AMMONIA EFFECTS ON HIGH-STRENGTH AUTOTROPHIC DENITRIFICATION OF SIMULATED INDUSTRIAL WASTEWATER

BY

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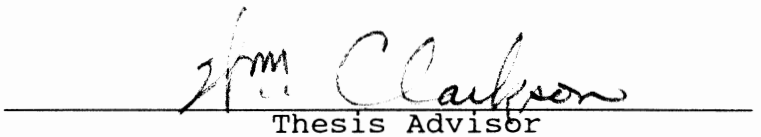

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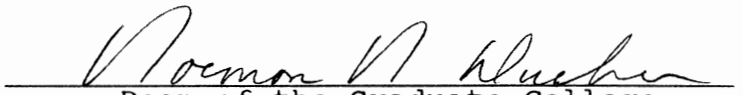

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CHAPTER I

INTRODUCTION

The Scope of the Research

Biological denitrification, as a method to control the nitrogenous compound discharge to the environment, has been studied with respect to biochemistry, microbiology and methodology. The basic mechanisms and certain applications are well known. During the previous research (Ross, 1989; Krishnamachari, 1990; and Clarkson et al., 1991) conducted in this department, loading rates as high as 17 kg NO_3^--N/m^3 d were applied to both heterotrophic and autotrophic denitrification. But more research is still needed for individual and site specific cases. However, the autotrophic process was found to have advantages in terms of stability as well as not requiring an organic carbon source (a cost-effective process). This research was conducted to investigate biological denitrification in the presence of high concentrations of ammonia nitrogen.

Agricultural Minerals Corporation (AMC) operates a nitrogen fertilizer plant at Catoosa, Oklahoma, which produces ammonium nitrate and urea ammonium nitrate fertilizer. Along with the fertilizer manufacturing, the process generates wastewater with widely varying flow rates (ranging from 42 to 2623 M^3/day (0.011 to 0.693 MGD) from limited available data). This wastewater concentrates a

great amount of both ammonia nitrogen and nitrate nitrogen, at a variable ratio of up to about 6:1. Ammonia nitrogen ranges from 1600 to 5300 mg NH_3-N/l, and nitrate nitrogen from 900 to 3000 mg NO_3-N/l. These represent a total nitrogen discharge of 100 to 11,100 kg N/day (200 to 24,500 lb N/day) (Clarkson, 1990).

Research Objectives

A proposed treatment process was to use a combined sequential high-rate autotrophic nitrification and denitrification treatment system to complete the conversion of all nitrogenous compounds to the harmless gaseous nitrogen form (Clarkson, 1990). But, as shown in Figure 1(a), with nitrification process as first stage, two problems could arise: (1) high concentration of nitrate in influent could interfere with nitrification, and (2) the sum of nitrate from influent and that from nitrification step could exceed the loading limit of denitrification process. One alternative (Figure 1(b)) could be a pre-denitrification step preceding the nitrification step in order to reduce the nitrate interference on nitrification and to reduce the nitrate loading on final denitrification step. Therefore, an investigation is needed to study the influent ammonia interference on the pre-denitrification process. The major goals of this research were to:

(1) investigate the influent ammonia effects on denitrification rate.

(2) determine the dilution factor of raw wastewater required for efficient conversion.

(3) observe the ammonia change through the process.

(4) study the biomass stability in denitrification reactor.

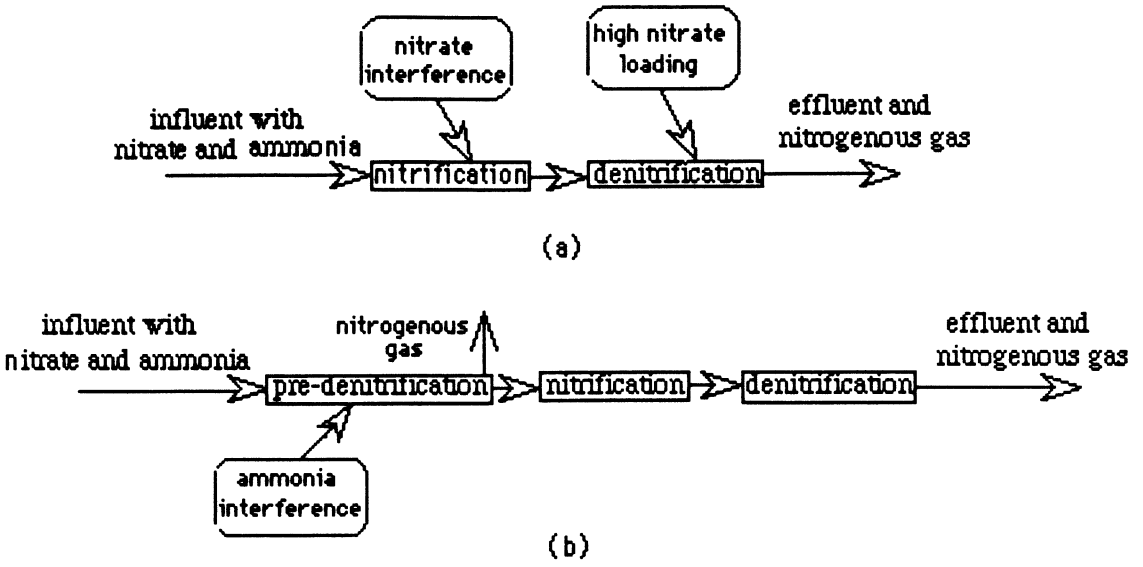

Figure 1. Treatment Sequences

Approach of Research

The anaerobic attached film expanded bed reactor was employed for the denitrification units, based on the success of previous research (Ross, 1989 and others). Diatomaceous earth was used as the support media for bacterial growth in the expanded bed reactors, as in previous bench-scale studies.

Nitrate nitrogen was tested at three different concentrations: 500, 750 and 1000 mg NO_3^--N, which were determined according to the previous research (Ross, 1989).

At these three nitrate nitrogen concentrations, ammonia nitrogen effects were examined at several concentration levels, ending with an ammonia-nitrate concentration ratio of 6:1, which is the maximum ratio of the raw AMC wastewater.

CHAPTER II

LITERATURE REVIEW

Scope of Review

Scope

Nitrates, produced from a variety of industries such as fertilizer, semiconductor, munition, and nuclear, are proven to be one of the major drinking water contaminants causing health problems. Methemoglobinemia in infants and some ruminant livestock is directly related to nitrate according to many researchers. Nitrites, produced from nitrate reduction, can inactivate the hemoglobin in blood and deduct its oxygen-transport capacity. The U.S. EPA established the standard of 10 mg/l nitrate nitrogen for drinking water supplies. Nitrate is also a major component causing eutrophication in lakes. Ammonia is toxic to fish and some plants at a concentration higher than 0.2-0.5 mg/l.

Available processes for nitrate removal are numerous. They include microbial denitrification, land application, selected ion exchange, electrodialysis, reverse osmosis, carbon adsorption, and filtration. From the environmental engineering point of view, microbial denitrification is preferable to this special case of high nitrate and ammonia concentrations because of its cost-effectiveness, tolerance

to high loading, and innocuous endproduct: nitrogen gas-the major component in air. Besides, other methods are not fully efficient if wastewater carries several forms of nitrogen at the same time and in the concentrations in excess of several hundreds mg N/l.

Biological denitrification is a natural process that uses microbial activity to convert the nitrate into gaseous nitrogen, eliminating its contamination. This natural process has been widely applied in municipal wastewater treatment plants for tertiary treatment. It has also been used widely for industrial wastewater treatment. Industrial wastewaters usually have a property of high strength and a characteristic of great variance in constituents, concentrations and flowrates. The process itself is subject to failure led by interferences coming from other existing components. As mentioned before, wastewater from the AMC nitrogen fertilizer producing plant contains a great deal of both ammonia and nitrate nitrogen. Thus, it is necessary to know how ammonia will affect microbial denitrification activity during practice. Little is presently known about the behavior of high-rate nitrification/denitrification systems with concentrated wastes, and less when the waste streams are mixed.

Development

Complete denitrification is relatively stable and easy to maintain compared with other processes, despite changes in system loading conditions and wastewater strength. Denitrification as first treatment stage for complex industrial wastewaters has been reported. Melcer et al.

(1988) used pre-denitrification for biological control of complex nitrogen wastewaters, and showed successful denitrification with a fluidized reactor, but at relatively low nitrogen loading rates.

Available information on ammonia interference is sparse. In a heterotrophic denitrification system with methanol addition as electron donor, ammonia showed no effects on denitrification rate, with the influent concentrations of ammonia nitrogen of 50-200 mg/l and nitrate nitrogen of 80-500 mg/l (Przytocka-Jusiak et al., 1984). This research was conducted for the treatment of nitrogen fertilizer industry wastewater by using packed-bed heterotrophic denitrification and rotating disk heterotrophic nitrification in a two-step purification system.

Biochemistry of Denitrification

Nitrate removing bacteria can use both organic and inorganic substrates as electron donors. In the case of organic utilization, heterotrophic denitrifiers can oxidize methanol, acetate, glucose, acetone, and a great number of other organic chemicals. Methanol is most commonly used for several reasons: low cost, high aqueous solubility, and easy biodegradation (Krishnamachari, 1990). However, the increasing cost of methanol and/or other organic compounds and their effluent residue problem make them increasingly undesirable as chemical additives. On the other hand, low biomass yield during autotrophic denitrification process can reduce the cost of sludge disposal. Lower attached biomass concentrations were reported in the autotrophic

process than in the heterotrophic process (Ross, 1989; Krishnamachari, 1990). Therefore, as an alternative method, autotrophic denitrification by adding inorganic compounds as electron donor, has attracted more attention in recent years.

Up to now, most inorganic chemicals used for high rate denitrification are reduced sulfur compounds such as elemental sulfur, sulfide, sulfite and thiosulfate because of their cost, electron donation potential and availability. Among these compounds, sulfide and sulfur have high electron contents and are relatively cheap on an electron equivalent basis (Driscoll & Bisogni, 1978). But they are difficult to handle since sulfur has a low aqueous solubility that could make it as a limiting factor in high rate denitrification process, and addition of sulfide causes odor problems, especially when added at high amount for high rate denitrification. Thiosulfate was chosen by many researchers because of its easy oxidation and high aqueous solubility compared with sulfur, odor-free use compared with sulfide, and high electron content compared with sulfite.

Microbiology

Autotrophic denitrification is a natural microbial activity, during which oxidized forms of nitrogen such as nitrate, nitrite and/or nitrous oxides are reduced to gaseous nitrogen by bacteria, coupled with the oxidation of other inorganic matter. Nitrogen compounds serve as the terminal electron acceptor. Energy produced by this biological oxidation-reduction is used by bacteria for cell

synthesis. Inorganic carbon sources are needed for this cell growth.

Bisogni and Driscoll (1977) used the formula which was developed by McCarty (1975):

$$R = R_d - f_e R_a - f_s R_c \qquad (1)$$

(where, f_e and f_s are the fractions of the electron donor used for energy maintenance and cell synthesis, respectively; R represents the overall stoichiometric reaction; R_d, R_a and R_c are the half reactions for electron donor, electron acceptor and cell synthesis, respectively.) to establish a stoichiometric relationship for autotrophic denitrification by using thiosulfate as energy source, ammonium as nitrogen source, and carbon dioxide and bicarbonate as carbon source:

$$NO_3^- + 0.844\ S_2O_3^= + 0.34\ CO_2 + 0.087\ HCO_3^- + 0.087\ NH_4^+ + 0.043\ H_2O = 0.087\ C_5H_7O_2N + 1.689\ SO_4^= + 0.5\ N_2 + 0.697\ H^+ \qquad (2)$$

Nitrate Reduction. In the absence of oxygen, bacteria use nitrate as an oxidant for respiration. During this dissimilatory reduction, nitrates are reduced to nitrites, and through several steps to nitrogen gas and nitrogenous oxides (Jeter & Ingraham, 1981):

$$NO_3^-\ \text{(nitrate)} \text{------>} NO_2^-\ \text{(nitrite)} \text{------>} \text{NO (nitric oxide)} \text{-----> } N_2O\ \text{(nitrous oxide)} \text{------> } N_2\ \text{(nitrogen gas)} \qquad (3)$$

Nitrate acts as the electron acceptor receiving the electrons from thiosulfate oxidation.

