HIGH-RATE AUTOTROPHIC DENITRIFICATION OF SIMULATED INDUSTRIAL WASTEWATER

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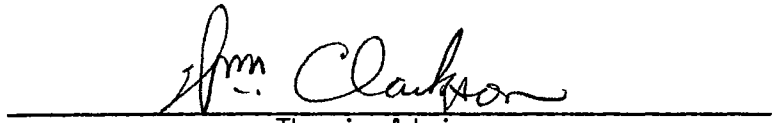

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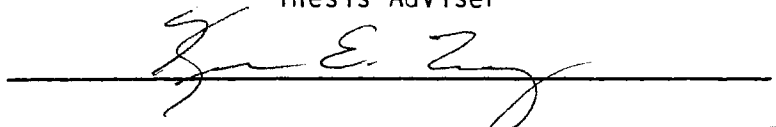

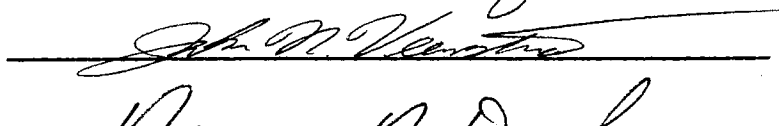

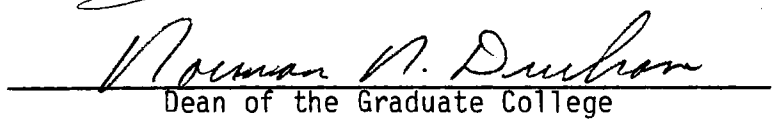

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CHAPTER I

INTRODUCTION

Although considerable research has been conducted on biological denitrification, relatively little effort has been directed at autotrophic denitrification and even less on high strength wastes. This research was undertaken to examine whether high-rate autotrophic denitrification was possible for treating simulated industrial wastewater.

High nitrogenous wastes are generated from industries such as munitions, nuclear, fertilizers, semiconductors, and meat and milk processing. Nitrate or ammonia is the principal form of nitrogen in these wastes. For such wastes, biological nitrification is an effective pretreatment to convert ammonia to nitrate. High concentrations of nitrate present either in raw or pretreated wastes may cause several types of problems. It may cause eutrophication if nitrate is the limiting nutrient in the receiving water, which can lead to algal blooms. Some species of algae impart turbidity, color, taste and odor to water. Nitrate concentration >45 mg/L can cause methemoglobinemia in infants and ruminant livestock.

Denitrification is one of several advanced treatments practiced to convert undesirable nitrate to nitrogen gas, an innocuous product making up the bulk of the atmosphere. Biological denitrification is a

microbial process in which denitrifying bacteria use nitrate as an electron donor. In the case of autotrophic denitrification, bacteria of the species Thiobacillus denitrificans use only inorganic chemicals for their carbon and energy requirements. Since organics are undesirable in receiving waters and it is difficult to obtain residual organic-free effluent from heterotrophic denitrification, autotrophic processes may possess advantages in this regard. Low biomass yield associated with this process can reduce the cost of sludge treatment and handling.

1.1 Objectives

This study investigated the potential of using autotrophic denitrification for nitrate removal from high strength wastewater. The specific objectives were twofold. The first objective was to evaluate nitrate removal efficiency in two treatment processes--the attached film expanded bed and the upflow sludge blanket. The second purpose was to determine stoichiometric parameters for systems using thiosulfate as an electron donor.

CHAPTER II

LITERATURE REVIEW

2.1 Nitrogen Removal From Wastewaters

In recent years, considerable attention has been devoted to controlling discharges to the environment of nitrogenous wastewaters. High concentration of nitrogen is present either as ammonia or nitrate in wastewater originating from industries such as munitions, nuclear, fertilizers, refineries, synthetic fiber, and meat and milk processing. Regenerant from ion exchangers used for the removal of nitrate from drinking water and wash-water from industries that clean with ammonia-containing compounds can also add significant quantities of nitrogen to the wastewater stream.

Ammonia nitrogen entering receiving water is objectionable for at least three reasons. It may serve as an important nutrient to support aquatic plants. Ammonia is toxic to fish at concentrations as low as 0.5 mg/L. Also, excessive nitrification of ammonia can exert an oxygen demand and exhaust the natural purifying capacity of receiving waters.

Nitrification, the first step in the overall nitrogen removal process, is an essential pretreatment for industrial wastes containing ammonia. This pretreatment is beneficial because the oxygen demand

associated with this oxidation process can be satisfied in a treatment plant outside the receiving waters. Nitrification does not remove nitrogen from wastes; it simply converts ammonical form to nitrate.

High concentration of nitrate present either in raw or pretreated wastes represents an unacceptable constituent. Nitrogenous compounds such as nitrates and nitrites in drinking waters have been a concern to authorities for public health reasons (Winneberger, 1982). The USPHS Drinking Water Standards of 1962 has set the limit of nitrates in drinking water at 45 mg/L. This is equivalent to 10 mg/L of nitrate nitrogen. This limit has been set to avert methemoglobinemia, a disease largely confined to infants less than three months old. Infants' characteristically high pH stomachs provide a suitable environment for nitrate-reducing bacteria which reduce nitrate to nitrite. Nitrite reacts with hemoglobin of blood to form methemoglobin. This altered form of hemoglobin no longer can carry oxygen from the lungs to the tissues. The physiological effects are anoxemia and sometimes death (Walton, 1951).

If nitrogen is the limiting nutrient in the receiving water, it may cause algal blooms. Such growths are unaesthetic and hinder recreational activities such as boating and swimming in lakes. They can exert high dissolved oxygen demand during the night and during senescence which may be detrimental to other aquatic forms of life. Algal photosynthesis and respiration can cause large diurnal variations in pH in surface waters (Sawyer and McCarty, 1967).

Algae in potable water can be objectionable from the aesthetic point of view. Consumer complaints would increase if large numbers of algae passed through the filters and created turbidity in the treated

water. Algae upon death and decay impart tastes and odors to water. If present in raw water, they can increase chlorine demand and clog filters. These problems necessitate treatments at additional costs to make the water suitable for human consumption.

2.2 Nitrogen Removal Process

Nitrate is highly soluble in water. It is not adsorbed to any significant extent on particulate matter. It is quite inert and does not precipitate with other chemicals (Montgomery, 1985). Of the several treatment techniques available, biological processes have been generally found to be the most economical means for controlling nitrogen in wastewater effluents (Barnes and Bliss, 1983). Biological denitrification is a process in which nitrate is converted to innocuous molecular nitrogen gas. As an important part of the nitrogen cycle, denitrification is considered necessary for recycling nitrogen to the atmosphere.

Based on metabolism, bacterial denitrifiers are either autotrophs or heterotrophs having the capability to use nitrate as a terminal electron acceptor in anoxic respiration. Autotrophs use inorganic chemicals for their carbon and energy requirements and heterotrophs use organic chemicals for their carbon and energy requirements. Generally, industrial wastewater of high nitrate content is poor in carbon and energy sources. The choice between heterotrophic and autotrophic denitrification processes depends mainly on cost of chemicals and problems arising from use of chemicals to meet the growth requirements of denitrifying bacteria.

Heterotrophic denitrification processes have been successful in many instances. A major disadvantage of this process arises from the use of organic chemicals. Although it may be possible to minimize effluent soluble organics by adjusting the carbon:nitrogen ratio below stoichiometric requirements, the process may not produce organics-free effluent (Dahab and Lee, 1988). Soluble organics in the effluent are objectionable because they can exert oxygen demand in the receiving waters. Dissolved organics would increase chlorine demand in water supplies. Also, they are potential precursors for organochloro compounds. These facts suggest that further treatment is necessary if dissolved organics are present in the denitrified effluent. This would increase the overall cost of heterotrophic denitrification processes. This consideration becomes increasingly important with higher nitrogen concentration in wastewater.

The rising cost of petrochemicals spurred researchers' interest in the autotrophic denitrification process (Bisogni and Driscoll, 1977; Batchelor and Lawrence, 1978; Driscoll and Bisogni, 1978). In autotrophic denitrification processes, nitrate serves as the terminal electron acceptor in the oxidation of an inorganic electron donor, such as hydrogen and reduced forms of sulfur, iron, etc. Carbon dioxide is the carbon source. An additional advantage claimed of autotrophic denitrification over heterotrophic denitrification is that the former is more stable under transient conditions (Batchelor and Lawrence, 1978). Momentary overdoses of thiosulfate and bicarbonate may not be as objectionable as organic carbon. Denitrification can be carried out autotrophically by Thiobacillus denitrificans.

2.3 Thiobacillus Denitrificans

Thiobacillus denitrificans was first isolated by Beijernick in 1904 (Taylor et al., 1971). This rod-shaped bacteria measures 0.5 by 1.0 μ and is highly motile, gram negative, and non-spore forming (Baalsrud and Baalsrud, 1954). T. denitrificans can grow efficiently in aerobic as well as anaerobic environments. Cell yield has been reported higher for aerobic growth (Justin and Kelly, 1978). This facultative anaerobe can derive its energy from the oxidation of reduced sulfur compounds using oxygen or nitrate as the terminal electron acceptor (Vishniac and Santer, 1957). T. denitrificans can utilize carbon from carbon dioxide, carbonates, and bicarbonates (Breed et al., 1957) and grows well near neutral pH values (Kuenen and Tuovinen, 1981).

2.4 Biochemistry of Denitrification

The biological process by which organisms pass electrons to a terminal electron acceptor when they oxidize organic or inorganic substrate is known as respiration. In aerobic respiration, molecular oxygen is used as the terminal electron acceptor whereas some bacteria use oxides of inorganic substances as the terminal electron acceptor when they carry out anaerobic respiration. Denitrification is a particular form of anaerobic respiration in which the electron acceptor nitrate or nitrite is reduced to nitrogen gas (Gaudy and Gaudy, 1988).

The ability to consume any oxide of nitrogen can vary from organism to organism. Thiosphaera pantotropha can utilize oxygen and nitrate simultaneously (Robertson and Kuenen, 1984). Thiomicrospira

denitrificans can use either nitrate or nitrite as an electron acceptor (Payne, 1981). Pseudomonas denitrificans grows well with nitrate, nitrite, or nitrous oxide as the terminal electron acceptor (Koike and Hattori, 1975). Thiobacillus denitrificans uses nitrate as an electron acceptor.

Bacteria that can use nitrate as an electron acceptor generally do so in an oxygen-free environment. In biological oxidation-reduction reactions, oxygen has more free energy than nitrate. Calculations presented by McCarty (1972) show that the free energy yield for the transfer of an electron equivalent from glucose to oxygen is 28.7 kcal and to nitrate is 19.45 kcal. Justin and Kelly (1978) say that the theoretical free energy for nitrate and oxygen-limited thiosulfate oxidation is 741 and 936 kJ per mole, respectively. This equals 22.1 kcal for nitrate and 27.9 kcal for oxygen for oxidation of one electron equivalent of thiosulfate.

From a thermodynamic point of view, oxygen respiration is favorable to the microorganisms. In general, not only is more ATP formed during aerobic respiration, it is also formed more efficiently (Grady and Lim, 1980). Justin and Kelly (1978) observed that the cell yield (g dry wt cell/mole thiosulfate) was 11.37 for nitrate and 14.69 for oxygen respiration.

The following biochemical sequence proposed by Payne (1973) has been accepted by many authors for the reduction of nitrate to nitrogen gas:

$$\text{Nitrate} \longrightarrow \text{Nitrite} \longrightarrow \text{Nitric oxide} \longrightarrow \text{Nitrous oxide} \longrightarrow \text{Nitrogen gas}$$

$$NO_3 \longrightarrow NO_2 \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

In denitrification, nitrate is reduced to nitrogen gas by a dissimilatory process. Basically, there are two major steps involved in this reduction. Nitrate is first reduced to nitrite by nitrate reductase and nitrite is further reduced to nitrogen gas by one or more enzymes via nitric oxide and nitrous oxide. The biochemistry of nitrite reduction is not well established.

In the competition between oxygen and nitrate for an electron donor, nitrate can take over for oxygen only in the presence of enzymes required for nitrate respiration. In the presence of oxygen, enzymes for nitrate respiration or denitrification are not usually synthesized (Haddock, 1977; Whatley, 1981). Oxygen produces a non-competitive inhibition of nitrate reduction. That is, the electron transport to oxygen is much easier than to nitrate. Therefore, in the presence of oxygen, no apparent denitrification takes place (Moore and Schroeder, 1970).

Electron transport chain pathways of oxygen and nitrate have flavins, quinones, and cytochrome b and c in common. Cytochrome a is found only in the aerobic pathway. In oxidative phosphorylation, it is believed that one mole of ATP is formed in association with the reoxidation of NADH by the flavin, another one in electron transfer from flavin to cytochrome b, and the third in the oxidation of cytochrome a. Since cytochrome a takes part only in aerobic respiration, this ATP-producing step is bypassed in denitrification. Therefore, a maximum of only two moles of ATP can be produced per mole of NADH during denitrification compared to three moles from aerobic respiration (Grady and Lim, 1980).

