third Edition, pp. 242-245

65 Sikora L. J. and D. R. Keeney, 1976, “Evaluation of a Sulfur Thiobacillus Denitrificans Nitrate Removal System,” *Journal of Environmental Quality*, vol. 5, No. 3, pp. 298-303

66 Skerman, V. B. and I. C. MacRae, 1957, “The Influence of Oxygen Availability on the Degree of Nitrate Reduction by Pseudomonas Denitrificans,” *Canadian Journal of Microbiology*, vol. 3, pp. 505-530

67 Smith, R. L., and J. H. Duff, 1988, “Denitrification in a Sand and Gravel Aquifer,” *Applied and Environmental Microbiology*, vol. 54, No. 5, pp. 1071-1078

68 Smith, R. L., M. L. Ceazan, and M. H. Brooks, 1994, “Autotrophic, Hydrogen-Oxidizing, Denitrifying Bacteria in Groundwater, Potential Agents for Bioremediation of Nitrate Contamination,” *Applied and Environmental Microbiology*, vol. 60, No. 6, pp. 1949-1955

69 Thurman, E. M., 1985, ”Organic Geochemistry of Natural Waters,” *Nijhoff-Junk, Dordrecht.*

70 Waki, T., K. I. Murayma, Y. Kawato, and K. Ichikawa, 1980, “Transient Characteristics of Paracoccus Denitrificans with Changes Between Aerobic and Anaerobic Conditions, *Journal of Fermentation Technology,* vol. 58, No. 3, pp. 243-249

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