Thiosulfate Oxidation. The path for the oxidation of thiosulfate to sulfate is not yet clear. One proposed path (Peeters & Aleem, 1970) is that under anaerobic conditions, thiosulfate is first cleaved by thiosulfate reductase into sulfide and sulfite moieties, and thus both couple with the electron transport chain. Another proposed path is that the thiosulfate is cleaved into sulfur and sulfite before entering the electron transport chain (Suzuki et al., 1974). The third proposed path which was referenced by more researchers is that tetrathionate is the first product of thiosulfate oxidation (Baalsrud & Baalsrud, 1954; Bergey's Manual, 1980). This oxidation path by whole cells is as follows:

$$S_2O_3^{=} \text{ (thiosulfate)} \text{ -----> } S_4O_6^{=} \text{ (tetrathionate)} \text{ -----> } S_3O_6^{=} \text{ (trithionate)} \text{ -----> } SO_3^{=} \text{ (sulfite)} \text{ ------> } SO_4^{=} \text{ (sulfate)} \quad (4)$$

Electron Pathway. Most researchers agreed that flavoprotein (fp), NAD, NADH, cytochromes b and c, and some other enzymes are involved in the electron transfer chain to transfer electrons from thiosulfate to nitrate (Baalsrud & Baalsrud, 1954; Justin & Kelly, 1978; Claus & Kutzner, 1985b; Bergey's Manual, 1980; Ross, 1989). A simplified conceptual electron transfer pathway for autotrophic denitrification using thiosulfate as electron donor is presented below (Sawhney and Nichloas, 1977; Ross 1989):

$$\begin{matrix} S_2O_3^{=} \text{ ---> Fp} - \\ NADH_2 \text{ ---> Fp} - \end{matrix} \text{--> Quin --> Cyt b --> Cyt c --> } NO_3^{-} \quad (5)$$

Energy Production. During this oxidation-reduction,

biological energy is produced in the form of ATP to support bacterial growth. According to Justin and Kelly (1978), oxidation of each mole of thiosulfate can produce 4-5 moles ATP: 1-2 moles produced through substrate level phosphorylation and 3-4 moles from oxidative phosphorylation, which represents about 741 KJ/moles theoretical free energy for nitrate-linked thiosulfate oxidation.

Carbon and Nitrogen Source. As a general rule, heterotrophic bacteria use organic matter for carbon source. In this case, most organic compounds can be utilized directly by bacteria into synthesis; for instance, glucose going through the substrate-level phosphorylation and TCA cycle under aerobic conditions or other pathway under anaerobic conditions, and finally an oxidative electron transport pathway (Gaudy & Gaudy, 1988; Grady & Lim, 1980). During the autotrophic denitrification by Thiobacillus denitrificans (T. denireificans), bacteria first need to convert the inorganic substrates into organic substrates by the fixation of carbon dioxide and/or bicarbonates. This fixation is completed through the Calvin cycle which utilizes ATP and NADPH2 generated by cells to reduce carbon to the oxidation level of carbohydrates. This is actually the reversal of a normal electron transport chain (Gaudy & Gaudy, 1988; Grady & Lim, 1980; Justin & Kelly, 1978; and Baalsrud & Baalsrud, 1954):

$$6CO_2 + 18ATP + 12NADPH_2 + 12H_2O \rightarrow$$
$$\text{glucose} + 18H_3PO_4 + 18ADP + 12NADP \qquad (6)$$

This consumption of energy for organic monomers required for synthesis of protein and lipids helps to explain why autotrophic processes produce less biomass than heterotrophic processes. (Senez, 1962; Stouthamer, 1977),

Ammonium is also utilized by bacteria for biosynthesis. Many researchers have pointed out that ammonium is preferred by bacterial use due to its reduced form. T. denitrificans can also use nitrate as growth nitrogen source, but nitrates have to be reduced by bacteria themselves first for this assimilatory purpose (Claus & Kutzner, 1978; and Peeters & Aleem, 1954).

Inhibitions and Interferences

Dissolved Oxygen. T. denitrificans can use both oxygen and nitrate as electron acceptor while oxidizing thiosulfate (Justin & Kelly, 1978; and Baalsrud & Baalsrud, 1954). If sufficient dissolved oxygen is available, aerobic respiration becomes predominant and denitrification rate decreases. This is because higher energy production exists during aerobic respiration, and bacteria tend to undergo metabolic reactions with higher energy output (Grady & Lim, 1980, and Krishnamachari, 1990). In the presence of oxygen, T. denitrificans carries out the incomplete oxidation of thiosulfate to tetrathionate only (Baalsrud, & Baalsrud, 1954). To eliminate the aerobic respiration effects in an anoxic reactor, McClintock et al. (1988) maintained the dissolved oxygen levels less than 0.5 mg/l in the feed and less than 0.1 mg/l in the reactor for an activated sludge system. In great excess of nitrate to oxygen, denitrification is predominantly a microbial

mechanism and oxygen effects are negligible.

Nitrite Accumulation. Nitrite accumulations during nitrate removal processes were widely reported by researchers (Claus & Kutzner, 1985a and 1985b; and Wilderer et al., 1987). Several mechanisms were proposed: (1) repression of synthesis of nitrite reductase, (2) selection and enrichment in favor of microorganisms capable of reducing nitrate but only to nitrite, and (3) the presence of more efficient nitrite oxidase of Nitrobacter sp. catalyzing the reverse reaction (Wilderer et al., 1987). Claus and Kutzner (1985a,b) reported that nitrite, an intermediate of the process, affected denitrification above 0.2 g/l, and that a minimal residence time was important to avoid nitrite accumulation.

Sulfate Inhibition. More important, autotrophic denitrifiers are very sensitive to sulfate, the product of thiosulfate oxidation. During the experiment by Claus and Kutzner (1985a), sulfate inhibition was observed at concentrations above 5 g/l, and above 20 g/l no denitrification activity was observed.

Ammonia Inhibition. Ammonia is present in water either in the form of ammonium ion (NH_4^+) or as dissolved ammonia gas (NH_3). They are in equilibrium, according to the following equation:

$$NH_4^+ = NH_3 + H^+ \qquad (7)$$

Therefore, the relative concentrations depend on the pH.

At high concentration, both forms are inhibitory to the anaerobic treatment system. At pH lower than 7.2,

inhibition is related to ammonium ion. Above that pH, it is related to ammonia gas, which becomes inhibitory at much lower concentrations. Table I shows the ammonia effects on anaerobic treatment.

TABLE I

AMMONIA EFFECTS ON ANAEROBIC TREATMENT

ammonia nitrogen concentration mg/l	effects on anaerobic treatment
50 - 200	beneficial
200 - 1000	no adverse effect
1500 - 3000	inhibitory at high pH
above 3000	toxic

(adapted from McCarty, 1964)

Other Chemicals. Other chemical compounds were also reported to be inhibitory at some high concentrations. They were alkalinity, chloride, nitrate, thiosulfate, and some cationic ions such as sodium and calcium (McCarty, 1964; and Claus & Kutzner, 1985a).

Autotrophic Denitrifying Bacteria

Mainly, there are two major species involved in autotrophic denitrification treatment processes:

Micrococcus denitrificans and Thiobacillus denitrficans. Micrococcus denitrificans use hydrogen as electron donor (Kurt et al., 1987), whereas Thiobacillus denitrificans use reduced sulfur compounds such as sulfides, sulfur, thiosulfate, polythionates, and probably sulfite as electron donors (Bergey's Manual, 1980). Kurt et al. (1987) used Micrococcus denitrificans in a fluidized-bed biofilm reactor for biological denitrification of drinking water. The process was very successful, but one problem remained. Due to the low solubility of hydrogen gas in water, experiments exhibited the accumulation of nitrite unless the residence time was long enough, especially at nitrate nitrogen concentrations higher than 25 mg/l.

As described in Bergey's Manual (1980), T. denitrificans are obligately chemolithotrophic and autotrophic. Optimum temperature for their growth is 28-32 ^{o}C, and optimum pH is 6.8-7.4. They are widely distributed in soil, mud, fresh water and marine sediments, especially under anoxic conditions. They are short rods, about 0.5 x 1.0-3.0 x 10^{-6} meters. They may be mobile by means of a polar flagellum and are gram negative, and non-spore forming (Baalsrud & Baalsrud, 1954).

Justin and Kelly (1978) measured the elemental composition of T. denitrificans and found the following percent composition: carbon, 47.40$\pm$1.02; hydrogen, 6.88$\pm$ 0.23; nitrogen, 12.70$\pm$0.79. C/N ratio, therefore, is about 3.6. As mentioned before, they grow anaerobically on reduced sulfur compounds by using nitrate, nitrite or nitrous oxide as terminal respiratory oxidant, and they utilize carbon from carbon dioxide, carbonate, and

bicarbonate (Breed et at., 1957; and Ross, 1989).

Denitrification Treatment Processes

The conventional aerobic processes have been used for many years to treat various industrial and municipal wastewaters. In the meantime, after some advances in the basic understanding of the microbiology, biochemistry and also hardware technology of anaerobic processes, many researchers showed that anaerobic processes, well known for the stabilization of municipal sewage sludge and animal residues and for solid waste reduction, may also be superior for the treatment of both dilute and concentrated soluble wastes. Energy consumption and cost effectiveness are important considerations in evaluating anaerobic processes (Switzenbaum, 1987).

The advantages of anaerobic processes are mostly : (1) no energy requirement for aeration, which saves operation cost; (2) low excess sludge production due to the slow growth rates of anaerobes, which saves much of the cost for sludge disposal. A third potential advantage, energy production from endproduct-methane (Jewell, 1985; and Switzenbaum, 1987), is not achieved from denitrification.

Though anaerobic processes work under one single metabolic constraint, the deficiency of dissolved oxygen, different process configurations work better for different wastewaters in terms of removal efficiency and cost effectiveness. Among these anaerobic processes, two major types of growth systems have been commonly employed for denitrification: suspended and fixed growth, which represent the most advanced and competitive processes.

Fixed growth systems usually have high surface area per unit of reactor volume for microbial growth, resulting in a higher biomass concentration. Two reactors, upflow sludge and expanded bed, have been shown to have greater potential as high-rate systems than any other anaerobic processes (Clarkson et al., 1991).

Sludge Blanket Reactor (UASB).

The UASB idea came from the 19th century biolytic tank concept and was more intensively developed in the middle 1970's (Jewell, 1985). The UASB relies upon the formation of aggregates of the bacteria (granules) in order to hold-up the biomass residence (Switzenbaum & Eimstad, 1987). In UASB, a biomass blanket is retained in suspension by both controlled upflow velocity of raw water and gas production of bioreaction. Slow mixing is essential to promote biomass agglomeration (Ross, 1989). Biomass can be the flocculent sludge or attached to inert medium particles. For the sake of better stability and settleability of the process, the latter seems to be recommended by most reporters (Lettinga et al, 1980). UASB-type reactors have become commercially available for full-scale use.

For denitrification, UASB were used by several researchers. Miyaji and Kato (1975) used nitrate as terminal electron acceptor for organic wastewater treatment in UASB. Klapwijk and Lettinga did a series of experiments in UASB systems to remove nitrate by adding sodium acetate (1975), alcoholic wastewater (mainly methanol and ethanol) (1981) and domestic wastewater (1981). Krishnamachari (1990) used UASB to achieve high rate nitrate reduction

with methanol as organic additive. The experimental results were very encouraging. More interestingly, Ross (1989) first successfully used UASB to achieve high rate autotrophic denitrification. At HRT of 0.8 hour, over 90 % removal efficiency was achieved at nitrate nitrogen concentrations above 500 mg/l.