2.5 Nitrite Accumulation

No unifying explanation has been found in the literature for the accumulation of nitrite during the process of denitrification. Fecal coliform bacteria can reduce nitrate to nitrite and cause nitrite to accumulate in systems fed with domestic sewage (Wilderer, et al., 1987). Some authors have concluded that nitrite buildup may result from the nitrite reduction rate falling below the rate of nitrate reduction (Betlach and Tiedje, 1981; Wilderer et al., 1987). Kuenen and Tuovinen (1981) report that enrichments for T. denitrificans often result in mixed cultures of nitrite-producing thiobacilli and heterotrophic bacteria.

Since nitrite is chemically very reactive, it can be potentially toxic to the bacterium (Cole, 1988). Baalsrud and Baalsrud (1954) have reported that nitrite accumulation could inhibit denitrification of T. denitrificans. However, inhibitory concentrations of nitrite appear to be rather high. Claus and Kutzner (1985a) have reported that nitrite above 200 mg NO_2^-/L affected autotrophic denitrification.

2.6 Biochemistry of Thiosulfate Oxidation

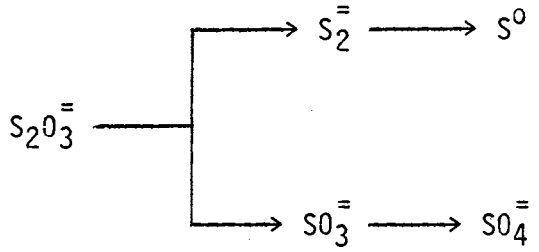
In nature, inorganic nitrogen and sulfur compounds exist in various forms. Interestingly, many reduced inorganic compounds are used by chemolithotrophic bacteria as the sole source of energy for growth. For denitrification by T. denitrificans, sulfur or its reduced compounds can serve as electron donors. Although considerable research has been performed, there is still much uncertainty regarding the biochemistry involved in the oxidation of thiosulfate by thiobacilli. Oh

and Suzuki (1980) have summarized the theories of thiosulfate oxidation under three categories.

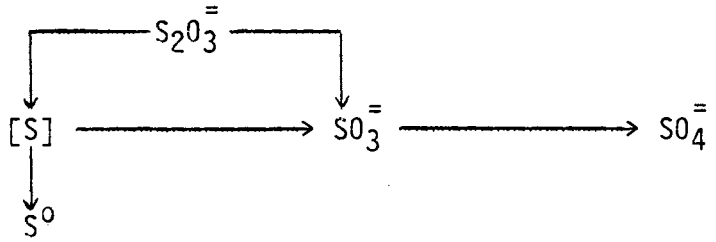
The first theory is that thiosulfate is oxidized to sulfate via tetrathionate and other polythionates (Vishniac and Santer, 1957):

$$S_2O_3^{=} \longrightarrow S_4O_6^{=} \longrightarrow S_3O_6^{=} \longrightarrow SO_3^{=} \longrightarrow SO_4^{=}$$

The second theory is that thiosulfate is first split to sulfate and sulfide. Sulfide is oxidized to sulfur whereas sulfite is oxidized to sulfate. Polythionates are not formed in this type of biochemical reaction (Peck, 1962; Peck and Fisher, 1962):



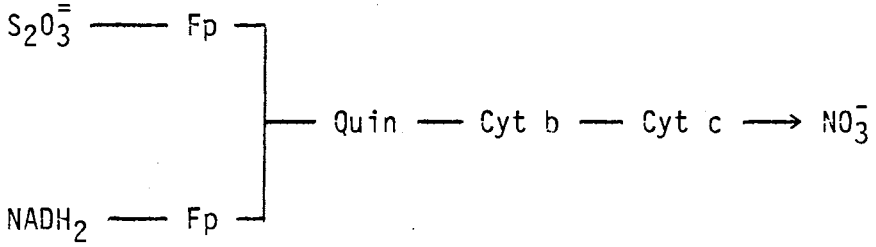
According to the third theory, thiosulfate is oxidized to sulfate following cleavage to sulfur and sulfite (Charles and Suzuki, 1966; Lyric and Suzuki, 1970; Suzuki, 1974). Sulfite is further oxidized to sulfate:



Several factors are involved in the thiosulfate electron transport system such as entry point of thiosulfate, generation of ATP, and cytochrome acceptor. Peeters and Aleem (1970) have found that aerobic

thiosulfate oxidation by T. denitrificans is unaffected by flavin inhibitors and antimycin A. According to these authors, under anaerobic conditions, thiosulfate is reductively cleaved by thiosulfate reductase into sulfite and sulfide which are able to couple to the electron chain at flavin.

The pathway proposed by Sawhney and Nicholas (1977) for thiosulfate oxidation is shown below. This is in agreement with the path adopted for nitrate reduction by Grady and Lim (1980). In both cases cytochromes b and c, flavoprotein (Fp), and a reduced form of nicotinamide adenine dinucleotide ($NADH_2$) are involved in the oxidation of thiosulfate or the reduction of nitrate:



2.7 Stoichiometry of Autotrophic Denitrification

The oxidation of thiosulfate and reduction of nitrate in the presence of an inorganic carbon source result in production of new cell mass, nitrogen gas, and other changes in the medium. This is well illustrated in the stoichiometric equation.

The stoichiometry of autotrophic denitrification can be theoretically derived based on the biological reactions in which T. denitrificans takes part. From the literature, it was not possible to find a theoretical stoichiometric equation for autotrophic denitrification using thiosulfate as the electron donor, bicarbonate as the carbon

source, and nitrate as the electron acceptor and nitrogen source. It is, however, possible to derive such an equation based on methods developed by McCarty (1975). This involves three half reactions: one for the cell material (R_c), one for the electron donor (R_d), and one for the electron acceptor (R_a). The overall stoichiometric equation R is given by

$$R = R_d - f_e R_a - f_s R_c$$

The terms f_e and f_s represent the fractions of energy coupled for electron transport and cell synthesis, respectively:

$$-f_e R_a = f_e \left[1/5\ NO_3^- + 6/5\ H^+ + e^- = 1/10\ N_2 + 3/5\ H_2O\right]$$

$$-f_s R_c = f_s \left[1/28\ NO_3^- + 5/28\ HCO_3^- + 34/28\ H^+ + e^- = 1/28\ C_5H_7O_2N + 16/28\ H_2O\right]$$

$$R_d = \left[1/8\ S_2O_3^{=} + 5/8\ H_2O = 1/4\ SO_4^{=} + 5/4\ H^+ + e^-\right]$$

The values of f_s and f_e have been calculated as 0.24 and 0.76 by Bisogni and Driscoll (1977) for an autotrophic system using thiosulfate as electron donor, nitrate as electron acceptor, and ammonia as nitrogen source. The following stoichiometric equation is derived based on the values of 0.24 for f_s and 0.76 for f_e:

$$NO_3^- + 0.79\ S_2O_3^{=} + 0.27\ HCO_3^- + 0.20\ H_2O = 0.47\ N_2 + 0.05\ C_5H_7O_2N + 1.56\ SO_4^{=} + 0.28\ H^+$$

The stoichiometric equation for a system that uses ammonia for cell synthesis and nitrate for electron acceptor (Bisogni and Driscoll, 1977) is given below:

$$NO_3^- + 0.84\ S_2O_3^= + 0.347\ CO_2 + 0.087\ HCO_3^- + 0.087\ NH_4^+$$
$$= 0.087\ C_5H_7O_2N + 1.689\ SO_4^= + 0.5\ N_2 + 0.697\ H^+$$

For a heterotrophic denitrification, the following equation has been used (Jeris et al., 1974):

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

Comparison of these three equations shows that biomass production in heterotrophic denitrification can be about 22% more compared to autotrophic denitrifying bacteria. Cell yield is also lower when nitrate serves as the electron acceptor and nitrogen for cell synthesis. Lower biomass yield will reduce cost of sludge treatment and disposal.

Autotrophic bacteria spend a large amount of energy for reducing oxidized inorganic carbon to organic monomers required for synthesis of protein and lipids (Senez, 1962; Stouthamer, 1977). This partly explains why cell production is less in autotrophic denitrification.

Among the very few studies made on autotrophic denitrification using thiosulfate as an electron donor, stoichiometric parameters are available where ammonia has been used for a nitrogen source. These parameters are given in Table I.

TABLE I

STOICHIOMETRIC PARAMETERS

Parameter	Value	Reference
$S_2O_3^{=}/NO_3^-$ (mol/mol)	0.84	Bisogni & Driscoll (1977)
	0.81	Claus & Kutzner (1985b)
	0.76	Baalsrud & Baalsrud (1954)
	0.75	Justin & Kelly (1978)
$SO_4^{=}/NO_3^-$ (mol/mol)	1.62	Claus & Kutzner (1985b)
$CO_2/S_2O_3^{=}$ (mol/mol)	0.21	Lu & Kelly (1983)

2.8 Denitrification Treatment Processes

In denitrification processes, the bacteria responsible for nitrate reduction are either suspended in the wastewater or attached to some inert media. Based on microbial growth, denitrification processes can be classified under two major categories as suspended growth processes and attached film processes.

2.8.1 Upflow Sludge Blanket

The successful work of Lettinga et al. (1980) with an anaerobic upflow sludge blanket (USB) reactor for treatment of industrial wastes encouraged some researchers to use the USB for denitrification. The USB is similar to the activated sludge process system in the sense

that bacteria are kept in suspension in both systems. Mixing in the USB is very slow. Slow mixing is essential to promote biomass agglomeration. Although the USB system does not use an inert carrier medium, the biomass grows as granules which are heavier than the biomass in an activated sludge system, but lighter than the attached biomass and carrier particles found in expanded or fluidized bed reactors. As a result of relatively low weight, these sludge granules can be fluidized at low linear velocities which minimizes shearing loss of the biomass.

2.8.2 Expanded Bed

Jewell (1981, 1985) has given the history of the attached film expanded bed from its inception as an aerobic process through various applications of the anaerobic expanded bed. Expanded and fluidized bed reactors are attached film processes. The expanded bed reactor differs from the fluidized bed in that bed expansion is set at about 10 to 20% rather than fluidization which occurs at >100% expansion. The use of small media in these reactors has several advantages, such as (a) greater surface area available for bacterial growth per unit reactor volume, (b) less chance of clogging, and (c) small head loss.

Any small inert particles can be used as biofilm support media. Sand, plastic, glass, carbon, anthracite, granular activated carbon, and diatomaceous earth can serve the purpose. However, lighter biofilm support media such as carbon and diatomaceous earth require lower energy for bed expansion. This must be considered while calculating operating costs.

Compared to the fluidized bed, the expanded bed operates under lower bed expansion as well as lower linear velocity. Thus the expanded reactor can offer not only lower capital cost but also lower operating cost.

2.9 Nitrate Removal Rates

Although much research has been reported on the denitrification process, relatively little information is available on denitrification with high nitrate concentrations. In the late 1970s, Bisogni and Driscoll (1977), Batchelor and Lawrence (1978), and Driscoll and Bisogni (1978) established that autotrophic denitrification could be used to remove nitrogen from wastewater. The studies, however, were confined to low nitrate concentrations of 20 to 50 mg NO_3^--N/L. A comparison of nitrate removal rates in denitrification systems treating concentrated wastes is given in Table II.

TABLE II

COMPARISON OF NITRATE REMOVAL RATES

Process	Reactor	Feed Conc. NO_3^-N mg/L	Removal Rate Kg-N Per m^3.d	Reference
Autotrophic	Static Filter	970	5.6	Claus & Kutzner (1985b)
Heterotrophic	USB	900	>7.2	Miyaji & Kato (1975)
Heterotrophic	USB	257	12.0	Klapwijk et al. (1981)

2.10 Inhibitors

Sulfate is one of the end products of autotrophic denitrification. According to Claus and Kutzner (1985a), sulfate in excess of about 5.0 g/L can inhibit denitrification. This concentration would be produced in a system converting 970 mg NO_3^--N/L. Therefore, wastewater with high nitrate concentrations may have to be diluted. Nitrite inhibition was noted by Claus and Kutzner (1985a) at NO_2^- concentrations >200 mg/L.

CHAPTER III

EXPERIMENTAL APPROACH

3.1 Purpose of Study

Autotrophic denitrification for treatment of low nitrate concentrations has been well established by several investigators. However, the suitability of this process for high strength nitrogenous wastewaters has for the most part remained untested.

Theoretically T. denitrificans in an anaerobic waste treatment system can reduce nitrate to nitrogen gas while oxidizing reduced sulfur compounds. Before implementing such a new system, it is necessary to identify a suitable reactor design and operating conditions. The purpose of this study was to determine the highest possible nitrate concentration and loading rates for autotrophic denitrification of industrial strength wastewaters.

3.2 Program of Study

Planning, design, and fabrication of experimental apparatus, establishment of attached biofilm and sludge granules, and conduction of experiments occupied approximately 17 months. Figure 1 shows the sequence of activities.