Expanded Bed Reactor (AAFEB)

The AAFEB idea was investigated by Jewell and co-workers at Cornell University beginning in the early 1970's. The AAFEB works on the concept of higher accumulation of biomass , better separation of liquid and solid phases, and good resistance to shock loading (Jewell, 1985; and Switzenbaum, 1987). Compared with fluidized flow pattern, expanded bed maintains a lower bed expansion, which is in the range of 15-25 %. In AAFEB, bed expansion is maintained by upward flow of recycle and feed, and by gas produced during bioreaction. Active bacteria grow on the surface of inert media as a thin film which assures a good substrate diffusion through the bacteria (Jewell, Switzenbaum, & Morris, 1981). Good support media should have the characteristics of high surface area per unit volume, low density, and high porosity, which allow the high microbial growth and easy bed expansion (Clarkson, 1986). Among commonly used medium materials, such as sand, activated carbon, anthracite coal, glass beads, plastic beads, gravel, and diatomaceous earth, carbon and diatomaceous earth are the most preferred for expanded bed because of their physiological priorities as mentioned above (Krishnamachari, 1990). Diatomaceous earth, used in

this study, is chemically inert, resistant to ignition at 550 ^{o}C, and low in cost (Clarkson, 1986).

Compared with other anaerobic processes, AAFEB has attained up to 10 times the mass per unit volume over suspended growth systems (Jewell, Switzenbaum & Morris, 1981), which has allowed the highest waste loading, in terms of both COD and nitrate nitrogen, on per unit bed volume basis (Switzenbaum, 1987; Stronach, 1987; Ross, 1989; and Krishnamachari, 1990). Because of the biomass attachment on the inert media, clear separation of biomass bed and supernatant liquid could be obtained and easy biomass wash-out could be prevented. On the other hand, recycle flow of effluent could ensure better mitigation against shock loading and help to dilute high strength wastes. Other reported advantages of AAFEB included small head loss through the bed, easier removal or/addition of active material, and better avoidance of short circuiting and clogging (Switzenbaum, 1987). The reported disadvantages of this system were the complexity of the flow pattern and additional cost for effluent recycle.

Although denitrification was conducted by several researchers in fluidized bed reactors, high rate denitrification was first conducted heterotrophically and autotrophically in AAFEB by Clarkson, Krishnamachari and Ross (1991). Ross achieved 98 % nitrate removal efficiency at the nitrate nitrogen loading of 17.5 kg/m^3.d within HRT of 1.1 hours (concentration of 773 mg/l). For consistent 3.3 hour HRT, he sustained high nitrate removal efficiency up to the nitrate nitrogen concentration of 1040 mg/l (loading rate of 6.66 kg/m^3 d) (Ross, 1989).

Other Processes

Packed bed reactors have been employed for primary studies of autotrophic denitrification. The upflow without recycle pattern was used in these researches. Lava stones, limestones and elemental sulfur granules were used as packing media for bacterial growth and/or utilization (Driscoll & Bisogni, 1978; Batchelor & Lawrence, 1986 and Claus & Kutzner, 1985b). Bisogni and Driscoll (1977) also operated their research in completely mixed semicontinuous flow mode, which was one sort of suspended growth system.

Lewandowski, Bakke and Characklis (1987) demonstrated the immobilization of autotrophic denitrifying cells (T. denitrificans) into calcium alginate gel beads. Cell entrapment in porous substrata or in polymer gels such as calcium alginate beads gives the same advantages as biofilm plus some additional benefits such as less sloughing and co-immobilization of nutrients and buffering agents with cells. The cell immobilized beads can be used in each pattern of packed bed, fluidized bed, or stirred tank. But, much research on immobilized cell systems still needs to be done with respect to the mechanism, application and operation.

Finally, it is worth being noted that many other anaerobic alternatives have been used for heterotrophic denitrification by many researchers. Some examples were anaerobic submerged filters (both up and down flow) (Bailey & Thomas, 1975), continuous flow stirred-tanks, and wash-out reactors (Bode, Seyfried & Kraft, 1987). However, for high rate denitrification, the upflow sludge blanket and

expanded bed reactor were more effective, mainly due to their higher biomass concnetration per unit of bed volume in reactors. Therefore, they were used by more researchers.

CHAPTER III

EXPERIMENTAL APPROACH

Scope of Study

Research on ammonia effects on denitrification (meaning autotrophic denitrification unless specified) can provide information on the capability of denitrifying bacteria to resist ammonia interference and on treatment of wastewater containing both nitrate and ammonia nitrogen, such as the wastewaters from nitrogen fertilizer manufacturing, munitions manufacturing, and nuclear fuel processing (Clarkson, 1990). These were the major concerns for this investigation.

The investigation was based on the previous research by Ross (1989). The previous study established background knowledge of high rate autotrophic denitrification. This research duplicated key results of the previous study and performed the interference study.

Therefore, the experiments were grouped in three major phases:

(1) start-up phase:
    a) biofilm establishment.
(2) Duplication of previous research;
    a) duplication study;
    b) preparation of next phase.
(3) ammonia effects phase:

a) at 500 mg/l of nitrate nitrogen;

b) at 750 mg/l of nitrate nitrogen;

c) at 1000 mg/l of nitrate nitrogen.

Planning, fabricating the experimental apparatus, establishing the biofilm, duplicating the experiments, and conducting the ammonia interference experiments occupied approximately ten and half months, as follows: planning and construction of apparatus for one month; establishment of biofilm for three months; repeating experiments for two months; and interference experiments for four and half months.

Experimental Apparatus and Materials

Figure 2 shows the anaerobic expanded bed reactor employed for this study. Three reactors were operated at the same time. Two were used by Ross (1989) (Reactor 2) and Krishnamachari (1990) (Reactor 3), while the third was newly constructed (Reactor 1). The reactors consisted of a styrene acrylonitrile Imhoff cone with a plastic cylindrical tube at the top. The total height of the reactor was 61.5 cm of which the tapered section made up 46.5 cm. The top diameter of cylindrical tube was 10.5 cm. A metal connector of diameter 0.55 cm was screwed and glued into the bottom, which served as the inlet for the reactors. Another bigger metal connector with diameter of 0.75 cm was screwed and glued into the cylindrical tube 10 cm away from the top (Reactor 2 and 3) and 20 cm away (Reactor 1), which served as the outlet. One more metal connector was screwed and glued into the cylindrical tube. It was 3.5 cm away from the top (Reactor 2 and 3), and 11

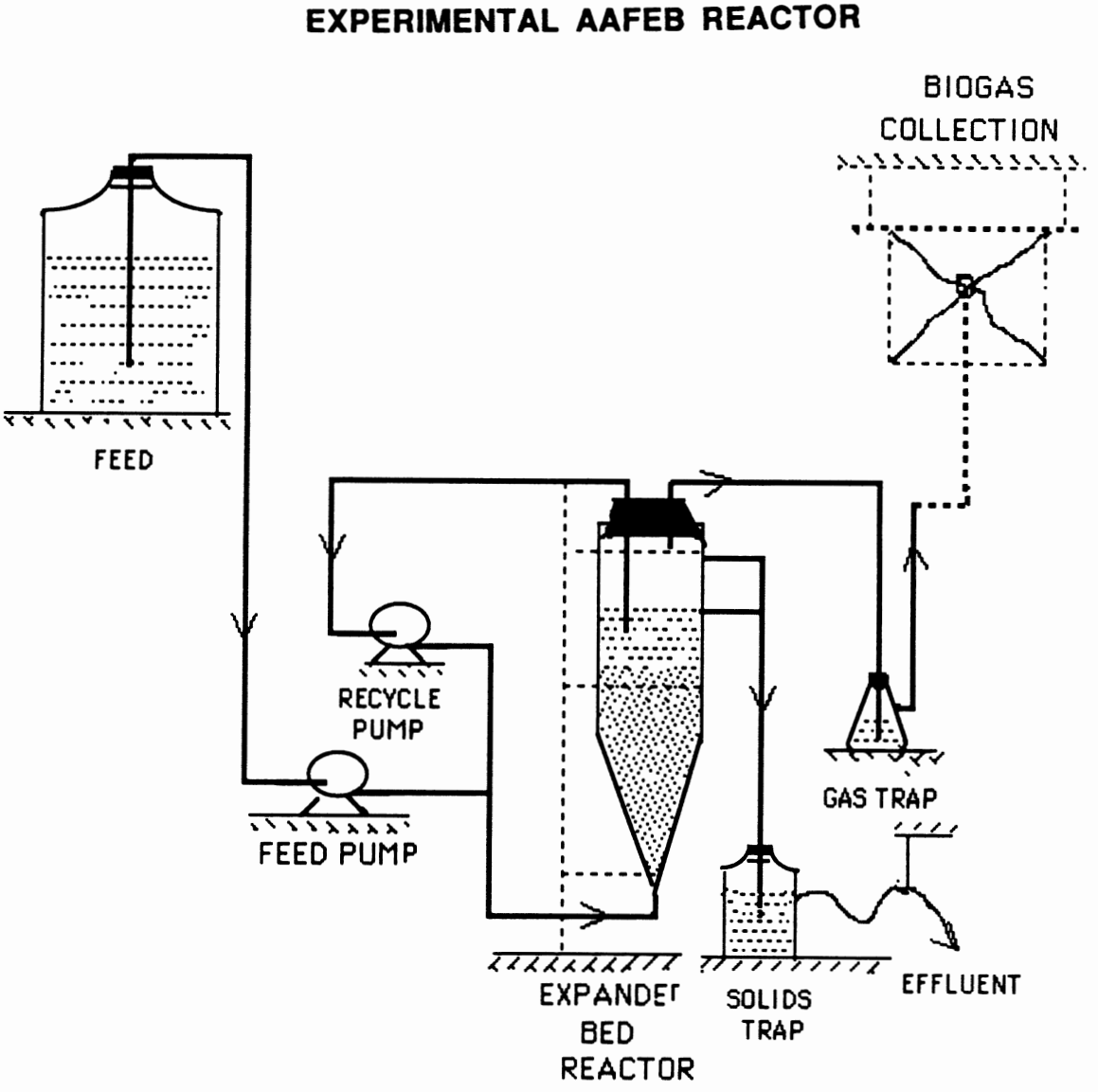

Figure 2. Schematic Diagram of AAFEB System

cm away (Reactor 1), which served as an emergency overflow outlet for effluent. The total liquid volume of the reactor was 1.6 L for Reactor 2 and 3 and 2.4 L for Reactor 1. For Reactor 2 and 3, a number 13 rubber stopper with larger diameter of 10.5 cm was placed (larger edge downward, smaller edge upward), on the top part of the reactor (larger edge was 1-1.5 cm away from top edge of the reactor). Water was poured along the larger edge to the top of the reactor to seal the system. Reactor 1 was sealed with a hard plastic lid of inner diameter about 11.5 cm, which was glued on the top part of the reactor and contained a threaded cap which could be unscrewed. Glass tubing was placed through the stoppers or lid for the reactor recycle outlet and gas outlet.

During the start-up phase, Reactor 1 was filled with 1200 ml of diatomaceous earth previously colonized by denitrifying biomass (Ross, 1989; and Krishnamachari, 1990). Reactor 2 and 3 were filled with 350 ml (122.5 g) of bare diatomaceous earth used as inert media to support the bacterial growth. The diatomaceous earth consists of porous, siliceous particles which are the skeletons or shells of diatoms (unicellular or colonial salt-and freshwater algae) (Clarkson, 1986). The specific surface area of used diatomaceous earth was about 5780 m^2/m^3, density about 0.35 g/cm^3 and softening point about 1430 ^oC (Yang, 1990).

The bed was expanded to 20-25 % above its static or unexpanded volume by recycling the supernatant into the bottom of the reactors using a Masterflex pump (Cole-Parmer model WZIK057 for Reactor 1; and model No. 7553-30

for both Reactor 2 and 3). The pumps were fitted with the standard pump heads (model No. 7015-20). The recycle tubing was connected to a glass tube through the top of the reactor. This glass tubing inlet level could be adjusted in the supernatant, and kept above the biomass bed.

The feed solution from 25 L glass bottles was pumped to join the recycle flow about 18 cm away from the bottom inlet of the reactors in order to get evenly mixed recycle and feed solution. The feed pumps were also Masterflex (Cole-Parmer model No. 7520-10 for Reactor 1; and model No. 7553-60 for reactor 2 and 3). The pump heads for feeding were of model No. 7014-20. Effluent was forced through the outlet port as feed was introduced to the reactors.

A solids trap bottle was connected with the effluent tubing from reactors. The effluent tubing from this bottle was kept in a U-shape (inverted siphon), letting the wasted effluent seal the flow path and isolate the system from outside atmosphere.

Gas outlet tubing from top of the reactors was placed through a number 7 rubber stopper into the water in a gas trap bottle, from where the gas was sent to the gas collection bag or wasted. In the case of gas collection, gas was finally collected in a Teflon gas bag. For the measurement of gas volume, a Cambro CW-C-30 gas meter was used. The gas was pulled from the bag by Masterflex pump (with pump head of model No. 7015-20) and pumped into the gas meter.

Experiments were run at room temperature at all times. Because of the controlled temperature in laboratory, the year-round room temperature in this

research would be about 24.5±2 ^{o}C.

Experimental Methods

Start-up Phase

The objectives of this phase were to establish the steady-state biofilm in all three reactors for the experimental phase, and obtain some information on the biofilm growth, attachment and establishment in AAFEB.

As mentioned before, about 1.2 l biomass was refrigerated for about six and four months, respectively, by Ross and Krishnamachari after their denitrification research (about 700 ml heterotrophic bacteria and 500 ml autotrophic bacteria). At the beginning of this research, the biomass-attached media were taken out and put under room temperature for three days. Then, they were mixed together and poured into Reactor 1, which was fed for one and half months with nitrate, thiosulfate, bicarbonate and other nutrients for autotrophic denitrification. The feed solution was prepared according to the recipe Ross (1989) provided. Three objectives were kept in mind while doing this incubating: (1) activating the bacteria; (2) establishing dominance of autotrophic bacteria; (3) providing seeding bacteria for other newly developed reactors. Operation of the reactor at this stage was the same as that of the experimental phase. The feeding rate was maintained at an intermediate level, about 4 ml/min. About 20 % expansion was maintained by both adjusting recycle rate and taking some bed volume out of the reactor from time to time.

Reactor 2 and 3 were filled with 350-400 ml (about 122.5-140 g) of new diatomaceous earth media. Diatomaceous earth was previously sifted through a No. 20 mesh sieve (W.S. Tyler Co.) to ensure a particle size less than 600 microns (Yang, 1990). Most particles were between 300-600 microns. Before being put into the reactors, it was ignited at 550±50 ^{o}C for one hour and cooled in a desiccator for another hour in order to eliminate volatile contaminants. Then, these two reactors were fed with 250 mg/l nitrate nitrogen and other necessary chemicals, which were mixed together in solution with half amounts of Ross's recipe (1989). They were also operated in the same way of the experimental phase. From the beginning of inoculating, 1-2 L effluent from Reactor 1 was fed into these two reactors almost every day. From time to time, varying amounts of biomass media were also taken from the incubating reactor and added into these two reactors, as inoculum. The amount added to each reactor was around 50 ml per month. These additions served the purpose of increasing biomass concentration in the reactor, promoting contact between the bacteria and media, and helping bacterial acclimation. During the early phase, feeding rate was kept at a relatively high level, about 5-7 ml/min. The objective of this procedure was to wash out poorly acclimated bacteria and poor film formers, while providing a steady supply of substrate to bacteria which would attach readily to the support particles (Clarkson, 1986). In the latter part of this phase, the feeding concentration of nitrate nitrogen was increased to 500, 750 and 1000 mg/l to meet the bio-growth needs. During the first few weeks

about 50 % bed expansion was controlled in the hope that a looser bed would give more chance for bacterial attachment, but about 20 % of expansion was maintained in latter weeks while good biofilm was observed on supporting media. During the start-up phase, the hydraulic retention time (HRT) was not strictly controlled because the bed volumes were subject to increase with time.

Duplicating Experiment Phase

High rate autotrophic denitrification was proven feasible by Ross (1989) at a nitrate nitrogen loading as high as 17 kg/m^3 d, and concentration as high as 1000 mg/l nitrate nitrogen, which was a range fitting the projected raw wastewater of this project. By doing this duplicating experiment, the biomass acclimation was tested and further promoted. System operation was compared with the previous research. The system was modified and conditioned for further investigation.

Loading of 750 mg NO_3^--N/l was conducted in only one reactor (Reactor 1) (first stage). After that, biomass from all three reactors was mixed together uniformly. About 700 ml well mixed media was then put into each reactor, and operated under same conditions for two more weeks before 1000 mg NO_3^--N/l level experiments were started.

Duplication experiments were conducted at three stages. Table II shows the list of stage conditions during the duplication experiment. Each stage was run for about 15-20 days until the system reached steady-state. Three steady-state operation was confirmed, the reactors were

shifted to the next stage.

TABLE II

EXPERIMENTAL CONDITIONS FOR DUPLICATION PHASE

feeding concentration (mg NO_3^--N/l)	loading (kg NO_3^--N/m^3.d)	bed volume (ml)	HRT (hrs)	bed expansion (%)
750	5.5	850	3.3	21.5
1000	8.5	850	2.8	21.5
1000	17	850	1.4	21.5

In the first two stages, the HRT was maintained at about 3 hours, and nitrate nitrogen concentration was changed from 750 mg/l to 1000 mg/l. During the third stage, nitrate nitrogen concentration was maintained at 1000 mg/l, but in two reactors (Reactor 2 and 3) the HRT was decreased from 2.8 hrs to 1.4 hrs (Reactor 1 maintained the same HRT). Through both of these changes, the nitrate loading per cubic meter of biomass bed each day increased from 8.5 kg NO_3^--N/m^3 d to 17 kg NO_3^--N/m^3 d. During the duplication phase, due to the continuous bacterial growth, biomass had to be taken out from reactors and stored to keep the reactor bed volume about 850 ml.

During the entire period of duplication stage, feed was made according to the recipe of Ross (1989), but both

thiosulfate and bicarbonate addition was experimentally and gradually reduced to the level at which much less thiosulfate and bicarbonate were left in the effluent. At all times, however, both thiosulfate and bicarbonate were added in excess of stoichiometric needs in order for nitrates to limit activity in the system. Table III shows the final feed recipe for both periods of duplication and ammonia effects.

TABLE III

FINAL FEED RECIPE FOR 1000 mg NO_3-N/l FEED SOLUTION DURING DUPLICATION AND AMMONIA EFFECTS PHASES

Ingredients	Concentration, g/l
Potassium	7.2
Sodium Thiosulfate	10.0
Sodium Bicarbonate	6.0
Monobasic Potassium Phosphate	0.20
Ferrous Sulfate	0.01
Magnesium Sulfate	0.01

Ammonia Effects Investigation Phase

Before the investigation began, the biomass media from three reactors were mixed together again and maintained under the same conditions to make certain the three

reactors had the same starting base. During the experimental period, three concentrations of nitrate nitrogen were tested against the ammonia interference. At each nitrate concentration, ammonia was tested at different concentrations. Ammonium sulfate was added as ammonia source in lower ammonia concentrations. Ammonium chloride was added in the latter part (higher ammonia concentrations), since chloride becomes inbihitory at a much higher concentration (Claus & Kutzner, 1985a; and McCarty, 1964). Table IV shows the both nitrate and ammonia nitrogen concentrations for each stage, which includes 16 ammonia levels and three nitrate levels.

Each ammonia level was determined experimentally. As mentioned before, the concentration ratio of ammonia nitrogen and nitrate nitrogen was about 6:1. Thus, the loadings at each stage ended with this ratio. Higher ammonia concentrations could have been used, but our primary goal was to test the projected wastewater from AMC fertilizer plant, not to find the ammonia limitation. The upper limit of ammonia interference on denitrification should be studied at a later time as an independent study.

At the first stage 500 mg NO_3^-N/l level, ammonia addition was started at a very low concentration, and then increased to relatively high concentrations. The purpose of this gradual increase was to try to get the bacteria well acclimated to the ammonia environment, then test at some higher level. Each time, when shifting nitrate from a lower level to a higher level, the ammonia was kept the same as the previous stage and only nitrate was increased. After a steady-state was reached and analysis was completed

TABLE IV

NITRATE AND AMMONIA CONCENTRATIONS FOR EACH STAGE

NO_3^--N, mg/l	NH_4^+-N, mg/l	
500	50	(started with $(NH_4)_2SO_4$)
	100	
	150	
	250	
	450	
	300	
	600	
	1000	
	1500	(started with NH_4Cl)
	2000	
	2250	
	3000	
750	2250	
	3000	
	3500	
	4500	
1000	3500	
	4500	
	5000	
	6000	

for this nitrate level, ammonia was increased again to a new level. For instance, when increasing nitrate nitrogen from 500 mg/l to 750 mg/l, ammonia nitrogen concentrations were kept at 2250 and 3000 mg/l, respectively, in two reactors. After steady-state and analysis, ammonia nitrogen concentrations were increased to 3500 and 4500 mg/l.

During this experimental phase, three reactors were operated in different ways in terms of amount of ammonia addition. One reactor (Reactor 1) played the leading role in the experiment, in which a higher amount of ammonia was always added at every ammonia stage. The second reactor (Reactor 2) was fed with nitrate nitrogen as Reactor 1, but ammonia was added at a level always somewhat less than Reactor 1, but greater than Reactor 1's previous ammonia stage. This reactor acted as a back-up and supplementary reactor. As a back-up reactor, it could have still worked if failure had happened in Reactor 1. To obtain supplementary data, it was tested with ammonia concentration between two concentrations tested in Reactor 1. The third reactor (Reactor 3) played the control reactor role for the experiment. During each stage, nitrate nitrogen was added in the same amount as that added to Reactor 1 and 2, but as a control reactor, ammonia was not added for the entire ammonia effects period.

During this phase, relatively stable hydraulic conditions (about 2.8 hours HRT) were maintained in the reactors by fixing the feeding rate at 5 ml/min. The expanded bed volumes were controlled at about 850 ml and bed expansion at about 21.5 % by adjusting both the biomass

and recycle flow rate.

Analytical Methods

Sampling and Analysis

Liquid and solid samples were taken for analyses. Liquid was collected from effluent tubing between the reactor outlet and solids trap. For each analysis, 500-600 ml effluent were collected from each of three reactors. The liquid sample was used to analyze the total suspended solids and volatile suspended solids first, and then for nitrate, ammonia, thiosulfate, alkalinity. Also, nitrite and sulfate in liquid samples were measured to check nitrite accumulation and sulfate production. Solids samples were taken from the center of the biofilm bed media to measure the attached biomass concentration in each unit. From time to time, the gas was collected in a gas collection bag and gas volume was measured every day.

Analytical Techniques

Scope. The analytical techniques used for this research were primarily based on the " Standard Methods of the Examination for Water and Wastewater " (APHA et al., 1985). For some other parameters such as thiosulfate and attached biomass, other methods used in similar research were used.