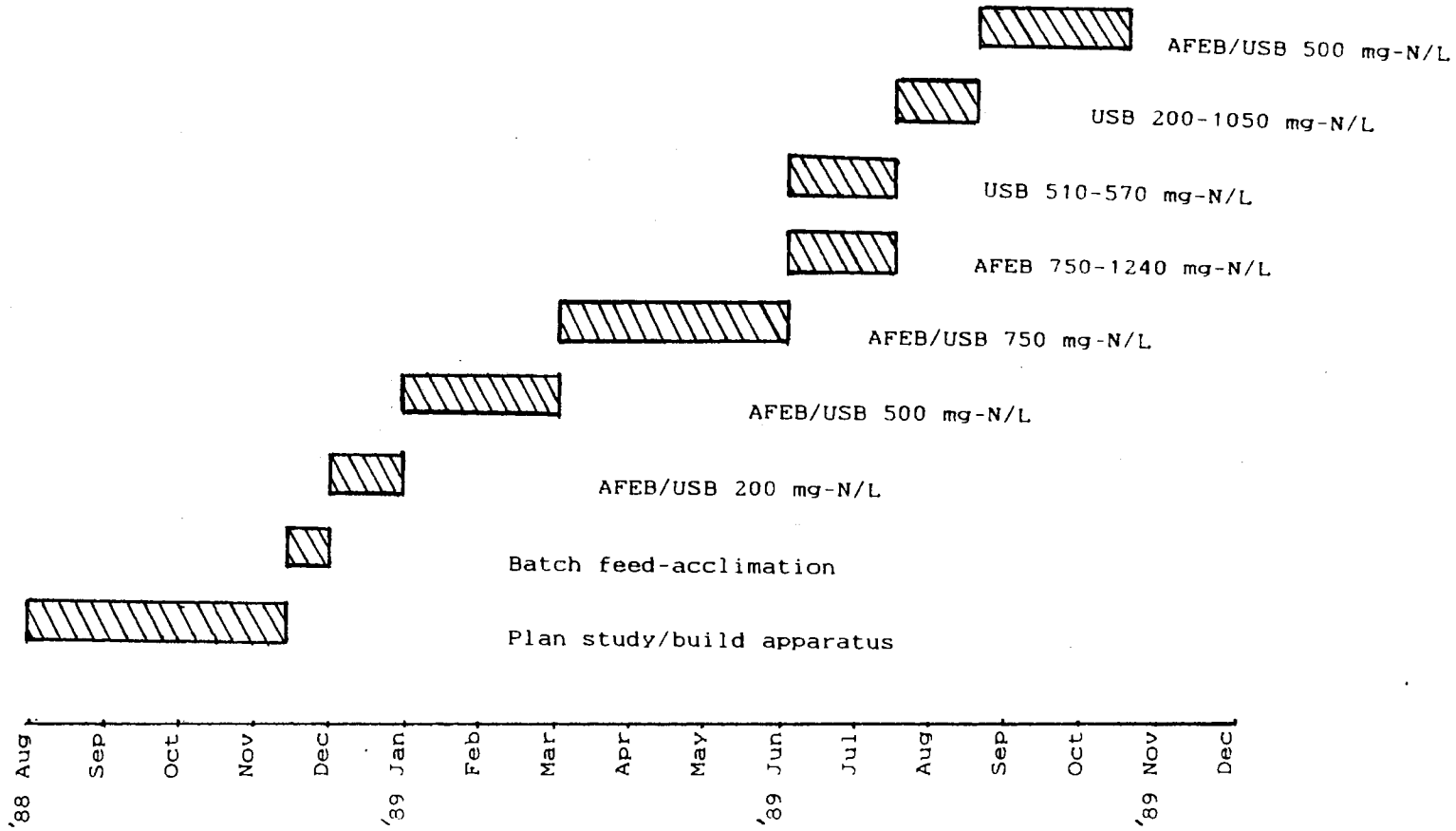


Figure 1. Schematic Diagram of Sequence of Activities

3.3 Materials and Methods

3.3.1 Experimental Apparatus

3.3.1.1 Anaerobic Attached Film Expanded Bed Reactor. A schematic diagram of the anaerobic attached film expanded bed (AFEB) system is shown in Figure 2 and a photograph of the bench-scale AFEB in Figure 3. The reactor consisted of a styrene acrylonitrile Imhoff cone with a plastic cylindrical tube at the top. The inner diameter increased from 1.5 cm at the bottom to 10.5 cm at the top. The total height of the reactor was 60 cm of which the tapered section made up 45 cm. A polyethylene tubing connector was affixed by melting a hole in the cylindrical plastic tube and gluing the connector in place with epoxy cement. This effluent overflow was 12 cm from the top. The reactor had a liquid volume of 1.6 L.

The reactor was filled with 300 mL support medium composed of diatomaceous earth. Diatomaceous earth consists of porous, siliceous particles which are skeletons or shells of diatoms. The inert particles were sieved dry through a 28 mesh (589 μm) screen and washed well to eliminate very fine particles and to produce particles of diameter between 300 and 600 μm. This material was selected because of its low cost, low density, and its ability to withstand high temperature. The last property is necessary to measure biofilm volatile organic matter at 550°C.

The bed was expanded to 20% above its static or unexpanded volume by passing the supernatant into the bottom of the reactor by a positive displacement Masterflex pump (model No. 7553-50, Cole-Parmer). This pump was fitted with a standard pump head (model No. 7015-20).

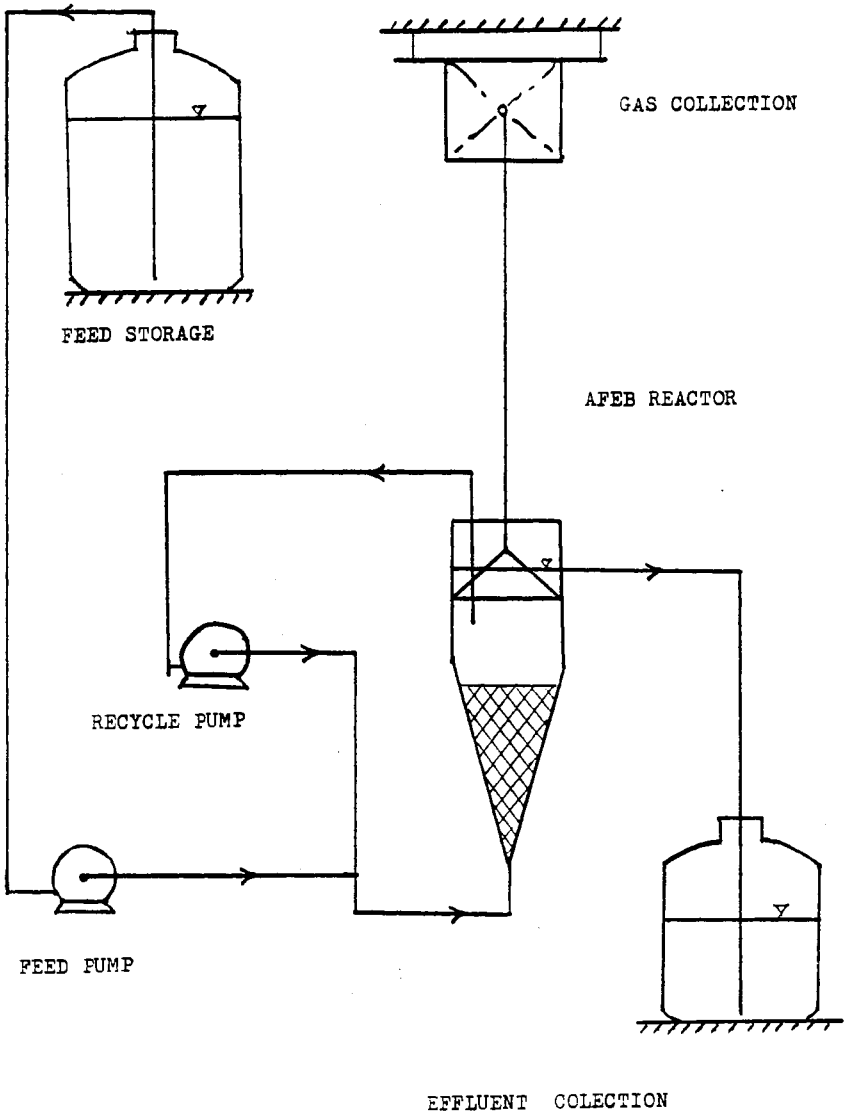


Figure 2. Schematic Diagram of the AFEB Reactor System

Figure 3. Photograph of the AFEB Reactor

The recirculation tubing inlet was placed much deeper inside the reactor than the feed outlet. Another Masterflex pump (model No. 7014-20) fitted with a Masterflex pump head (model No. 7553-60) was used as the feed pump. Throughout this study only Masterflex tubing was used for these pumps. An inverted funnel was kept below the top wastewater level to provide a water seal for gas collection. This funnel was connected to a Teflon gas storage bag.

3.3.1.2 Sludge Blanket Reactor. The sludge blanket reactor consisted of a glass cylinder with a hemispherical bottom and an outer cylinder at the top. The configuration of this reactor is shown in Figure 4 and a photograph of the reactor in Figure 5. The diameter of the inner cylinder was 10.0 cm and that of the outer cylinder was 14.0 cm. The volume of the inner cylinder was 2.5 L. Feed solution was pumped through a Masterflex pump (model No. 7553-60, Cole-Parmer). The pump was fitted with a standard pump head (model No. 7016-20). Feed was introduced through a fitting at the bottom of the reactor, and treated effluent overflowed from the top of the inner cylinder into the outer cylinder and escaped through the outlet.

The reactor was mixed by a 20 x 20 cm Sargent-Welch magnetic stirrer. A suspended stir bar with cage (Fisher Scientific) was placed inside the reactor. The mixing rate was set by observation to produce a well mixed suspension. Stirring was continuous. A hollow circular plate (10 cm outer diameter and 8.5 cm inner diameter) was fixed at 3.0 cm below the top of the reactor. This arrangement minimized gas escape through the periphery of the inverted funnel placed above it. The funnel was connected to a Teflon gas storage bag.

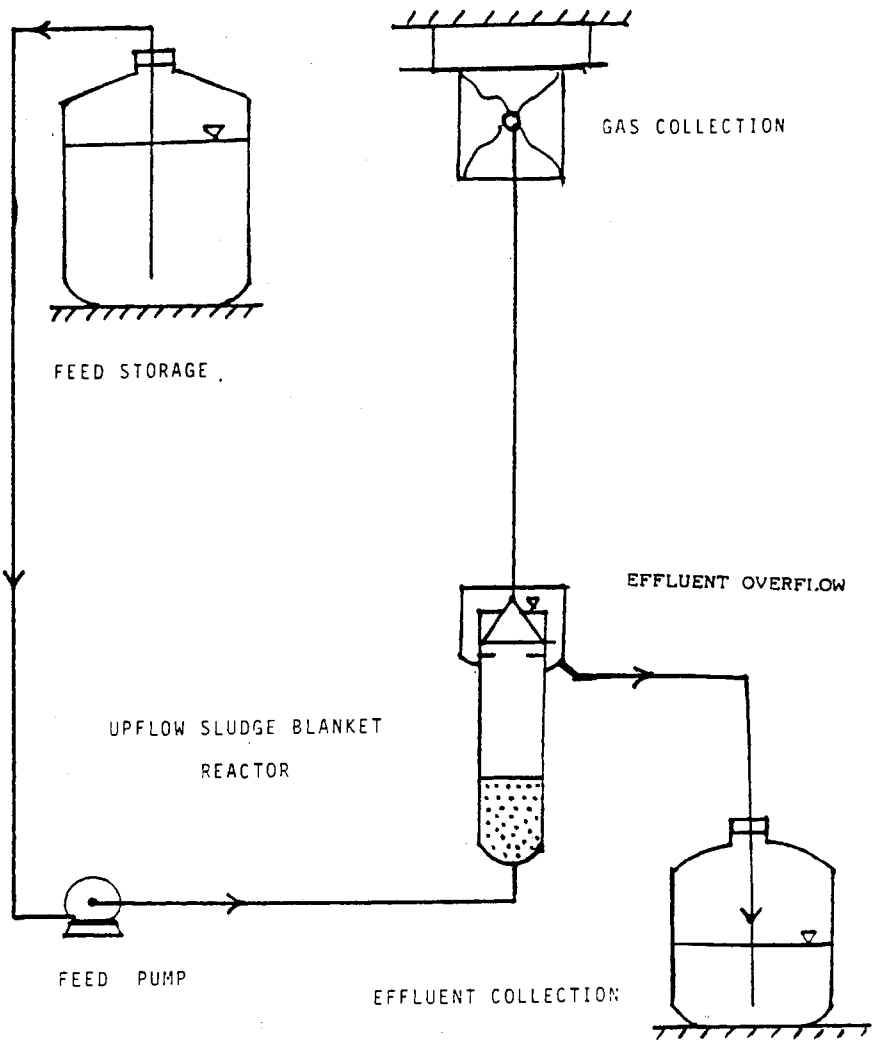


Figure 4. Schematic Diagram of the USB Reactor System

Figure 5. Photograph of the USB Reactor

3.3.1.3 Gas Measuring Apparatus. Gas was collected from reactors in Teflon bags. A schematic diagram of a simple apparatus to measure gas volume collected is shown in Figure 6 and a photograph in Figure 7. The apparatus consisted of a calibrated plastic cylinder inside a glass cylinder containing water. Gas from the Teflon bag was let inside the floating plastic cylinder through a rigid glass tube that passed through the bottom of the outer cylinder. This rigid tube was connected to two plastic tubes with plastic valves. These two tubes served as inlet and outlet to the floating chamber. Two strings were fixed to the cylinder which passed through two smooth arms as shown in Figures 6 and 7. To make the cylinder buoyant and to balance it at any height, counterweights were attached to the end of the strings.