Total Suspended Solids. The determination of total suspended solids (TSS) was done according to the procedures described in Standard Methods, Section 209 C.3 (APHA et al., 1985). As mentioned before, 500-600 ml effluent were

collected from each of three reactors, and each was mixed well. For each reactor, the analysis was doubled by splitting collected effluent into two parallel samples. Each sample took 200 ml known volume of effluent for solids analysis. Samples were filtered in Whatman glass microfiber filters (4.25 cm), ignited at 103 ^{o}C in oven overnight, cooled in desiccator for usually 1.5 hours, and then weighed.

Volatile Suspended Solids. The determination of volatile suspended solids (VSS) was done according to Standard Methods, Section 209 D.3 (APHA et al., 1985). Sample was also doubled for VSS analysis. The finished samples for TSS analysis were, then, ignited at 550 ^{o}C in muffle furnace, cooled in desiccator and then weighed.

Attached Biomass. The attached biomass concentration was determined according to the procedures described by Clarkson (1986). Ross (1989) used the same method for the feasibility study of high rate autotrophic denitrification. A similar method was briefly described in the Standard Methods, Section 802 G.4 (APHA et al., 1985) for biomass monitoring.

The determination of the attached biomass concentration was done in duplicate, too. About 10-15 ml biomass attached media samples were taken from the center of the media bed in each reactor with wide mouth glass tubing. Then, a portions of the biomass samples were put into 10 ml wide bore graduated cylinders, which were then gently tapped and spun several times to pack the biomass media. During the process of compaction, particles were

added or subtracted and the tamping procedure continued until each sample contained exactly 5.0 ml of the packed bed particles. Supernatant was decanted and the sample was transferred to an ashed, prewighed porcelain drying dish by sluicing it out with a stream of distilled water from a wash bottle. In the dish the 5.0 ml biomass sample was vigorously agitated by the jet of water from squeezing wash bottle. The purpose was to loosen the entrapped solids from attached biomass. This procedure was repeated until no further loose solids could be washed off. Care was taken to keep the granular media particles in dish, only removing the loose solids. By doing this, the amount of attached biomass per 5.0 ml bed was obtained, and entrapped biomass was excluded. At the same time, two blank samples were obtained by taking the same amount of biomass-free diatomaceous earth from blank sample storage and undergoing the same procedures. The blank sample was diatomaceous earth as that in the reactor, stored in buffer solution at room temperature and kept in the dark to eliminate microbial growth.

All samples and blanks were subjected to drying at 103 ^{o}C, cooling back to room temperature and weighing, and igniting at 550 ^{o}C, cooling and weighing again, a procedure which was similar to the procedure for the total solids. Blank samples were necessary to account for hygroscopically bound water in the diatomaceous earth in performing the solids calculations. After final igniting, cooling and weighing, the particles were rehydrated with distilled water, transferred to graduated cylinder, and compacted again, to measure the final volume of nonvolatile media and

other substances for calculation of attached volatile biomass solids.

Thiosulfate. The determination of thiosulfate was mainly done according to the classical method in Pierce and Haenisch (1947). This is a exclusive method to determine the soluble thiosulfate in a solution, which uses iodine (an oxidant) to titrate the thiosulfate and starch to indicate the endpoint, at which excess iodine and starch react to form blue color complex. Each time, a standard thiosulfate solution was prepared to check the strength of the iodine titration solution. The 0.1 N iodine solution was used for this research, and 20 ml effluent sample from the TSS filtrate was used to measure the thiosulfate consumption by bacteria, which was the concentration difference of influent and effluent. The iodine solution and starch solution were made according to the procedure described in the above reference.

Ion chromatography was also found capable to quantify the thiosulfate during this research. This method was not recorded for previous treatability studies in the literature. The comparison of experimental results for three samples of thiosulfate measurement is shown in Table V.

From the research point of review, chemical titration is easier to get an accurate answer, and is good for the determination of high thiosulfate concentration. Using the ion chromatography, the analyst can simultaneously determine several chemicals with one sample, for instance, nitrate, nitrite, sulfate, thiosulfate.

TABLE V

THIOSULFATE MEASUREMENT BY TWO METHODS FOR REACTOR 3

sample No.	calculated from feed (g/l)	titration method (g/l)	ion chromatography (g/l)
1	10.21	10.26	10.00
2	10.21	10.28	10.10
3	10.21	10.22	10.10

Alkalinity. The procedure for alkalinity determination was described in the Standard Methods, Section 429.4 (APHA et al., 1985). Like thiosulfate, alkalinity consumption was measured by the difference of influent and effluent alkalinity. Twenty ml samples were taken from the TSS filtrate, and titrated with 0.05 N sulfuric acid. The endpoint was determined with bromcresol green-methyl red reagent pillows (Hach Chemical Co.).

Nitrate Nitrogen, Nitrite Nitrogen and Sulfate. The procedure for the determination of anions with ion chromatography was given in Standard Methods, Section 429.4 (APHA et al., 1985). The Dionex ion chromatograph, series 2000 i/sp was used for this research. Samples from TSS filtrate were diluted with distilled water according to the assumed concentration, then injected into the IC. The actual concentration of each sample was read according to standard curves. The standard curves for these anions were

made from the prepared standard solutions and obtained by computer linear regression with a coefficient of correlation about 0.90-0.99, at each time for sample measurement.

Ammonia Nitrogen. The determination of ammonia nitrogen was conducted according to Standard Methods, Section 418 A and B-Nesslerization Method (Direct and Following Distillation). The Hach nessler reagent was used for determination. The ammonia nitrogen concentrations were read directly on a DR/3 spectrophotometer (Hach, model No. 4200-10). The deionized water from reverse osmosis was used for dilution and reagent preparation.

pH. All samples from TSS filtrate were subjected to pH measurement before any other further treatment. An Accumet pH meter (Fisher Scientific, model No. 900) with a single electrode was used to determine the pH value.

CHAPTER IV

RESULTS AND DISCUSSION

Scope

The overall objective of this research was to investigate ammonia interferences on the high rate autotrophic nitrate reduction. It was conducted to treat the high concentrations of both ammonia and nitrate existing in the targeted waste stream from the AMC fertilizer manufacturing plant. As one alternative, denitrification was proposed, at the first stage, as a pretreatment to reduce the nitrate strength and/or interferences on the latter processes. Pre-denitrification has also been shown to be valuable for other industrial wastes (Bridle, Bedford & Jank, 1980; and Melcer & Nutt, 1988).

It has been proven by Przytocka-Jusiak et al. (1984) that the denitrifying bacteria could tolerate ammonia at some degree. For higher ammonia levels, such as in the wastewater from the AMC nitrogen fertilizer plant, the tolerance ability of autotrophic bacteria was tested during this research. From the results of this tolerance test, the dilution factor of raw waste could be determined.

The first part of the research was to promote well attached biomass film on the diatomaceous earth support media. Both autotrophic and heterotrophic bacteria were

used as seed bacteria and acclimated to the new environment in one reactor. In the other two reactors, bacteria were grown on new support media. With constant bed volume and bed expansion, the second part of the research was to duplicate autotrophic denitrification at the three high loading rates and two HRT levels studied by Ross (1989). Thus the bacteria were acclimated to high nitrate levels, and the reliability of autotrophic denitrification was shown in this application. Third, the major part of the research, was to test the bacterial ability to tolerate high concentrations of ammonia. It was conducted at 16 ammonia levels and three nitrate levels with constant bed volume, hydraulic retention time and percent bed expansion. For each nitrate concentration, experiments were run up to a 6:1 ammonia nitrogen and nitrate nitrogen concentration ratio. Analytical results of denitrification rates were given for each ammonia and nitrate concentration tested in combination.

The time sequence of entire experimental period is shown in Figure 3. Most of the experimental results were curve-fitted by computer with a correlation coefficient range of 0.65-0.93. However, these curves represent only a trend for each case.

Start-up Phase

There were two major parts of work involved in this phase: promoting and stabilizing the previously left biomass-attached media in one reactor and establishing new biomass media in the other two reactors. Data collection was limited for this stage, but some observations and

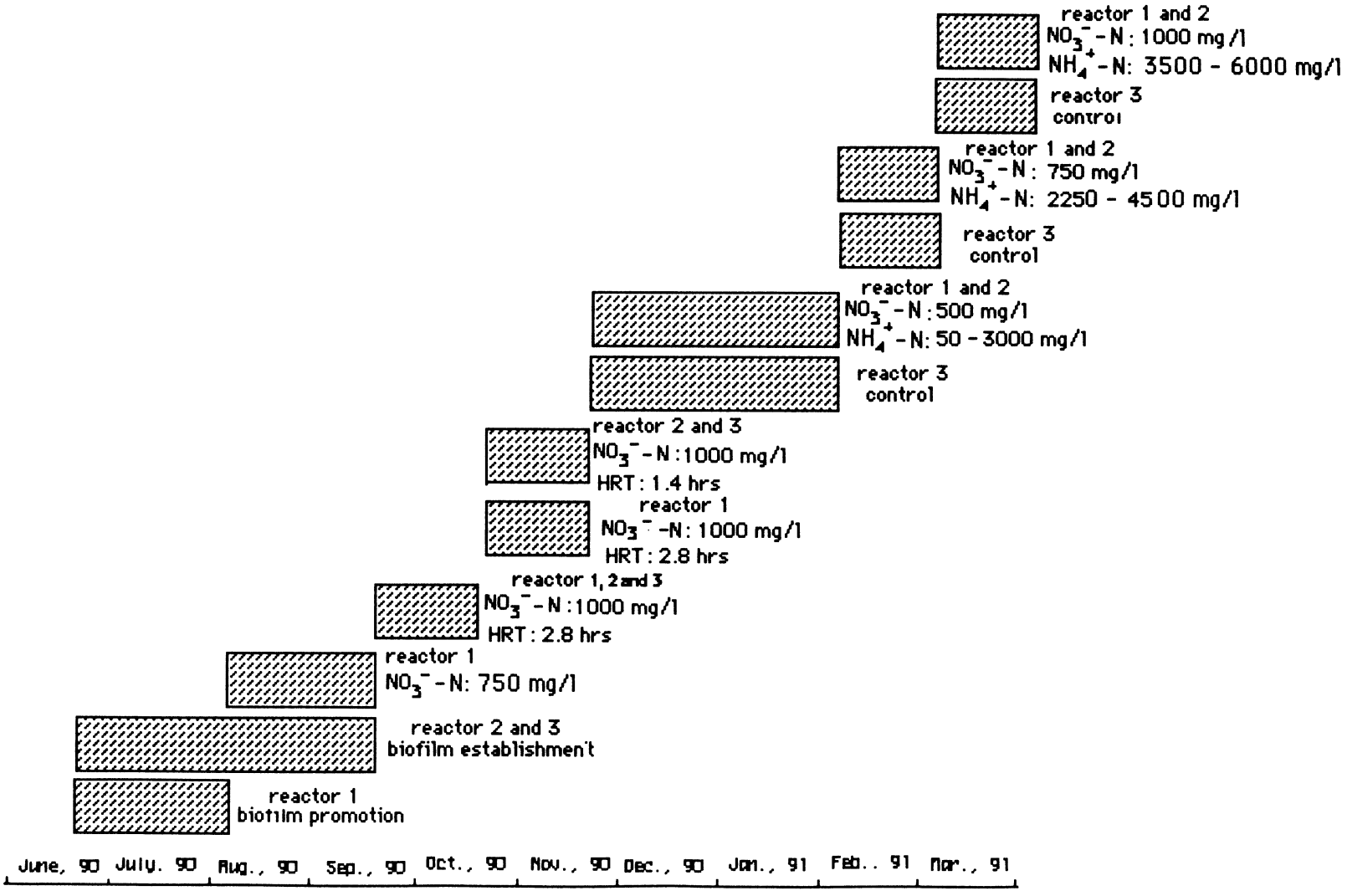

Figure 3. Time Sequence Diagram for Entire Experimental Period

biomass concentration measurements were made.

Biomass Stabilization

Stabilizing the previously left biomass media in Reactor 1 took about one and a half months. During this time, observed biomass growth was tremendous. The bed volume increased from 1.2 L to 1.6 L, though some biomass was continually taken out. Attached biomass concentration increased, too, but not very significantly. The growth of autotrophic microorganisms was promoted and the enzymatic activities of heterotrophic microorganisms were regressed under autotrophic conditions. Then, this reactor was adjusted, maintained and shifted to the duplication study at 750 NO_3^--N mg/l level.

Establishment of New Biofilm

This part lasted for about 100 days from beginning in both Reactor 2 and 3. After the three months, a mature biofilm had accumulated on the bare media. Then, the reactor contents from all three reactors were mixed and maintained in preparation for the 1000 mg/l levels of nitrate nitrogen duplication experiments.

For this part, the feeding of nitrate nitrogen concentration was increased from 250 mg/l to 500, 750 and finally to 1000 mg/l. During the starting period, 250 mg/l of nitrate nitrogen solution was fed into these two reactors at a relatively high rate, about 7 ml/min (corresponding loading rate, about 10.08 kg/m^3 d). Additions of effluent and biomass media from the more mature reactor (Reactor 1) and the relatively high feeding

rate helped the growth considerably. In about 40 days, biofilm was observed on the media. Bed volume increased from about 350 ml to 450-500 in these two reactors. Then the feeding concentration was increased to 500 mg/l of nitrate nitrogen, resulting in rapid increases of bed volume and attached biomass concentrations. Analytical results showed complete nitrate conversion and stoichiometric thiosulfate and alkalinity consumptions. The feeding rate was decreased to a normal value, about 5 ml/min, from now on. After some time, feed concentration was increased to 750 and furthermore to 1000 mg NO_3^--N/l (corresponding loading rates, about 6.4 and 8.0 kg/m^3 d, respectively). The loading rate increase resulted in the bed volume increasing to about 900 ml in both reactors and attached biomass concentration to about 55 mg/l, in the end of this phase. But as in Figure 4, the attached biomass concentration increase was not very significant in the later part of this period. During this study period, the size increase of media granules was obvious.

Duplication Experiment Phase

Experiment

As mentioned before, the duplication tests were conducted at three levels of nitrate nitrogen loading: 750 mg/l and HRT 3.3 hours; 1000 mg/l and HRT 2.8 hours; and 1000 mg/l and HRT 1.4 hours. These corresponded to the nitrate nitrogen loading of about 5.5, 8.5 and 17 kg/m^3 d, respectively. For each nitrate level, at least three data sets were collected after the steady-state conditions were

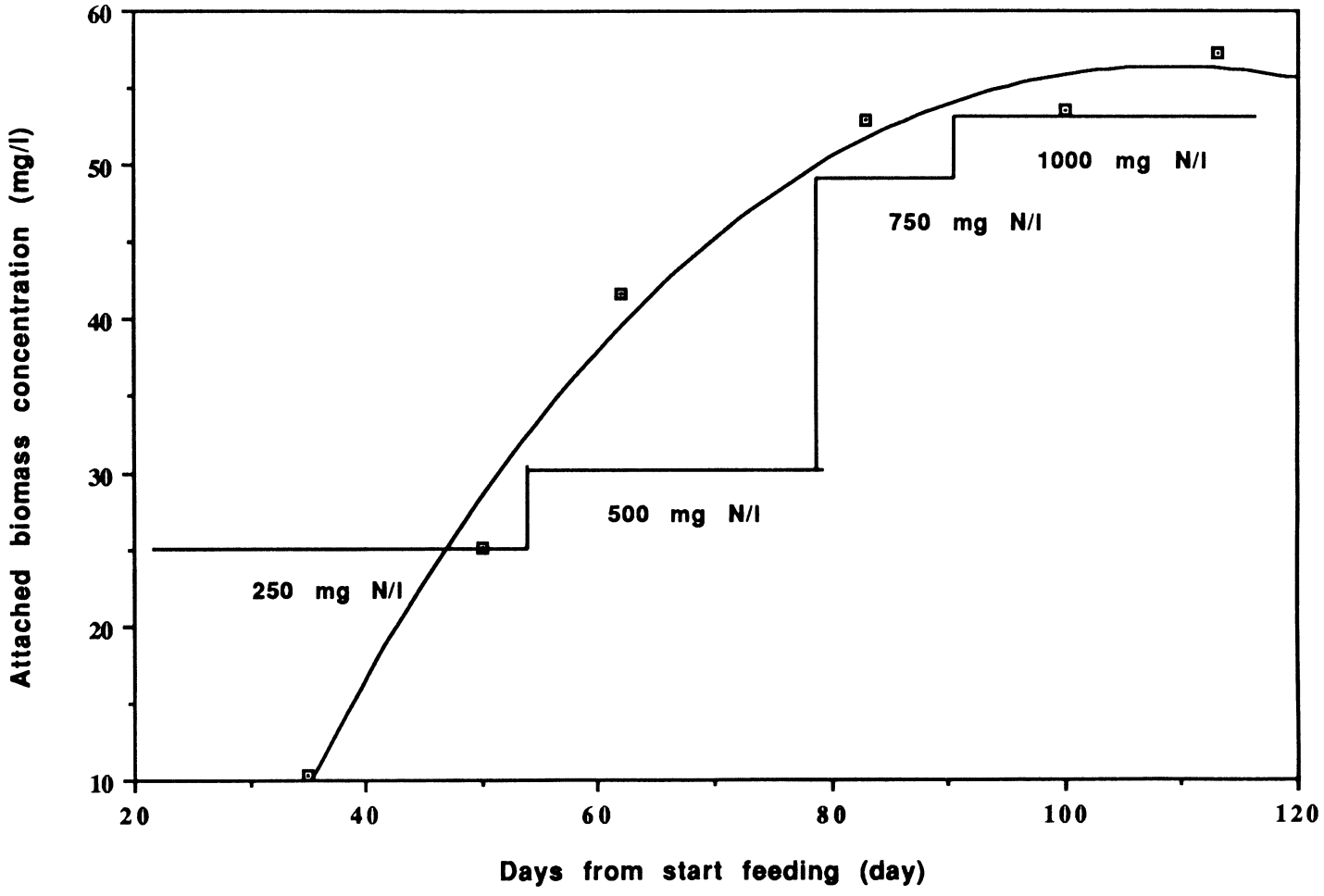

Figure 4. Biomass Concentration during Startup Phase

established. A steady state was considered to be reached if system removal efficiency and other parameters such as thiosulfate and the alkalinity consumptions were stabilized, which was usually after more than 50 hydraulic retention periods. Like previous research (Ross, 1989), calculations of the loading rates and hydraulic retention time were based on the total expanded bed volume without making any correction for the volume occupied by support media.

Results and Comparisons

Table VI gives the results for this phase, and the comparison with Ross's results. Complete conversion of nitrate was accomplished in both the previous experiment and this duplication experiment, at relatively high nitrate nitrogen loading rates. Data in Table VI demonstrate the agreement of analytical results from both experiments. The thiosulfate and especially the alkalinity consumptions in this experiment were relatively higher than that of the previous study, possibly due to the higher attached biomass concentration in this experiment.

As a result, autotrophic denitrification could be duplicated at these high loading rates. Under the nitrate nitrogen loading rate of 17 kg/m^3 d and HRT of 1.4 hours, 1000 mg/l of nitrate nitrogen in influent could be converted almost completely. But some individual parameters of each system could vary from case to case.

Serious operational difficulties were not encountered during the experimental phase. As the result of bacterial growth, biomass had to be taken out continually to maintain

TABLE VI

DUPLICATION RESULTS AND COMPARISONS

items	stage 1	stage 2	stage 3	Ross (1989)
Loading (kg Ne*/m^3 d)	5.5	8.5	17	5.3-17.46
Concentration (mg Ne/l)	770	992	1000	747-1242
HRT (hrs.)	3.3	2.8	1.4	3.8-1.1
Biomass volume (ml)	850	850	850	815-1000
Removal efficiency (%)	100	99.5	99.9	66.7-99.7
$S_2O_3^=$ consumed (mg/l)	4580	6066	6098	4216-5926
Alkalinity consumed (mg $CaCO_3$/l)	1300	1807	1910	1180-1250
Attached biomass (mg/l)	55.6	52.8	51.0	20-27
$SO_4^=$ produced (mg/l)	7620	10456	11633	7434-9453
Gas production (l/d)	5.6	8.4	13.7	5.3-11.7
$S_2O_3^=$/Ne (mol/)	0.76	0.76	0.76	0.72
Ne/alk (mol/ $CaCO_3$)	4.1	3.9	3.8	4.5
$S_2O_3^=$/alk ($CaCO_3$) (mol/)	3.13	3.00	2.86	3.23
Influent pH	8.1	8.1	8.1	/
Effluent pH	7.2	7.2	7.2	/

* Ne expressed as NO_3^--N, nitrate nitrogen

a bed volume of 850 ml. The bed volume increase was obvious when the nitrate nitrogen loading was increased. But as Figure 5 shows, it was interesting to know that per unit volume, the attached biomass concentration slightly decreased or was relatively constant. This phenomenon was possibly explained by the fact that pore space of the bed slightly increased due to the bigger media beads.

Ammonia Effects

Experiment

The significance of investigating ammonia interferences involves the possibility of treating waste streams highly loaded with both nitrate and ammonia. In the case of treatment of wastewater from a nitrogenous fertilizer industry, denitrification may be used as a pretreatment to protect the nitrification step, as well as a second-stage treatment of nitrification effluent which is not completely oxidized.

Hydraulic retention time of about 3 hours was chosen as the experimental HRT, since it was more commonly used by many researchers. This value is representative of the HRT range for most practical systems.

During this phase, denitrification rate (nitrate removal efficiency), biomass stability (attached biomass, VSS and TSS), and fate of ammonia were monitored. At least three steady state data sets were collected for each ammonia concentration. For each stage, nitrate removal efficiency, thiosulfate and alkalinity consumptions were measured. Attached biomass concentration, and total and