3.4 Start-Up Procedure

The inoculum for autotrophic denitrification was collected from an activated sludge unit of the sewage treatment plant of Ponca City, Oklahoma. Return sludge from one of the activated sludge units was collected in a 25 L glass bottle. Every day the supernatant was drained and replenished with feed solution containing 2.0 g KNO_3/L, 2.0 g $NaHCO_3$/L, and 4.0 g $Na_2S_2O_3 \cdot 5H_2O$/L. This batch feed continued for two weeks. The purpose of the batch feed was to select for autotrophic denitrifiers.

After the end of two weeks, the USB and AFEB reactors were filled to about two-thirds of their volume with biomass taken from the acclimated seed culture. The reactors were started with a feed concentration of about 250 mg NO_3^--N/L and HRT of 6 hours. A small quantity

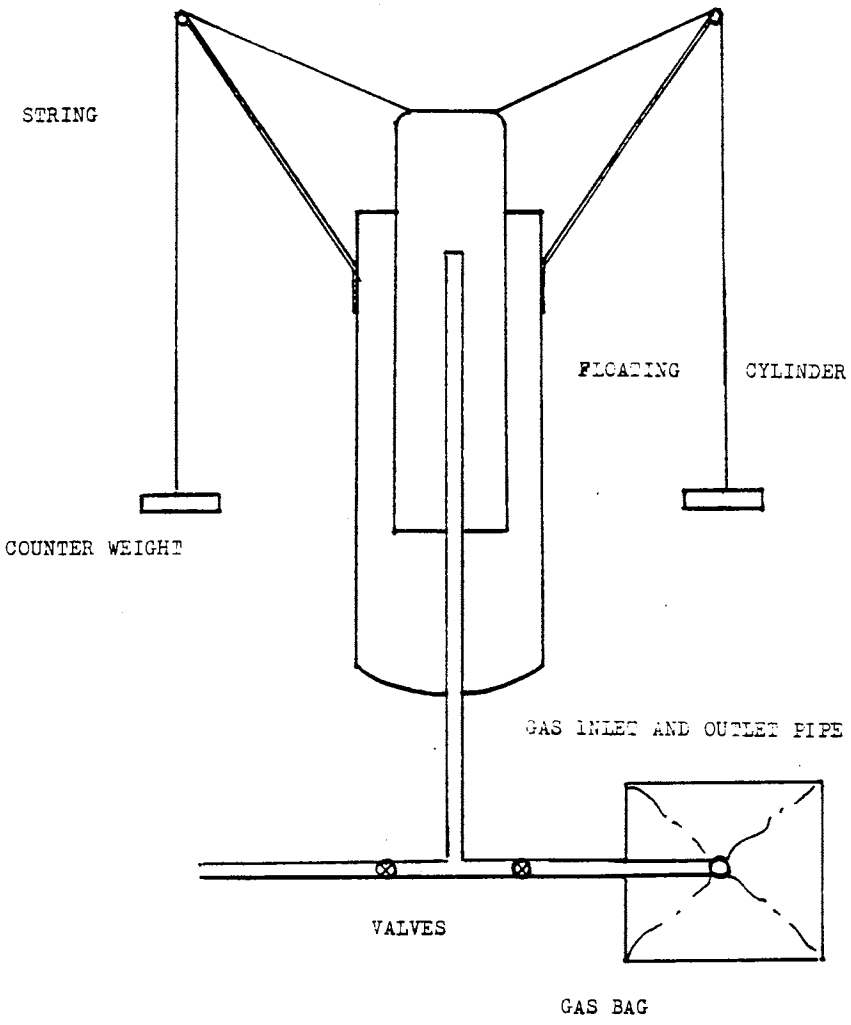


Figure 6. Schematic Diagram of the Gas Measuring Apparatus

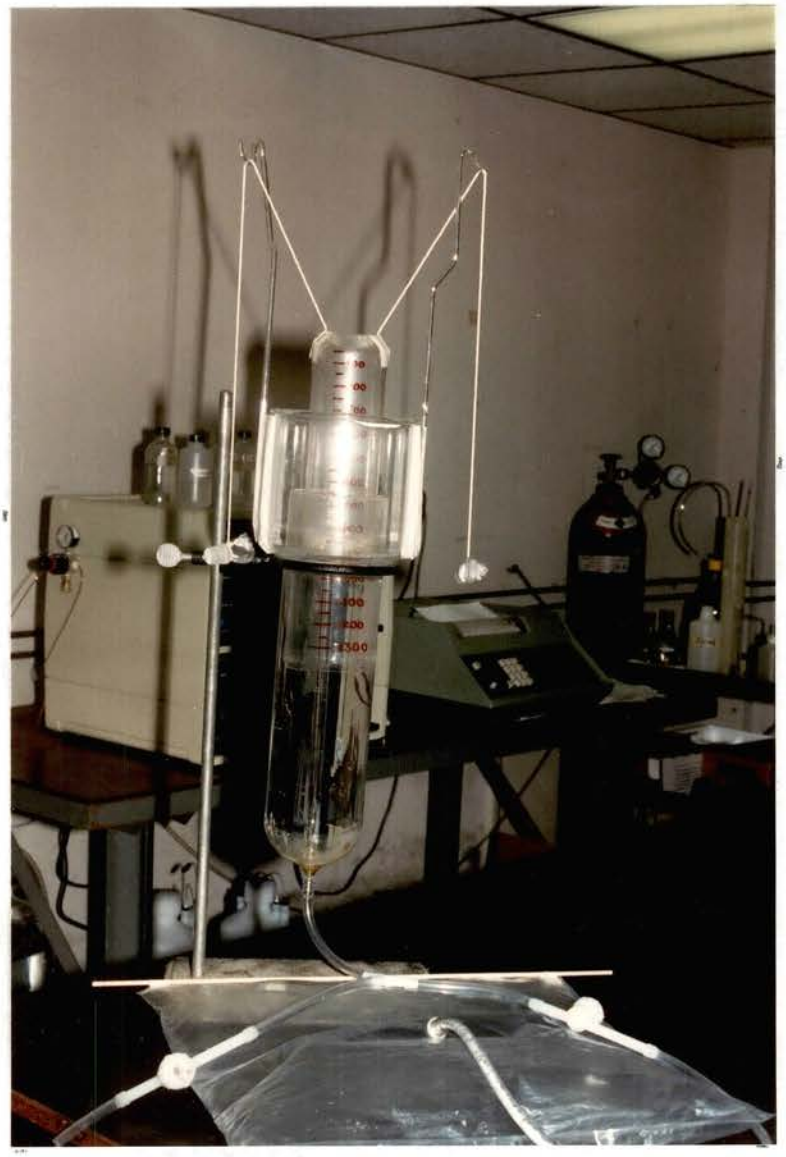


Figure 7. Photograph of the Gas Measuring Apparatus

of acclimated sludge from the batch reactor was added daily to the continuous feed reactors to make up the daily biomass loss from the reactors. By the end of six weeks, granular sludge began to accumulate and the floc-type sludge disappeared. The granular sludge had good settling properties and occupied the lower regions of the USB reactor. In the AFEB reactor, expanded bed volume began to increase as a result of biofilm attachment to the media. Within six weeks all diatomaceous particles were fully coated with biofilm. The expanded bed volume increased from 350 to 450 mL during this time.

3.5 Feed Solution

Feed solution for each reactor was prepared daily. Potassium nitrate, sodium thiosulfate, sodium bicarbonate, magnesium chloride, ferrous sulfate, and monobasic potassium phosphate were dissolved separately in tap water and then mixed well in a 25.0 L glass bottle. Thiosulfate and bicarbonate were added at greater than the stoichiometric requirements so that these chemicals did not become limiting substrates. For this reason nitrate, thiosulfate, and bicarbonate were used in the ratio of 1:2:1 on a weight basis. The composition of 500 mg NO_3^--N/L feed solution is given in Table III.

3.6 Experimental Operation

3.6.1 Attached Film Expanded Bed Reactor

The feed solution required for the AFEB reactor was prepared daily and stored in a 25 L glass bottle. From this bottle the reactor was fed continuously. The expanded volume of the reactor slowly

TABLE III

RECIPE FOR 500 MG NO_3^--N/L FEED SOLUTION

Ingredients	Concentration, g/L
Potassium Nitrate	3.600
Sodium Thiosulfate	7.200
Sodium Bicarbonate	3.600
Monobasic Potassium Phosphate	0.100
Ferrous Sulfate	0.010

increased from 350 to 450 mL in six weeks. At the seventh week the volume suddenly increased from 450 to 550 mL in two days. Such a large change has not been reported in the literature. During the two days there was considerable change in the size and shape of the attached biomass. The small spherical particles changed into a bigger and irregular biomass with an apparently fluffy consistency. Biomass retention in the AFEB, however, was unaffected by the physical transformation and remained virtually complete.

The AFEB reactor was tested with various nitrate concentrations ranging from 500 to 1050 mg NO_3^--N/L at a constant HRT of 3.3 hours. When the concentration was increased to 1250 mg NO_3^--N/L, nitrogen removal efficiency began to decrease and reached as low as 41% on the tenth day. Gas bubbles evolving from the reactor at this loading rate began to lift the attached biomass particles. Because of entrapped gas, the biomass did not settle easily and began to accumulate at the top of the reactor. It was therefore decided not to increase feed concentration above 1250 mg NO_3^--N/L.

The reactor performance was studied for various hydraulic retention times ranging from 3.3 to 0.8 hours for a constant feed concention of 760 mg NO_3^--N/L. When the HRT was changed from 1.1 to 0.8 hours, the system collapsed without any prior indications. All of the biomass in the reactor was washed out overnight.

3.6.2 Upflow Sludge Blanket Reactor

The performance of the USB reactor was studied for various feed concentrations. During the course of this study, nitrogen removal followed a predictable pattern except for one instance when efficiency

dropped suddenly for unknown reasons at 500 mg NO_3^--N/L. The system regained its high nitrogen removal efficiency when the reactor was fed for 10 days with low nitrate feed (200 mg NO_3^--N). Then the reactor was subjected to various feed concentrations in the range of 500 to 1100 mg NO_3^--N/L for a constant HRT of 6.0 hours. As a result of large biomass escape at 1100 mg NO_3^--N/L, it was decided not to try any higher concentration. After the above maximum feed concentration was reached, the system was brought back to a lower concentration of 500 mg NO_3^--N/L. In this second series of experiments, the HRT was varied over a range of 6 to 0.59 hours but the feed concentration was kept constant at 500 mg NO_3^--N/L.

3.6.3 Analytical Techniques

3.6.3.1 Total Suspended Solids. The technique used for determination of total suspended solids was according to the procedures given in Standard Methods (APHA et al., 1985), Section 209 C.3. About 300 ml of the effluent from the reactor was collected. This was well mixed and a known volume, usually 200 mL, was taken for solids analysis. Only grab samples were collected for analysis.

3.6.3.2 Volatile Suspended Solids. Volatile suspended solids were measured according to the procedures given in Standard Methods (APHA et al., 1985), Section 209 D.3. The residue from total suspended solids determination (section 3.5.3.1) was used for the determination of volatile suspended solids.

3.6.3.3 Attached Biomass. The procedures described by Clarkson (1986) were followed to determine the attached biomass. Samples were

taken from the center of the expanded bed reactor using a wide mouth pipet and transferred to 10 mL wide bore graduated cylinders. The cylinders were then tapped and spun several times to consolidate the sample. During this process of consolidation, particles were added or removed and the tamping procedure followed until each sample contained exactly 5.0 mL of packed particles. Supernatant was decanted and the sample was transferred to an ashed, preweighed porcelain drying dish by sluicing it out with a stream of distilled water from a wash bottle. The jet of water from the wash bottle was used to agitate the particles vigorously. The supernatant containing loose solids was transferred to other drying dishes. Care was taken not to remove support particles from their original dish. This process was repeated until further washing produced no further loose biomass.

The dishes containing these samples were subjected to the total solids procedure described in Section 3.5.3.1. Blanks consisted of biomass-free diatomaceous earth particles which had been sieved and prepared along with those which were used for the expanded bed but stored in a buffer solution at room temperature. Blank samples were necessary to correct for hygroscopically bound water in the diatomaceous earth in performing the solids calculations. After the samples were ashed finally, the particles were rehydrated with distilled water, transferred to the graduated cylinders, and the final volume of each bed sample was taken. The samples were tamped well before the final volume of the rehydrated sample was recorded.

3.6.3.4 pH. Samples were measured using an Orion digital ion-

alyzer (model No. 501). This pH meter had a single electrode and digital display.

3.6.3.5 Thiosulfate. Concentration of thiosulfate was measured by titration with iodine solution. A standard thiosulfate solution was prepared daily to determine the strength of the iodine solution. The standardized iodine solution was then used to determine the concentration of the thiosulfate solution. This classical method is described in Quantitative Analysis (Pierce and Haenisch, 1948, p. 242).

3.6.3.6 Alkalinity. Alkalinity was measured by titration with sulfuric acid of 0.025 N. The end point of titration was determined with an Orion digital ionalyzer (model No. 501). The procedures described in Standard Methods, Section 403.4.c (APHA et al., 1985) were followed.

3.6.3.7 Nitrate, Nitrite, and Sulfate. Nitrate, nitrite, and sulfate were measured with a Dionex ion chromatograph, series 2000 i/SP. These anions were measured according to the procedures outlined in Standard Methods, Section 429.4 (APHA et al., 1985).

3.6.3.8 Gas Volume. Gas produced in the reactors was collected in Teflon gas storage bags. The volume of gas collected was measured by a simple fill and draw method. The displacement of gas took place in the floating chamber of the apparatus described in Section 3.3.1.3. The Teflon bag containing gas was connected to the inlet pipe of the gas measuring apparatus. The outlet valve was closed first and then the inlet valve was opened. This allowed the gas to move from the bag

to the floating chamber. To facilitate quick measurement, small additional weights were added to the counterweights. When the chamber reached the top position, the additional weights were removed and the volume of gas was noted. After this the inlet valve was closed and the outlet valve was opened to allow the gas to escape from the chamber. To make the chamber move downward, small weights were kept on the top of the chamber. These weights were removed when the chamber reached the bottom position. This cycle was repeated until all gas in the bag was measured.

CHAPTER IV

EXPERIMENTAL RESULTS

4.1 Research Objectives

The overall scope of this research was to establish operating performance parameters and to evaluate suitability of autotrophic denitrification for high strength nitrogenous wastewater. The experiments were divided into two parts.

Throughout the discussion, the term nitrogen equivalent (N_e) will be used. N_e is the sum of nitrogen available in nitrate plus nitrite. A small amount of nitrite has been found in the feed solution, although no nitrite was added while preparing the feed solution. It is possible that nitrite appeared as a result of some minor chemical reaction.

The first part of the research study was to determine the effect of different nitrogen equivalent concentrations on the AFEB and USB systems when they were operating under constant hydraulic retention time. The second part of the study was to observe the behavior of the reactors at various hydraulic retention times with constant N_e feed concentration.

Thus the research was focused on two parameters, feed concentration and hydraulic retention time, which together determine volumetric

rates. AFEB and USB reactors were compared to determine maximum denitrification rates.

Data collected in this study are presented in Tables IV, V, VI, and VII. Each value in these tables represents an average value for a particular laoding rate. Each loading rate was generally tested for a minimum period of one week and a minimum of three readings were taken. Whenever the loading rate was changed, readings were taken only after 10 detention times. This time interval was provided to avoid any transients which would result from altered loading rates. A steady state was assumed when the reactor performance in terms of denitrification remained almost constant. The time interval specified as well as the steady state condition defined above have been found in the literature (Mulcahy, 1980).

4.2 Attached Film Expanded Bed

4.2.1 Data Summary

The results of the experiments on the AFEB with varying N_e feed concentration are given in Table IV. The reactor in this phase of the study was operated under a constant HRT of about 3.3 hours. The N_e loading rates were varied from 5.4 to 7.9 $kg/m^3.d$ by changing the feed concentrations from 741 to 1242 mg N_e/L.

The calculations on loading rates and hydraulic retention time have been made on the basis of total expanded bed volume without making any correction for the volume occupied by support particles and biofilm. This approach is in agreement with previous studies of the AFEB process (Clarkson, 1986). Although this method results in low

TABLE IV

SUMMARY OF RESULTS OF THE AFEB AT CONSTANT HYDRAULIC RETENTION TIME

Feed Concentration mg-N_e/L	N_e Consumed mg/L	%	Biomass Volume mL	HRT hr	Gas Volume L/d	Loading Rate kg $N_e/m^3.d$	Removal Rate kg $N_e/m^3.d$	TSS mg/L	VSS mg/L	Attached Biomass mg/mL	$S_2O_3^{=}$ Consumed mg/L	Alkalinity Consumed mg as $CaCO_3$	$SO_4^{=}$ Formed mg/L	$S_2O_3^{=}/N_e$ mol/mol	$SO_4^{=}/N_e$ mol/mol	N_e/Alk mol/mol
741	738	99.6	875	3.4	5.8	5.42	5.40	53	40	23						
892	879	99.7	1000	3.8		5.65	5.63	67	45	27						
1041	1026	98.6	1000	3.8	6.9	6.66	6.57	52	34	21	5926		9453	0.71	1.32	
1242	828	66.7	1000	3.8		7.95	5.30	121	85		5577	1250	9045	0.63	1.35	2.38

TABLE V

SUMMARY OF RESULTS OF THE AFEB AT CONSTANT N_e FEED CONCENTRATION

Feed Concentration mg-N_e/L	N_e Consumed mg/L	%	Biomass Volume mL	HRT hr	Gas Volume L/d	Loading Rate kg $N_e/m^3.d$	Removal Rate kg $N_e/m^3.d$	TSS mg/L	VSS mg/L	Attached Biomass mg/mL	$S_2O_3^{=}$ Consumed mg/L	Alkalinity Consumed mg as $CaCO_3$	$SO_4^{=}$ Formed mg/L	$S_2O_3^{=}/N_e$ mol/mol	$SO_4^{=}/N_e$ mol/mol	N_e/Alk mol/mol
741	738	99.6	875	3.3	5.3	5.42	5.40	53	40	23						
771	767	99.5	900	1.7	8.3	10.96	10.91	52	41	21	4276	1204	7621	0.70	1.45	2.33
747	740	99.1	850	1.3	11.7	13.53	13.41	54	41	20	4216	1198	7521	0.71	1.48	2.21
773	763	98.7	850	1.1		17.46	17.23				4328	1180	7434	0.71	1.43	2.29

TABLE VI

SUMMARY OF RESULTS OF THE USB AT CONSTANT HYDRAULIC RETENTION TIME

Feed Concentration mg-N_e/L	N Consumed mg/L		Gas Volume L/d	HRT hr	Loading Rate kg $N_e/m^3.d$	Removal Rate kg $N_e/m^3.d$	TSS mg/L	VSS mg/L	$S_2O_3^=$ Consumed mg/mL	Alkalinity Consumed mg as $CaCO_3$	$SO_4^=$ Formed mg/L	$S_2O_3^=/N_e$ mol/mol	$SO_4^=/N_e$ mol/mol	N_e/Alk mol/mol
277	265	95.3	3.35	6.0	1.11	1.05	N/T	N/T	1648	407	2379	0.78	1.51	2.32
496	487	97.9	6.87	6.0	1.98	1.98	39	26	2792	844	5005	0.71	1.48	2.10
758	756	99.7		6.0	3.03	3.02	33	23	4331	1104	7427	0.72	1.40	2.25
801	761	95.0	7.52	6.0	3.20	3.05	52	37	4245	1238	7248	0.69	1.39	2.23
939	923	98.3	7.82	6.0	3.76	3.69	N/T	N/T	5432	1416	9095	0.74	1.45	2.33
1086	864	79.6		6.0	4.34	3.46	N/T	N/T	5583	N/T	8441	0.81	1.41	

TABLE VII

SUMMARY OF RESULTS OF THE USB AT CONSTANT N_e FEED CONCENTRATION

Feed Concentration mg-N_e/L	N Consumed mg/L	N Consumed %	Gas Volume L/d	HRT hr	Loading Rate kg $N_e/m^3.d$	Removal Rate kg $N_e/m^3.d$	TSS mg/L	VSS mg/L	$S_2O_3^{=}$ Consumed mg/mL	Alkalinity Consumed mg as $CaCO_3$	$SO_4^{=}$ Formed mg/L	$S_2O_3^{=}/N_e$ mol/mol	$SO_4^{=}/N_e$ mol/mol	N_e/Alk mol/mol
510	491	96.3		6.00	2.04	1.06			2845	791	5045	0.72	1.50	2.21
476	470	98.7	7.53	3.00	3.81	3.76	53	37	2807		4687	0.75	1.45	
486	469	96.5		2.00	5.83	5.62	106	90	2621	750	4812	0.70	1.50	2.23
490	468	95.5	24.61	1.25	9.41	8.98	114	84	2673	726	4657	0.71	1.45	2.41
497	476	96.2	40.91	0.83	14.30	13.72	115	86	2845	776	4534	0.72	1.39	2.22
492	157	36.2	18.52	0.59	20.10	6.40	123	92	963	228	2151	0.76	1.98	2.65

estimates of hydraulic retention time, it is considered to be the most practical and useful in comparing systems.

Figure 8 was drawn from the data taken from Table IV. Each point on the graph represents the average value of the parameter for a particular loading rate. Low loading rates produced high treatment efficiencies. The highest N_e removal rate obtained in this phase of study for steady state conditions was 6.6 kg/m^3.d at 98.6% removal efficiency. Figure 9 shows denitrification efficiency for various loading rates at a constant HRT of 3.3 hours.

The experiment could not be continued beyond a volumetric loading rate of 7.9 kg N_e/m^3.d for two reasons. At this volumetric loading rate (corresponding to 1242 mg N_e/L and HRT 3.3 hours) vigorous gas evolution created operational problems. The gas bubbles attached themselves to the expanded bed biomass media and carried them to the top of the reactor. At the liquid-gas interface, the gas bubbles did not break easily and quickly. As a result of this, biomass began to accumulate at the top of the reactor. Also, denitrification began to decrease. The average N_e removal efficiency for this period was only 66.7%.

Further experiments on the AFEB were conducted to meet the objectives of determining the performance under various hydraulic loading rates with constant N_e concentration. The results of the experiments for this mode of operation are given in Table V.

The reactor was fed with an average N_e concentration of about 750 mg/L. The HRT was varied from 3.3 to 0.8 hours. It can be seen from Table V and Figures 10 and 11 that the reactor maintained a very high N_e removal efficiency for HRTs from 3.3 to 1.1 hour. The maximum N_e