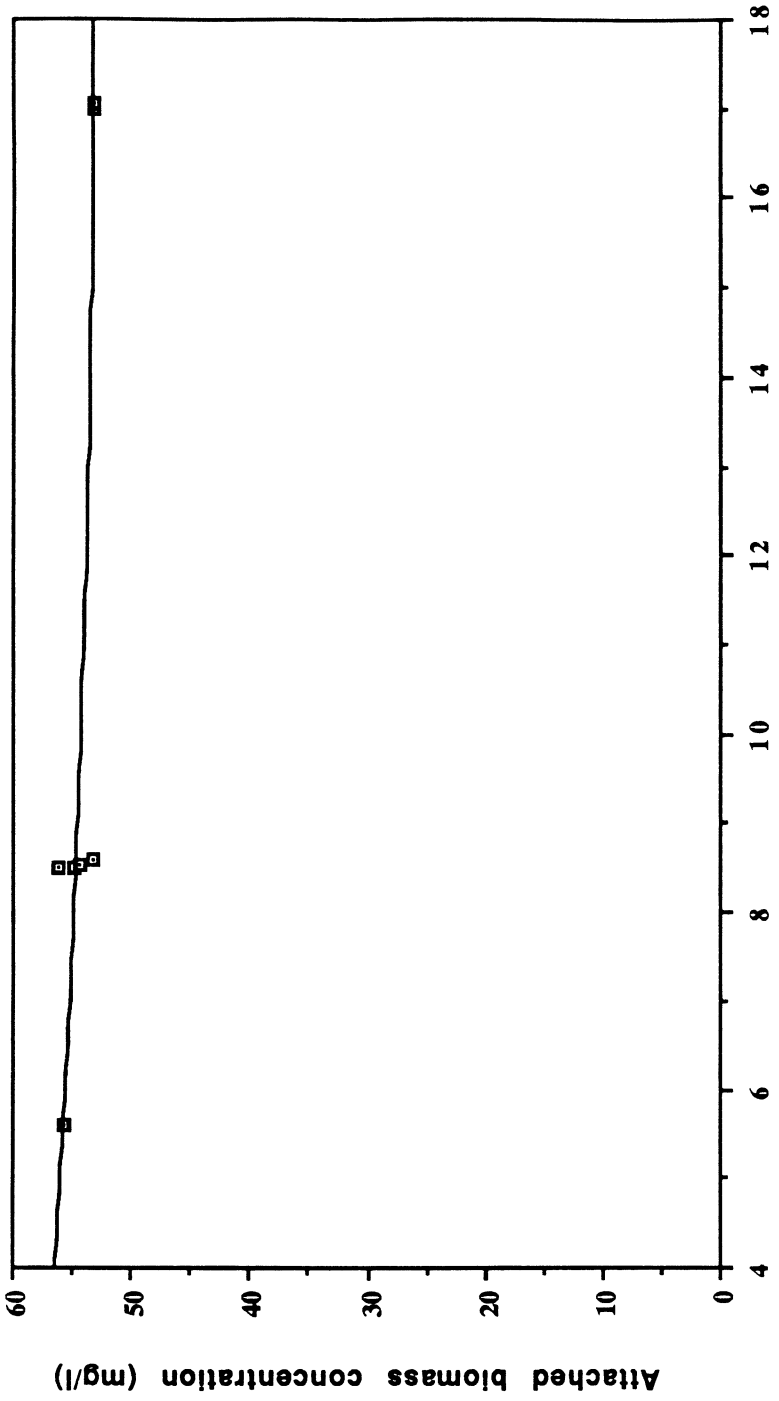

Figure 5. Attached Biomass Concentration during Duplication Phase

volatile suspended solids were also measured. All the data were compared with the data from a parallel control reactor (Reactor 3) not receiving ammonia. Ammonia concentration was routinely monitored in the influent and effluent.

At constant HRT of 2.8 hours, nitrate nitrogen concentrations of 500, 750 and 1000 mg/l (corresponding to the loading rates of 4.2, 6.4 and 8.5 kg NO_3^--N/m^3 d) were tested against 16 ammonia nitrogen concentrations (Table IV), under a fixed bed volume of 850 ml.

Denitrification Rate

At the beginning, one (Reactor 2) of the two reactors with ammonia addition was loaded with 50 mg/l of ammonia nitrogen, and another one (Reactor 1) with 100 mg/l. Denitrification rates decreased to 92 % in Reactor 1 within the first few days, but recovered back to 100 % after 9 days (Figure 6). This reduction did not exist in Reactor 2 and with further ammonia additions up to 3000 mg NH_3-N/l. The reduction might be due to the fact that denitrifying bacteria took some time to get acclimated to the ammonia environment, and after that, ammonia did not exert interferences on the systems greatly for this stage. Table VII shows the results of this stage. Overall, 100 % nitrate removal efficiency was still maintained.

A conclusion drawn from the above observation was that, at nitrate nitrogen concentration of 500 mg/l and up to 3000 mg/l of ammonia nitrogen, no decrease in denitrification rate was observed. The complete nitrate conversion could be achieved even though some time might be needed to get the system stabilized.

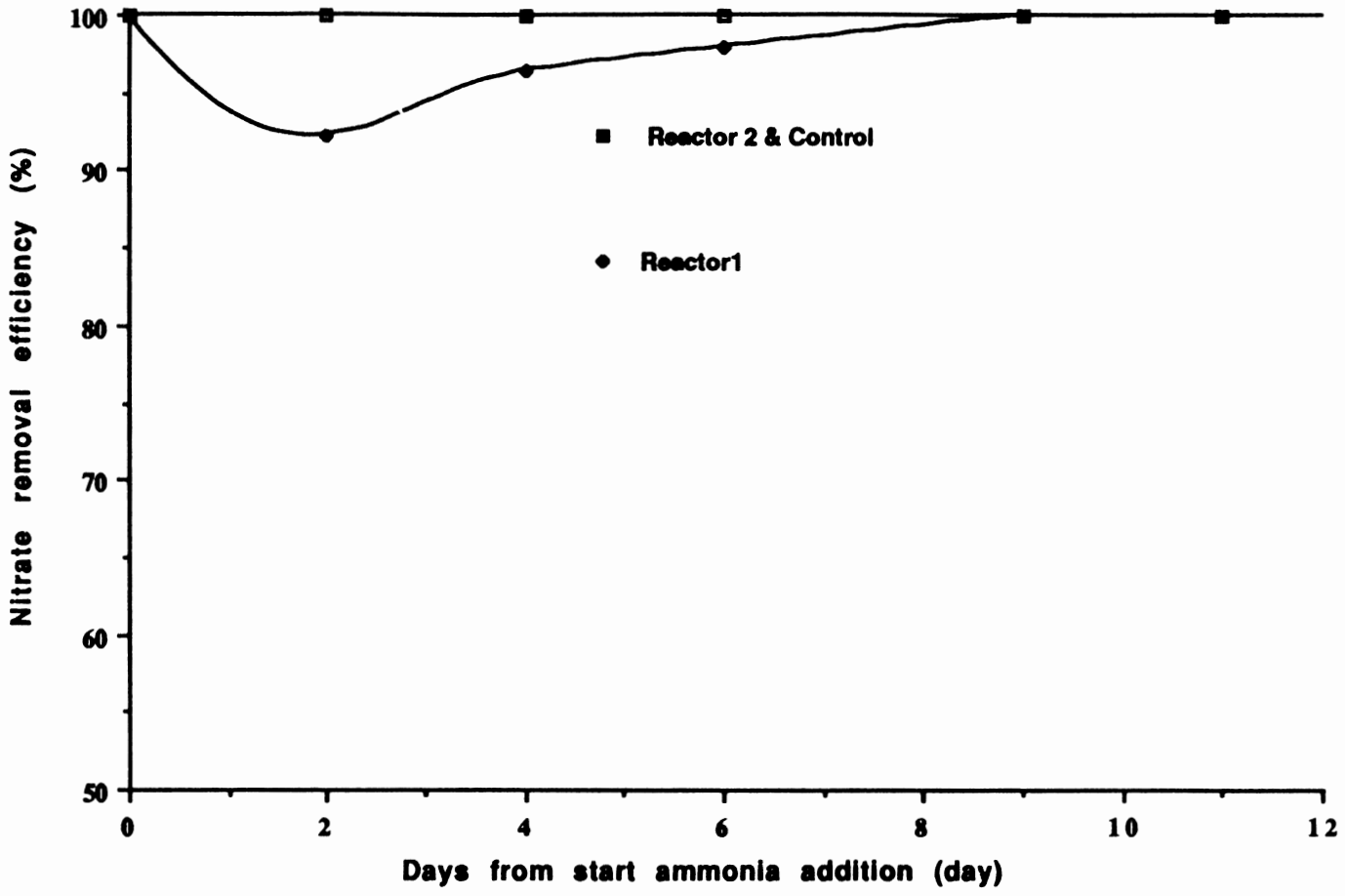

Figure 6. Denitrification Rate during during Starting-up 100 mg/l Ammonia Nitrogen Addition Phase (reactor 1)

TABLE VII

SUMMARY OF 500 MG/L NITRATE NITROGEN PHASE

ammonia concentration (mg Ne**/l)	denitrification efficiency (%)		thiosulfate consumption (mg/l)		alkalinity* consumption (mg/l)		thiosulfate/ nitrate N (mol/mol)		nitrate N/ alkalinity* (mol/mol)		thiosulfate/ alkalinity* (mol/mol)	
	test	control	test	control	test	control	test	control	test	control	test	control
50	100		3612.7		1120.1		0.90		3.19		2.88	
100	100	100	3326	3153.9	1158.8	909.5	0.83	0.80	3.08	3.90	2.56	3.10
150	100		3001		989.6		0.76		3.57		2.71	
250	100	100	3211	2989.6	1036	935.5	0.83	0.73	3.32	3.87	2.77	2.85
300	100		3498.6		1053		0.87		3.03	2.97		
500	100	100	3383.3	3555.3	1053	1205.1	0.85	0.89	2.94	2.95	2.87	2.63
600	100		3612.6		1075		0.90		3.38		3.01	
1000	100	100	3727.3	/	1050	/	0.93	/	3.35	/	3.17	/
1250	100		3211.3		850		0.80		4.22		3.37	
2000	100	100	3096.6	3006.9	800	823	0.75	0.78	4.58	4.23	3.46	3.26
2250	100		2981.9		850		0.75		4.20		3.13	
3000	100	100	3154	2838.5	775	700	0.79	0.71	4.61	5.10	3.63	3.62

* alkalinity expressed as calcium carbonate

** Ne representing ammonia nitrogen

After 500 mg/l of ammonia nitrogen level, the nitrates were tested at 750 and 1000 mg/l levels by simply increasing the nitrate concentration in feeding solution. The ammonia addition was continually increased. Table VIII shows the experimental results for these two stages.

The denitrification was still complete while nitrate nitrogen concentrations were increased from 500 to 750 mg/l and ammonia nitrogen concentrations were still kept at 2250 and 3000 mg/l. It was obvious that above 3500 mg/l ammonia nitrogen level, denitrification rates dropped, and continued to decrease somewhat with increasing both ammonia additions and nitrate concentrations. At the ammonia nitrogen concentration of 6000 mg/l and nitrate nitrogen concentration of 1000 mg/l, the denitrification rate dropped to about 82.5 % (Figure 7). The first line on top represents the control reactor without ammonia addition, the second line is the denitrification under 750 mg NO_3^--N/l, and the third line is the denitrification under 1000 mg NO_3^--N/l. They indicate the bacterial capacity to tolerate ammonia interferences under this strength of both ammonia and nitrate nitrogen.

Even though the denitrification rate dropped to some degree, the thiosulfate and alkalinity consumption ratios per unit nitrate nitrogen removed were still consistent with that of the control reactor, duplication experiment, and previous experimental results (Ross, 1989). It indicated that the system could be stabilized at a certain nitrate removal rate after some time. As mentioned before, for each stage, three stabilized data sets were collected. Table IX shows the original data of removal efficiency of

TABLE VIII

SUMMARY OF 750 AND 1000 MG/L NITRATE NITROGEN PHASE

1 nitrate conc. (mg/l)	2 ammonia conc. (mg/l)	3 denitri. effici. (%)		4 thiosu. consum. (mg/l)		5 alka. consum. (mg/l)		6 thiosu./ nitrate N (mol/mol)		7 nitrate N/ alka. (mol/mol)		8 thiosu./ alka. (mol/mol)	
		test	con.	test	con.	test	con.	test	con.	test	con.	test	con.
750	2250	100		4329.5		1175.0		0.73		4.56		3.33	
	3000	100	100	4670.6	4286.9	1287.5	1057.5	0.78	0.71	4.16	5.07	3.24	3.62
	3500	96.1		4381.4		1083.3		0.76		4.75		3.61	
	4500	93.5	100	4211.4	4655.4	1073.3	1191.7	0.75	0.78	4.67	4.50	3.50	3.49
1000	3500	90.0		5506.4		1337.5		0.76		4.81		3.68	
	4500	83.4	100	5177.6	6185.7	1287.5	1462.5	0.78	0.77	4.63	4.88	3.59	3.78
	5000	85.1		4956.4		1276.1		0.73		4.76		3.47	
	6000	82.5	100	4838.8	5817.4	1215.1	1367.6	0.73	0.73	4.85	5.22	3.57	3.80

1 - nitrate nitrogen concentration
2 - ammonia nitrogen concentration
3 - denitrification efficiency
4 - thiosulfate consumption
5 - alkalinity consumption
6 - unit thiosulfate consumption
7 - unit alkalinity consumption
8 - thiosulfate and alkalinity consumption ratio
test - test reactors
con. - control reactor