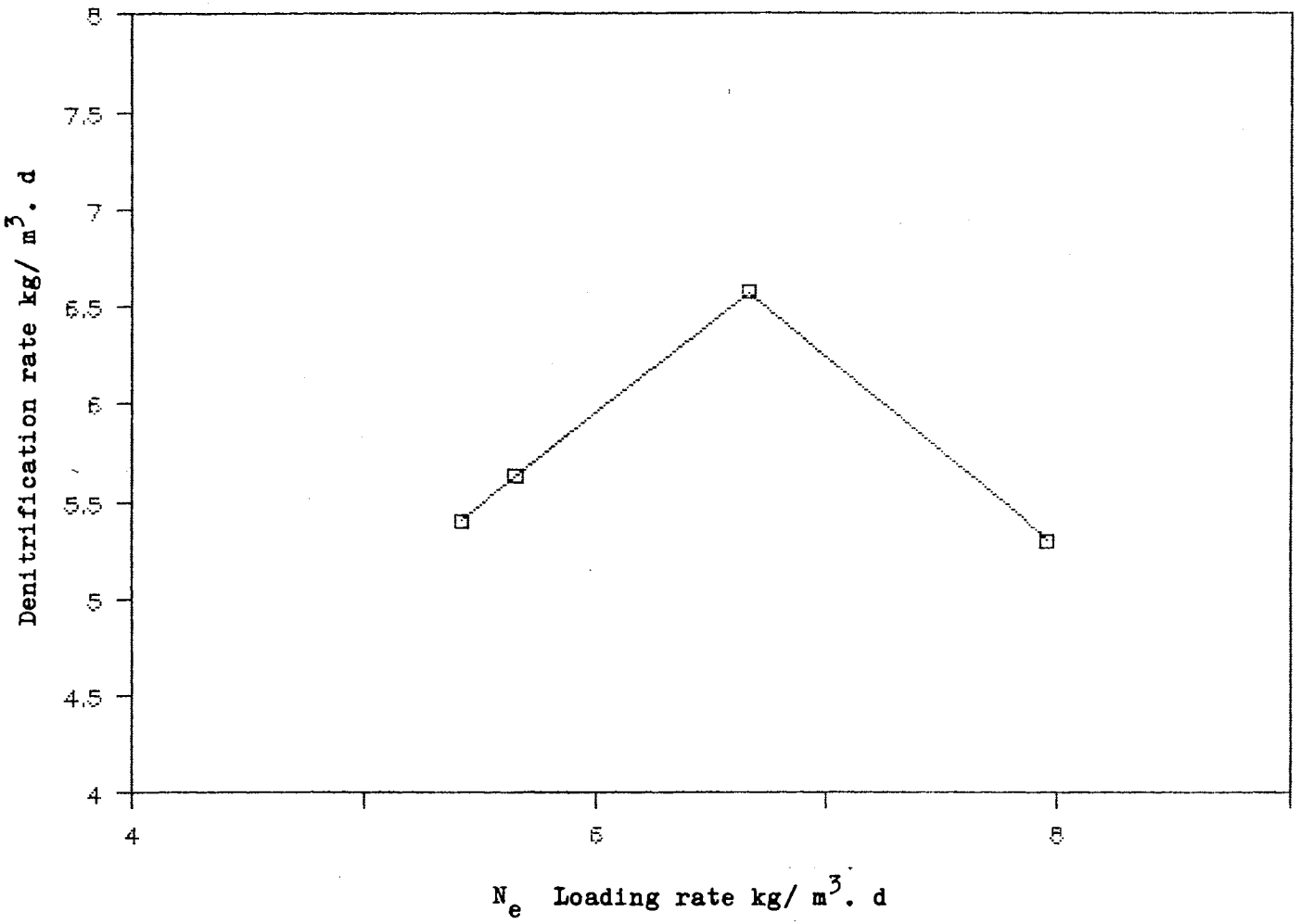


Figure 8. Denitrification Rates in the AFEB Reactor for Constant 3.3 Hour HRT

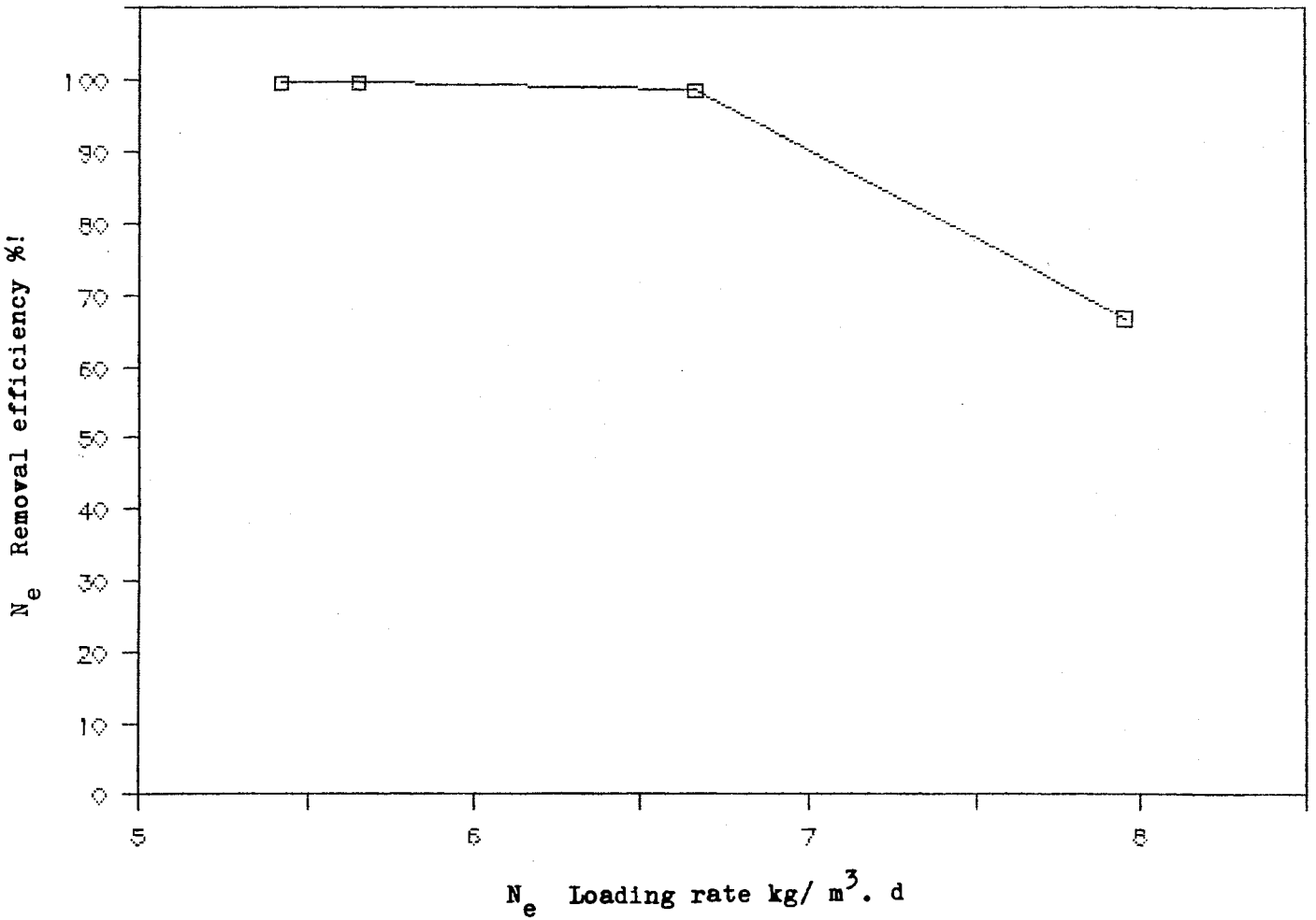


Figure 9. Denitrification Efficiencies in the AFEB Reactor for Constant 3.3 Hour HRT

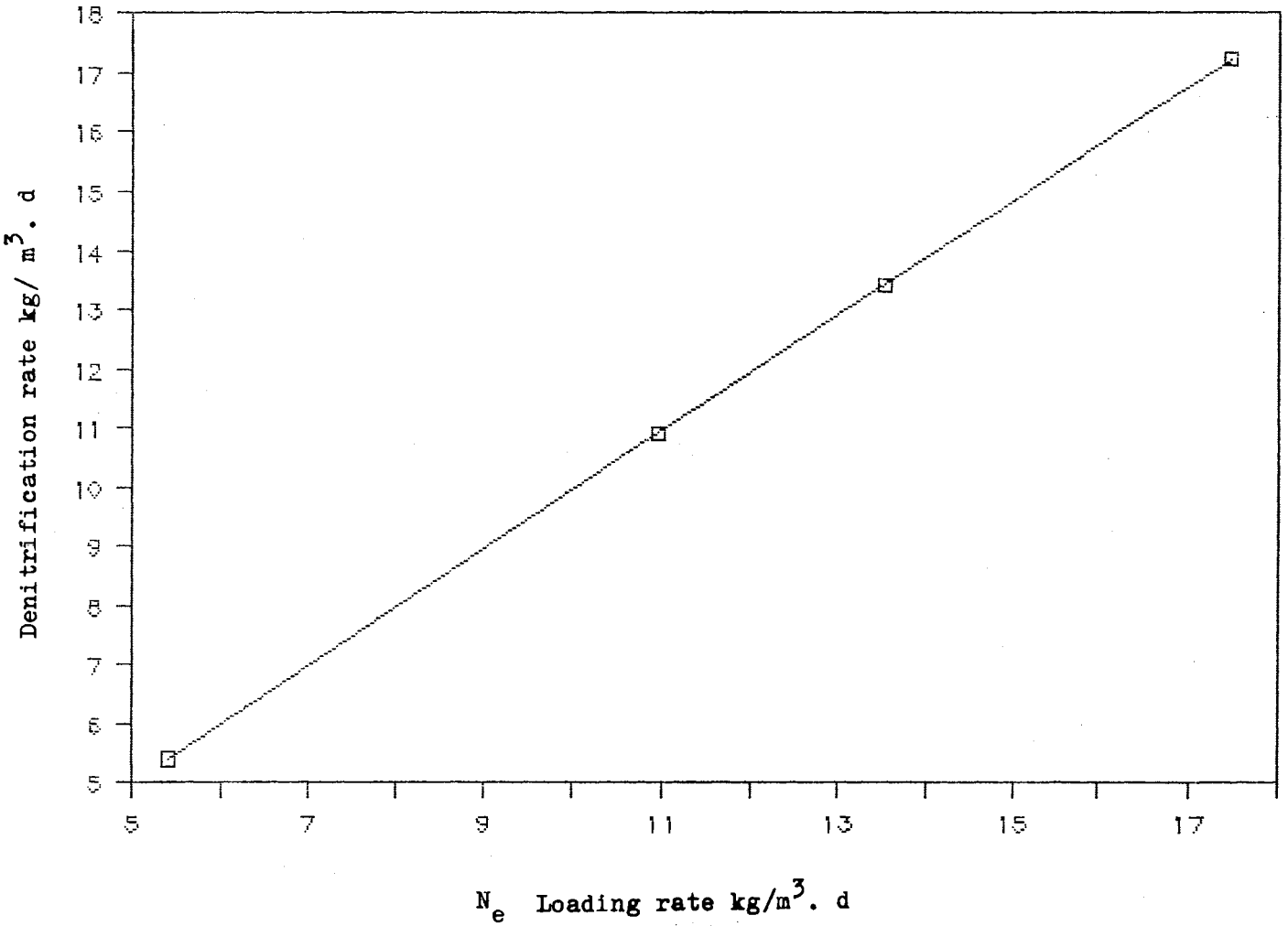


Figure 10. Denitrification Rates in the AFEB Reactor for Constant 750 mg Ne/L Feed Concentration

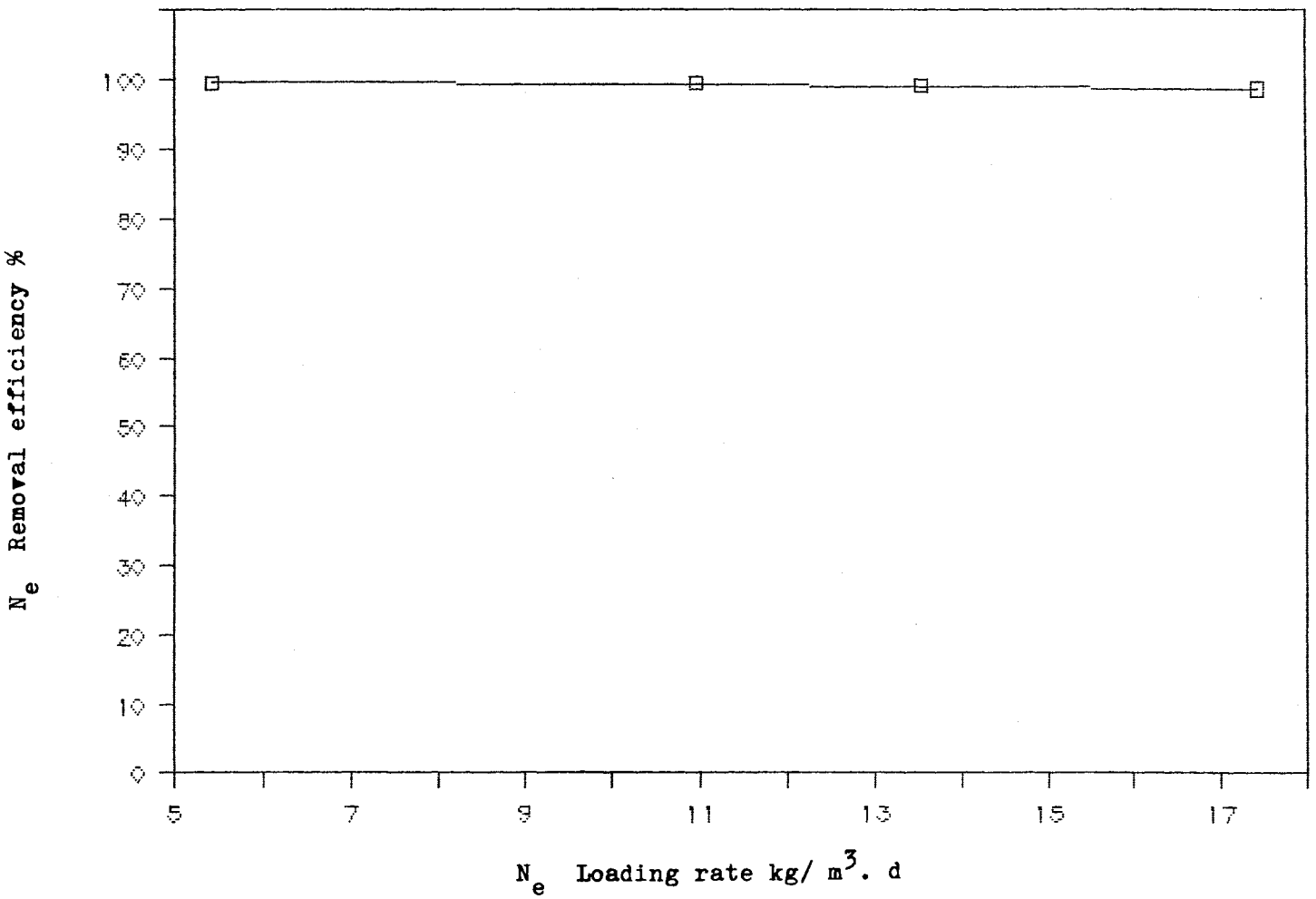


Figure 11. Denitrification Efficiencies in the AFEB Reactor for Constant 750 mg Ne/L Feed Concentration

removal rate achieved under steady state condition at a constant feed concentration of 758 mg N_e/L was 17.23 kg/m^3.d. The system, however, collapsed when the HRT was changed from 1.1 to 0.8 hour. Within 12 hours, 90% of the diatomaceous earth with the attached biomass escaped from the reactor.

Reasons for massive expanded bed media escape have not been reported in the literature. It might be due to vigorous gas evolution at high N_e loading rates or a sudden release of large amounts of entrapped gas in the bed, carrying biomass media to the top of the reactor. If the bubbles do not break when they reach the top of the reactor, the biomass can escape from the reactor with the effluent instead of settling back into the expanded bed.

4.3 Upflow Sludge Blanket

4.3.1 Data Summary

The results of experiments on the USB with varying N_e feed concentration from 277 to 1086 mg/L and a constant HRT of about 6.0 hours are summarized in Table VI. Each value of the table represents the average reading taken for any one loading rate. The N_e removal rate varied from 1.05 to 3.69 kg/m^3.d. The experiment could not be continued beyond 4.3 kg/m^3.d because excessive biomass escape was observed at this N_e loading rate. Figures 12 and 13 were drawn from data taken from Table VI. These figures show removal rate and efficiency for various N_e loading rates for constant HRT.

The results obtained from operating the USB under constant N_e concentration of 500 mg/L but varying HRTs from 6.0 to 0.59 hour are

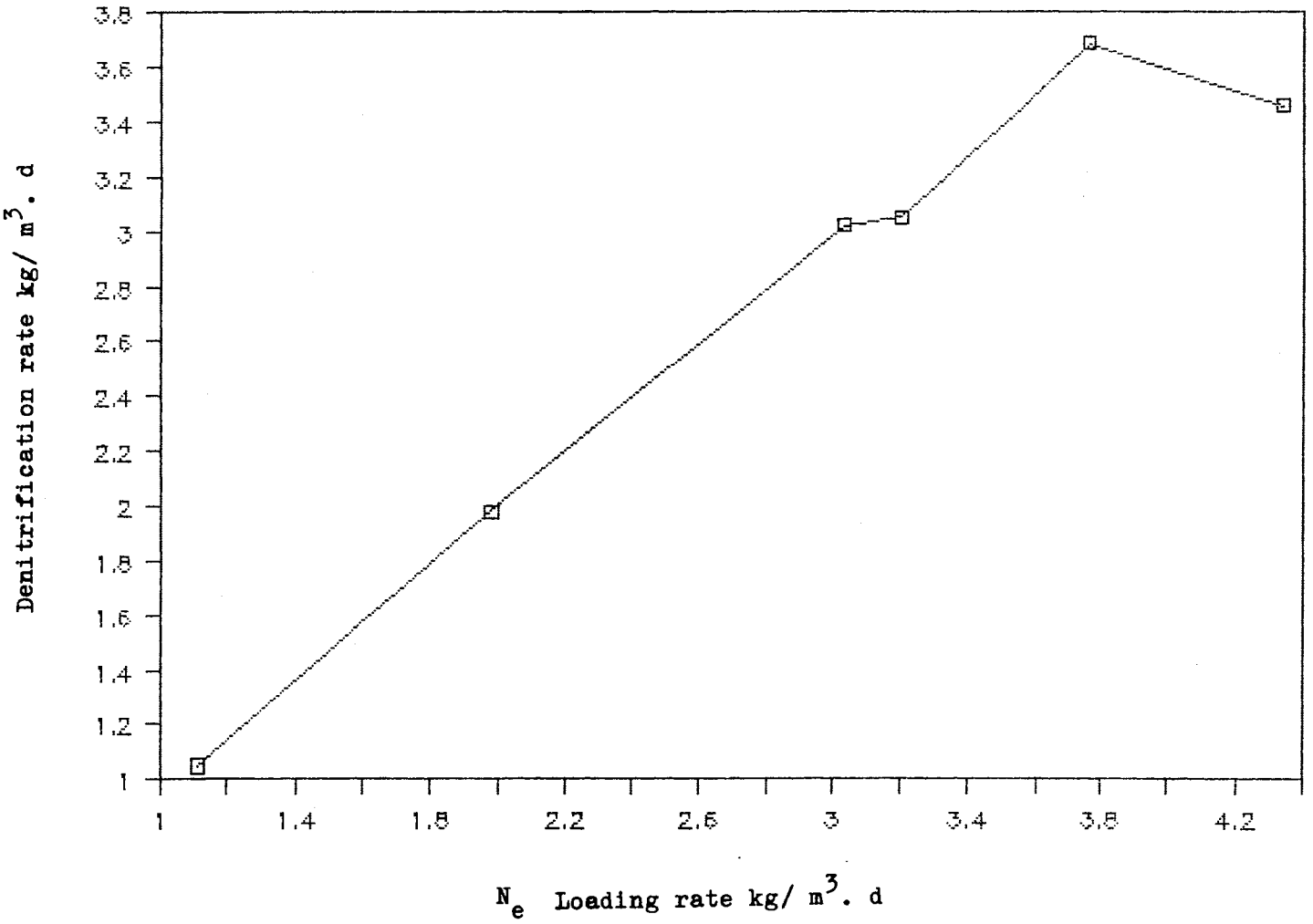


Figure 12. Denitrification Rates in the USB Reactor for Constant 6 Hour HRT

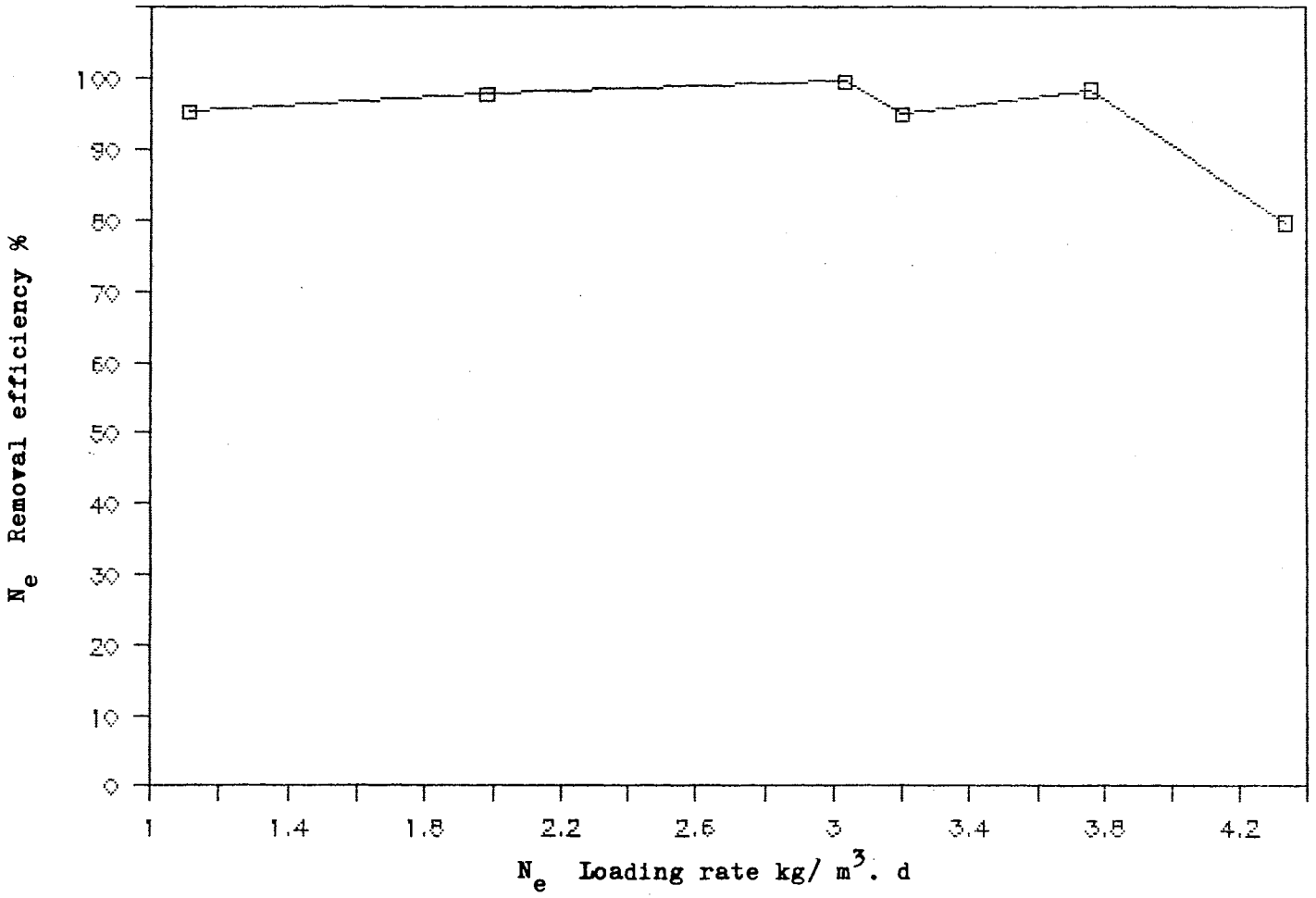


Figure 13. Denitrification Efficiencies in the USB Reactor for Constant 6 Hour HRT

presented in Table VII. Figures 14 and 15 are drawn from data taken from Table VII. The maximum N_e removal rate achieved in the USB at steady state conditions was 13.7 $kg/m^3.d$ for a loading rate of 14.3 $kg/m^3.d$. When the loading rate was increased from 14.3 to 20.1 kg $N_e/m^3.d$, the USB began to lose large amounts of biomass. It was observed that the density or the compactness of the sludge granules was reduced with time. To avoid total biomass escape, it was decided to stop the experiments at this stage.

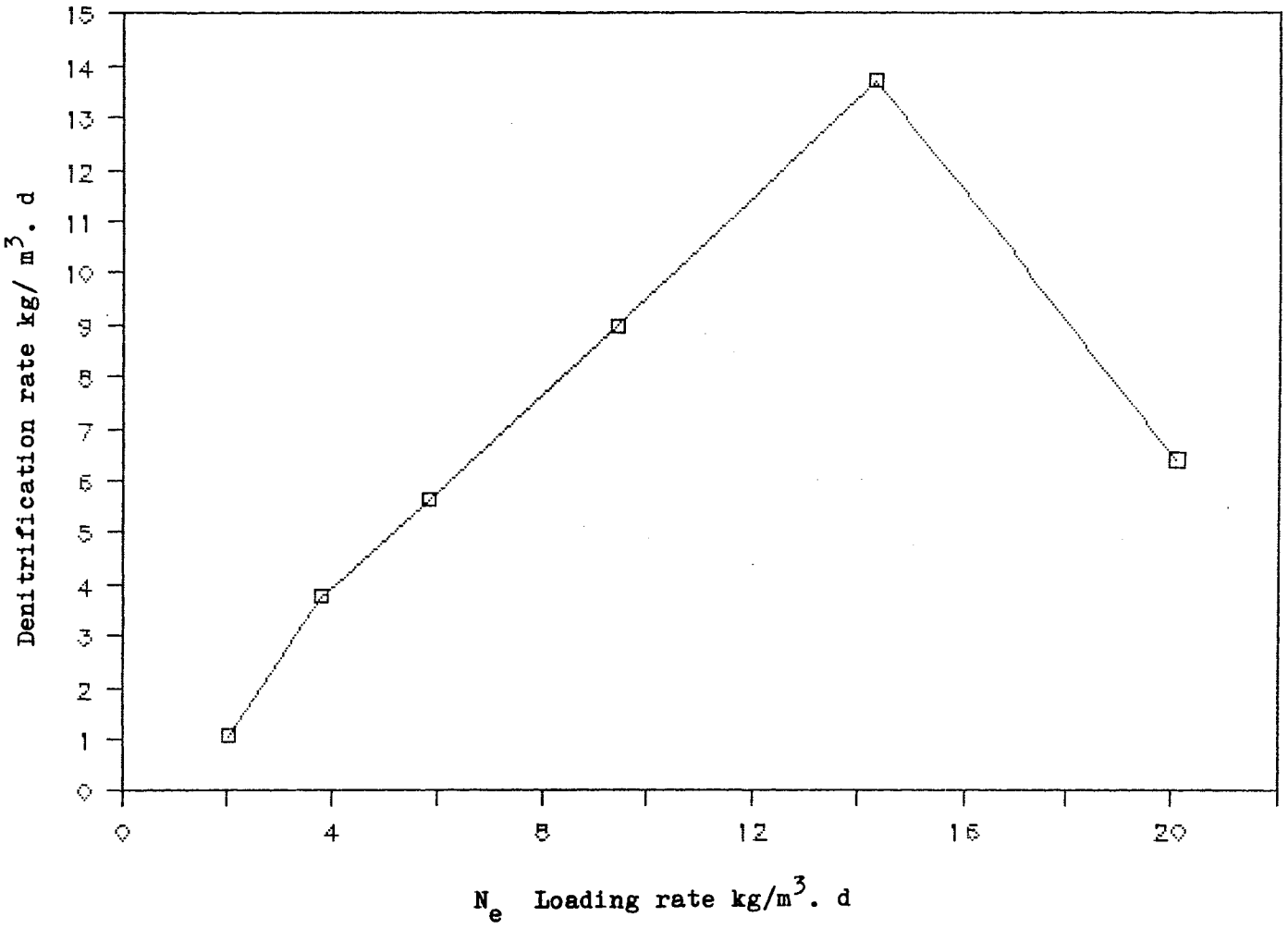


Figure 14. Denitrification Rates in the USB Reactor for Constant 750 mg Ne/L Feed Concentration

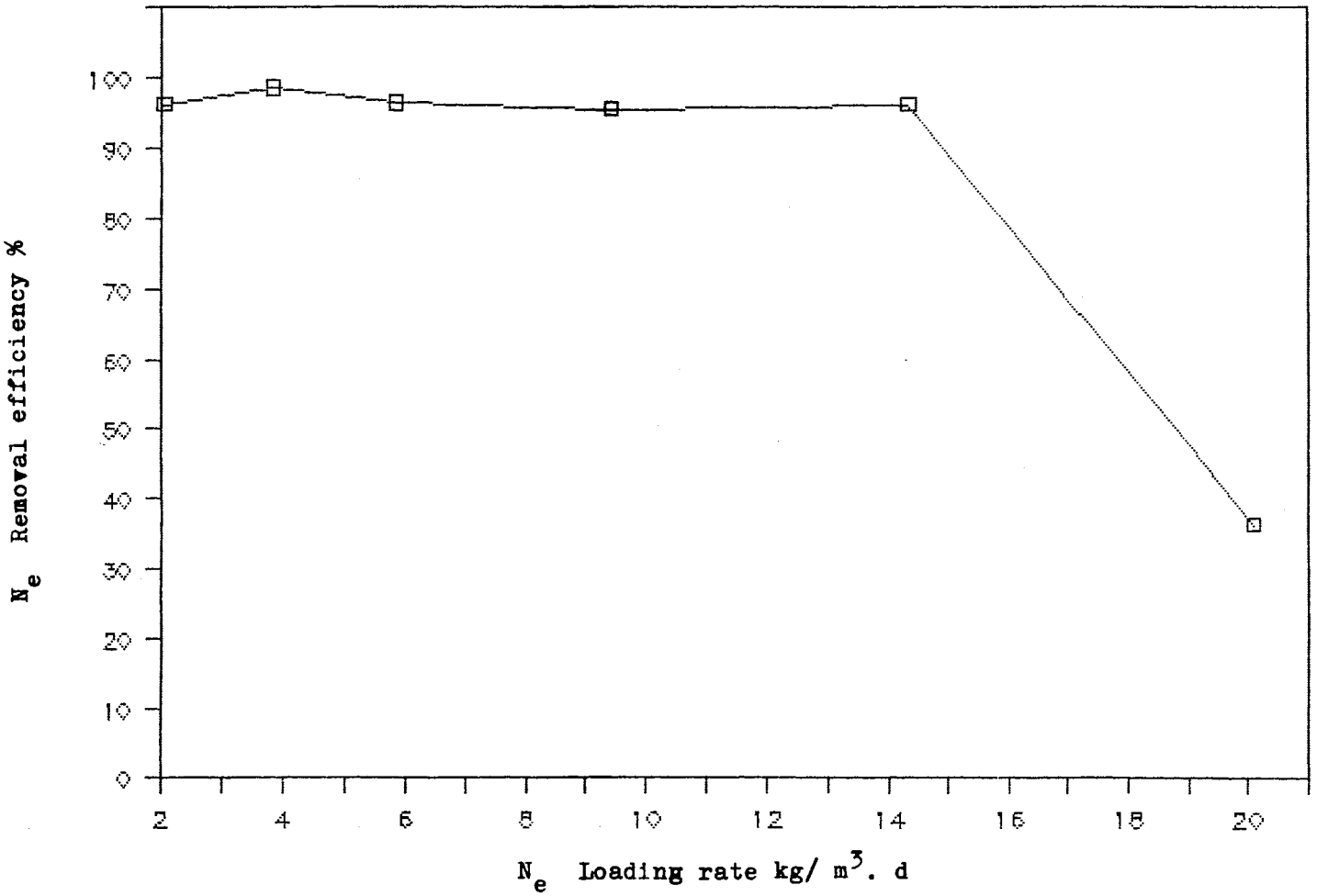


Figure 15. Denitrification Efficiencies in the USB Reactor for Constant 750 mg Ne/L Feed Concentration

CHAPTER V

DISCUSSION OF RESULTS

The primary objective of this research was to investigate the feasibility of autotrophic denitrification for high strength industrial wastes and gather information that may assist in the implementation of this process in the field of wastewater treatment. This involved experimentation with two systems, the AFEB and USB. The two systems were tested for two operating parameters--N_e feed concentrations and hydraulic retention times. The results of the experiments can be used to identify a process as well as operating conditions for autotrophic denitrification.

5.1 Evaluation of AFEB

Figure 8 shows the performance of the AFEB when it was operating under varying N_e feed concentrations but at constant HRTs. Figure 10 shows the reactor operating at constant N_e feed concentration but at varying HRTs. The best way to evaluate the systems would be to examine the two systems for various N_e loading rates. From Figures 8 and 10 it can be seen that the maximum N_e removal rate of 17.23 $kg/m^3.d$ occurred when the loading rate was 17.46kg/m^3. Thus the AFEB maintained high denitrification efficiency (>98%) with N_e concentration of

about 770 mg/L at 1.1 hour HRT. The system, however, collapsed when the HRT was changed from 1.1 to 0.8 hr.

For a constant 3.3 hour HRT, the AFEB sustained high N_e removal efficiency (>98%) up to the N_e feed concentration of 1040 mg/L. When the influent N_e concentration was increased from 1040 to 1240 mg/L, removal efficiency began to decrease drastically. The minimum denitrification observed was 35% for this highest feed concentration. The maximum denitrification was only 6.7 $kg/m^3.d$ for this mode of operation.

In the AFEB reactor, when the N_e loading rate was increased, the rate of gas evolution also increased. Higher gas production was due to increased denitrification rates. Gas bubbles lifted the biomass and carried it to the top of the reactor. As there was no mechanism to break the gas bubbles at the liquid-gas interface, the biomass began to accumulate instead of settling. As a result of accumulation, the biomass began to escape in the reactor effluent. The system failed not because of biological incapability but because of insufficient physical means to manage the gas produced. Thus, the maximum denitrification rate achieved in this study (17.23 $kg/m^3.d$) could have been increased had there been arrangements in the reactor to handle problems associated with gas evolution.

5.2 Evaluation of USB

Figures 12 and 14 represent the behavior of the USB under various N_e feed concentrations as well as HRTs. An examination of Figure 12 shows that the maximum N_e removal rate of 13.7 $kg/m^3.d$ occurred when the loading rate was 14.3 $kg/m^3.d$. This maximum N_e removal rate

occurred when the reactor was fed with 500 mg N_e/L and the HRT was 0.8 hour.

At a constant 6.0 hour HRT, the maximum feed concentration studied in the USB was 1086 mg N_e/L. The reactor operated at 79.6% denitrification efficiency and N_e removal of 3.46 kg/m^3.d at this feed concentration.

As discussed in Section 5.1., the gas produced in the reactor created problems. As in the case of the AFEB, there was no arrangement in this reactor to break gas bubbles which carried the biomass to the top of the reactor.

5.3 Attached Biomass

The attached biomass in the AFEB averaged 22.6 g VS/L with a range of 20.2 to 27.1. These values were measured for most of the steady state conditions. A value of 26.7 g/L was measured after 11 weeks the reactor was started with continuous feed. No values are available in the literature for the attached biomass for autotrophic denitrification.

5.4 Total and Volatile Suspended Solids

For total and volatile suspended solids measurement, only grab samples were collected. Invariably, all effluent samples were very clear. Low suspended solids results were in agreement with the physical observations. A portion of the suspended solids always attached themselves to the inverted funnel and only the balance escaped with the effluent. This happened in both the AFEB and USB.

5.5 Stoichiometric Parameters

The observed N_e/alkalinity value varies greatly from the stoichiometric value. No values are available in the literature for comparison. The ratio of N_e/Alk (mol/mol) has a stoichiometric value of 3.7 and the value obtained in this study was 2.24. Higher alkalinity consumption observed in this study could be due to reaction of hydrogen ions present in the water as well as ions dissociated from monobasic potassium phosphate. Formation of H_2SO_4 can also result in higher comsumption of alkalinity (Sikora and Keeney, 1976). Table VIII shows a comparison of observed stoichiometric parameters.

TABLE VIII

COMPARISON OF OBSERVED AND CALCULATED STOICHIOMETRIC PARAMETERS

Parameter	Value	Reference
$S_2O_3^-/N_e$	0.84	Bisogni & Driscoll (1977)
	0.81	Claus & Kutzner (1985b)
	0.76	Baalsrud & Baalsrud (1954)
	0.75	Justin & Kelly (1978)
	0.75	Stoichiometric Value (See Section 2.7)
	0.72	Observed Value in This Study
$SO_4^=/N_e$	1.62	Claus & Kutzner (1985b)
	1.56	Stoichiometric Value (See Section 2.7)
	1.44	Observed Value in This Study
$CO_2/S_2O_3^=$	0.21	Lu & Kelly (1983)
$HCO_3^-/S_2O_3^=$	0.34	Stoichiometric Value (See Section 2.7)
	0.32	Observed Value in This Study
N_e/Alk	3.70	Stoichiometric Value (See Section 2.7)
	2.24	Observed Value in This Study

CHAPTER VI

ENGINEERING SIGNIFICANCE

Results of this study indicate that the AFEB has significant potential for autotrophic denitrification of high strength nitrogenous wastes. At a feed concentration of 758 mg N_e/L, the process is stable and it maintains high N_e removal efficiency of 98.7% for loading rates up to 17.46 kg/m^3.d. Minimum hydraulic retention time at which stable operation occurs is 1.1 hour.

The volumetric loading rate depends on N_e concentration and HRT. A high loading rate can be achieved either with low HRT and low N_e concentration or with high HRT and high N_e concentration. If volume of biomass remains constant, the feed pumping rate must increase as the HRT decreases. This is an important factor to be considered in the design of a reactor. Low HRTs will increase the capital cost as well as the operating cost of pumps. However, the capital cost of the reactor will be small for small HRTs. For best results, data on costs must be collected for various feed concentrations and HRTs.

The AFEB reactor needs two pumps: one for the feed solution and another one for recirculation. The USB requires only one pump. Treatment cost should be based on capital as well as operating cost.

To achieve maximum possible removal efficiency, the system should be fed with thiosulfate and bicarbonate in quantities in excess of

stoichiometric requirements so that they will not be rate limiting. When N_e feed concentration is high, either sulfate (one of the end products of denitrification) or thiosulfate and bicarbonte (added in excess of stoichiometric requirements) may be present in unacceptable concentrations in the effluent from the reactor. It may become necessary to dilute the effluent in such cases. It is important to decide where dilution should be made--in the influent or effluent of the denitrification process.

CHAPTER VII

CONCLUSIONS

This study is the first to focus on high rate autotrophic denitrification using the AFEB and USB. For the range of loading rates tested, the AFEB maintained very high N_e removal efficiency of >98.7% for HRTs as low as 1.1 hour. The maximum N_e removal rate in this reactor was 17.2 $kg/m^3.d$ for a feed concentration of 760 mg N_e/L. This conversion rate is higher than any value reported in the literature to date for autotrophic denitrification, and was achieved at a volumetric N_e loading of about 17.4 $kg/m^3.d$.

For an N_e feed concentration of 500 mg/L and HRT of 0.83 hour, the USB removed 13.7 kg $N_e/m^3.d$ when the N_e loading was 14.3 $kg/m^3.d$. Although the denitrification rate was lower in the USB compared to AFEB, the maximum N_e removal obtained in this study seems to be the highest rate achieved for autotrophic denitrification using the USB.

In the AFEB reactor, the attached biomass varied between 20.2 and 27.1 mg/mL (kg/m^3). The molar ratio of thiosulfate consumed to N_e consumed was 0.70 and 0.73 for the AFEB and USB, respectively. For the AFEB and USB, the molar ratio of sulfate produced per mole of N_e utilized was 1.41 and 1.46, respectively. In the USB, sulfate concentration up to 9100 mg/L did not have any inhibitory effect on

denitrification; the feed concentration was 939 mg N_e/L and the N_e removal efficiency was 98.3%.

This bench-scale research indicates that autotrophic denitrification is technically feasible for high-strength nitrogenous wastes. However, to implement this process in the field, it must be shown to be economically viable. This will be enhanced if thiosulfate and bicarbonate are available inexpensively as industrial by-products, in the same way that many organic by-products have been shown to be acceptable substrates for heterotrophic denitrification.

CHAPTER VIII

RESEARCH NEEDS

This is the first study conducted to determine whether high rate autotrophic denitrification is feasible with the AFEB and USB. Though the results indicate high potential for autotrophic denitrification, additional data are required to implement application-oriented engineering of these processes:

1. Evaluation of sulfite, sulfide, and sulfur as electron donor based on technical feasibility and economical suitability.
2. Investigation of the effect of HRTs for a wider range of N_e feed concentrations.
3. Design of suitable mixers for the AFEB and USB. A high torque low speed motor with a suitable impeller is suggested.
4. Analysis of composition of gas collected from the reactors.
5. Investigation of the mechanism and effect of nitrite accumulation in the reactors.
6. Determination of precise pH, temperature, and nutritional optima for autotrophic denitrification.
7. Collection of basic cost information on pumps, energy, dilution, and chemicals for making rational engineering design decisions, and choosing between the AFEB and USB systems.

8. Suitability of autotrophic denitrification for potable water treatment.
9. Engineering design solution to the problem of biomass carry-over due to gas evolution.

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