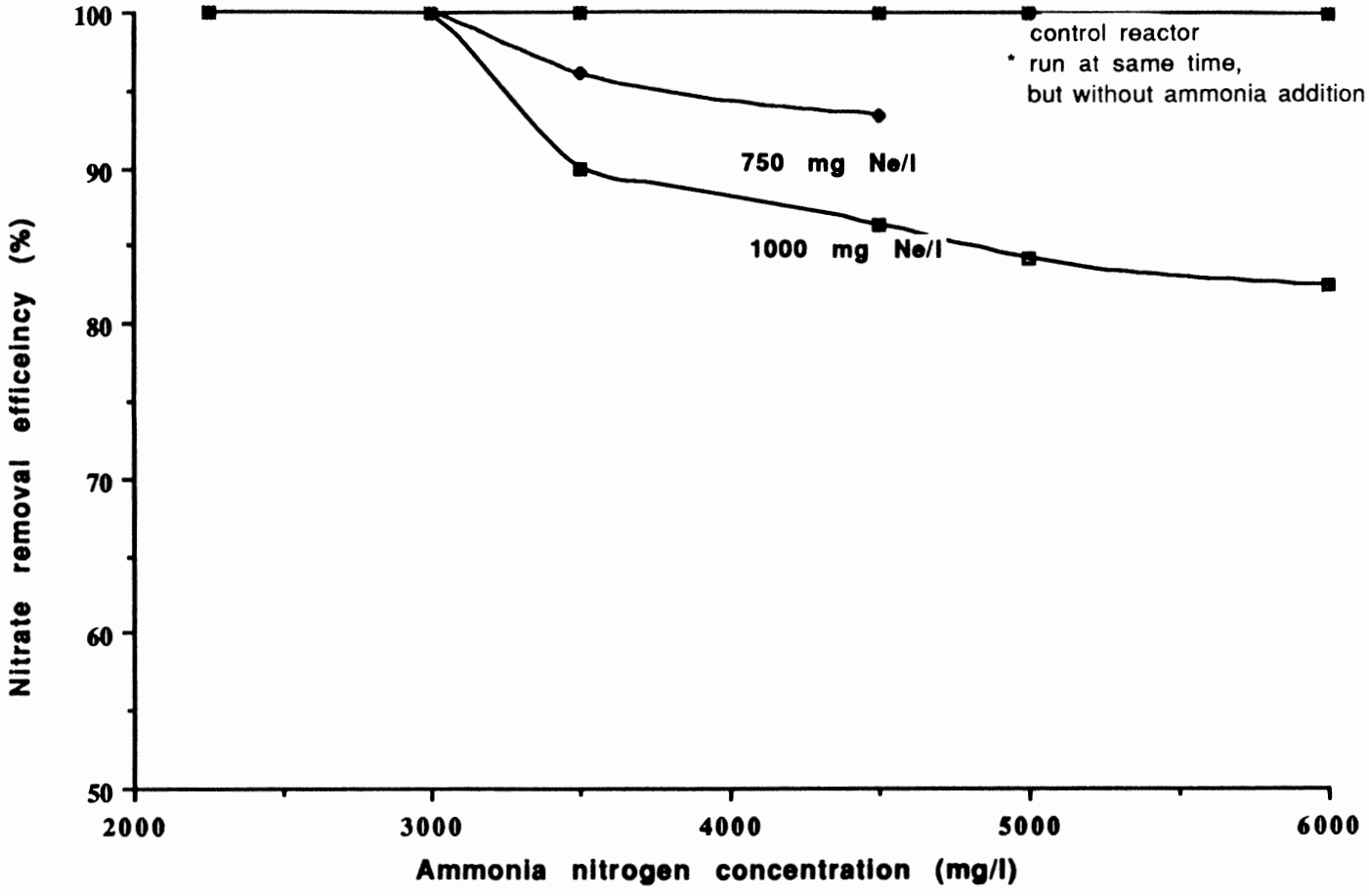

Figure 7. Denitrification Rate under High Ammonia Addition

three stages in Reactor 1. Therefore, system performance was still stable, even at these high ammonia additions.

TABLE IX

STABILIZED DENITRIFICATION EFFICIENCY FOR THREE STAGES IN REACTOR 1

nitrate nitrogen (mg/l)	ammonia nitrogen (mg/l)	nitrate removal efficiency (%) data 1	2	3
750	4500	91.4	94.8	94.2
1000	4500	79.0	85.0	86.2
1000	6000	84.3	80.7	82.1

Overall, this entire experiment of ammonia investigation basically matched the categories McCarty (1964) suggested (Table I). That is, ammonia nitrogen over 3000 mg/l is toxic to anaerobic bacteria. Below 3000 mg/l, there are no obvious adverse effects at middle pH range, or ammonia may be beneficial to bacterial activity.

Ammonia Loss

Figure 8 shows the ammonia loss (ammonia nitrogen concentration in feed minus concentration in effluent) through the process. Up to 1500 mg/l ammonia nitrogen, the more ammonia was added, the more was lost. Figure 8

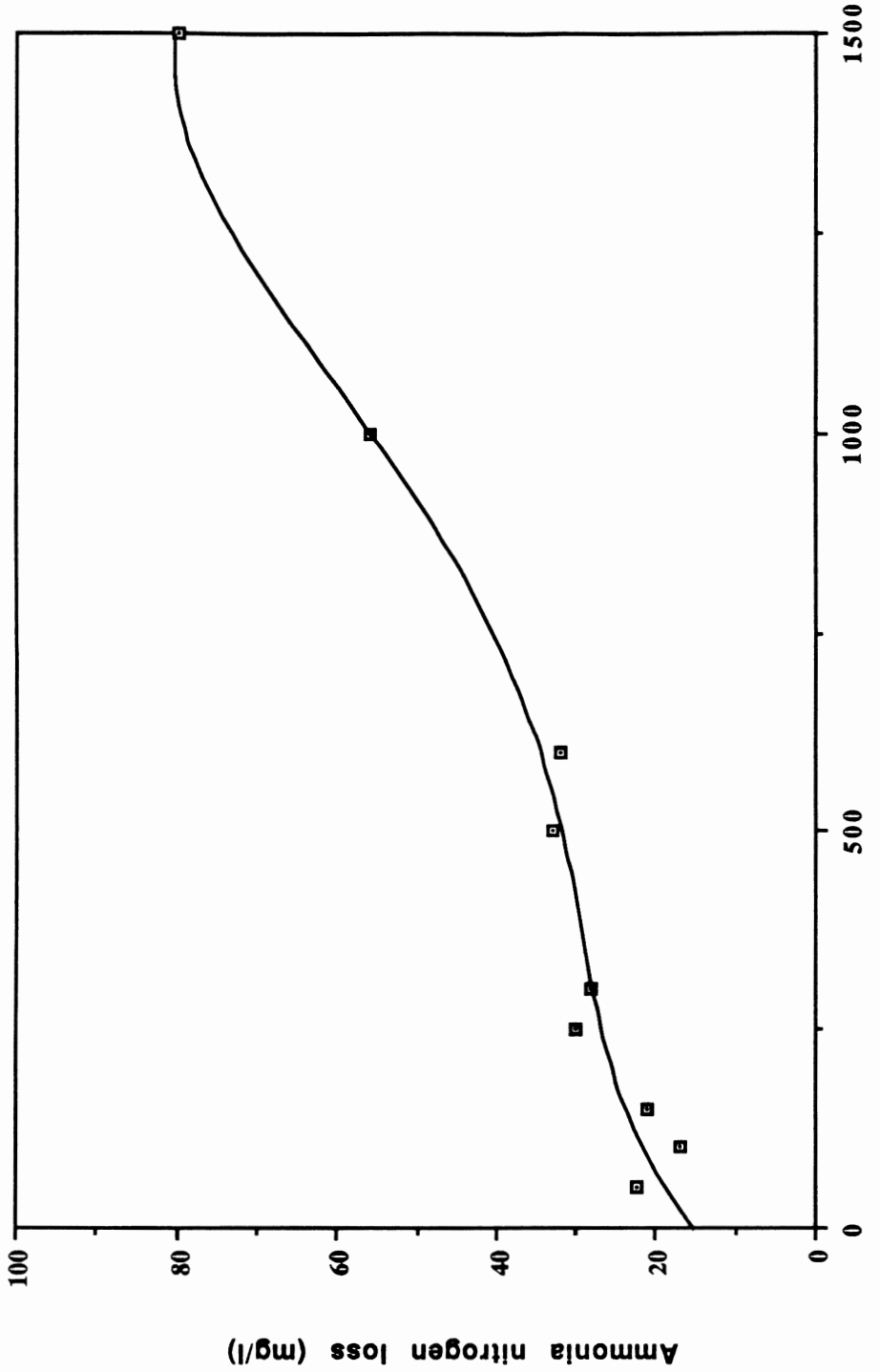

Figure 8. Ammonia Loss during 500 mg/l Nitrate Nitrogen Phase

appears to be like a breakthrough curve, but many factors could have contributed to the loss, such as microbial utilization, adsorption by bed media, and loss to the atmosphere.

Bacteria are more likely to use a reduced form of nitrogen compounds such as ammonia as their nitrogen source for synthesis. But under the absence of ammonia the bacteria would be able to use nitrate as nitrogen source for synthesis. Therefore, in Reactor 1 and 2 , the bacteria would use ammonia as nitrogen source and denitrify all the nitrate while consuming more thiosulfate and alkalinity, instead of using a portion of nitrate for synthesis, as in Reactor 3 (control reactor).

During the 500 mg NO_3^--N/l stage, ammonia loss varied significantly with the ammonia concentrations. The loss increased slowly during the first several ammonia nitrogen additions, up to the feeding concentration of about 1000-1500 mg/l. Then, the system exhibited negative ammonia loss (effluent ammonia concentration higher than influent) at the higher ammonia feeding concentrations. The ratio of ammonia loss per unit of nitrate conversion followed the same pattern as that of ammonia loss. The stoichiometric value for this ratio was 0.087 mol NH_3 utilized/NO_3^--N removed (Bisogni and Driscoll, 1977). The ratio during these experiments increased from 0.043 to 0.16 with increasing ammonia addition.

The ammonia nitrogen concentrations varied considerably during the higher ammonia addition period, but mostly were higher in effluent than in feed solution. It was realized that the magnitude of the negative ammonia

concentration loss was directly proportional to the volatile suspended solids concentration in effluent; the lower the VSS concentration, the lower the negative ammonia loss (Figure 9). It is possible that desorption from both the sloughing of biomass and biomass media surface might have taken place to contribute the extra ammonia in effluent, along with some biomass decay. Figure 9 represents the situation in Reactor 2 at ammonia nitrogen of 3500 mg/l and nitrate nitrogen of 750 and 1000 mg/l. The situations were similar in other stages and other reactors. The negative ammonia loss (top line) went down with the VSS concentration decrease.

The ammonia sorbed onto the microbial granular media was determined roughly by a short side-experiment. The side-experiment was conducted by taking 100 ml biomass media out from the three test reactors. The media were put into three expanded bed reactors which were similar to the test reactors but smaller and in a recycle pattern for six hours each time , fed continually with a solution of nitrate, thiosulfate, bicarbonate and other necessary nutrients, but without ammonia. As shown in Table X, a great amount of ammonia from the control reactor. The data are from two measurements taken on the first day of the side experiment. Ammonia was desorbed from the bed media of the two reactors previously loaded with ammonia, and only a very small amount from the control reactor. Ammonia utilization due to the bacterial growth can be derived from the higher thiosulfate and alkalinity consumptions as in Table VII and VIII. In most cases, the thiosulfate and alkalinity consumptions in ammonia-loaded reactors (Reactor

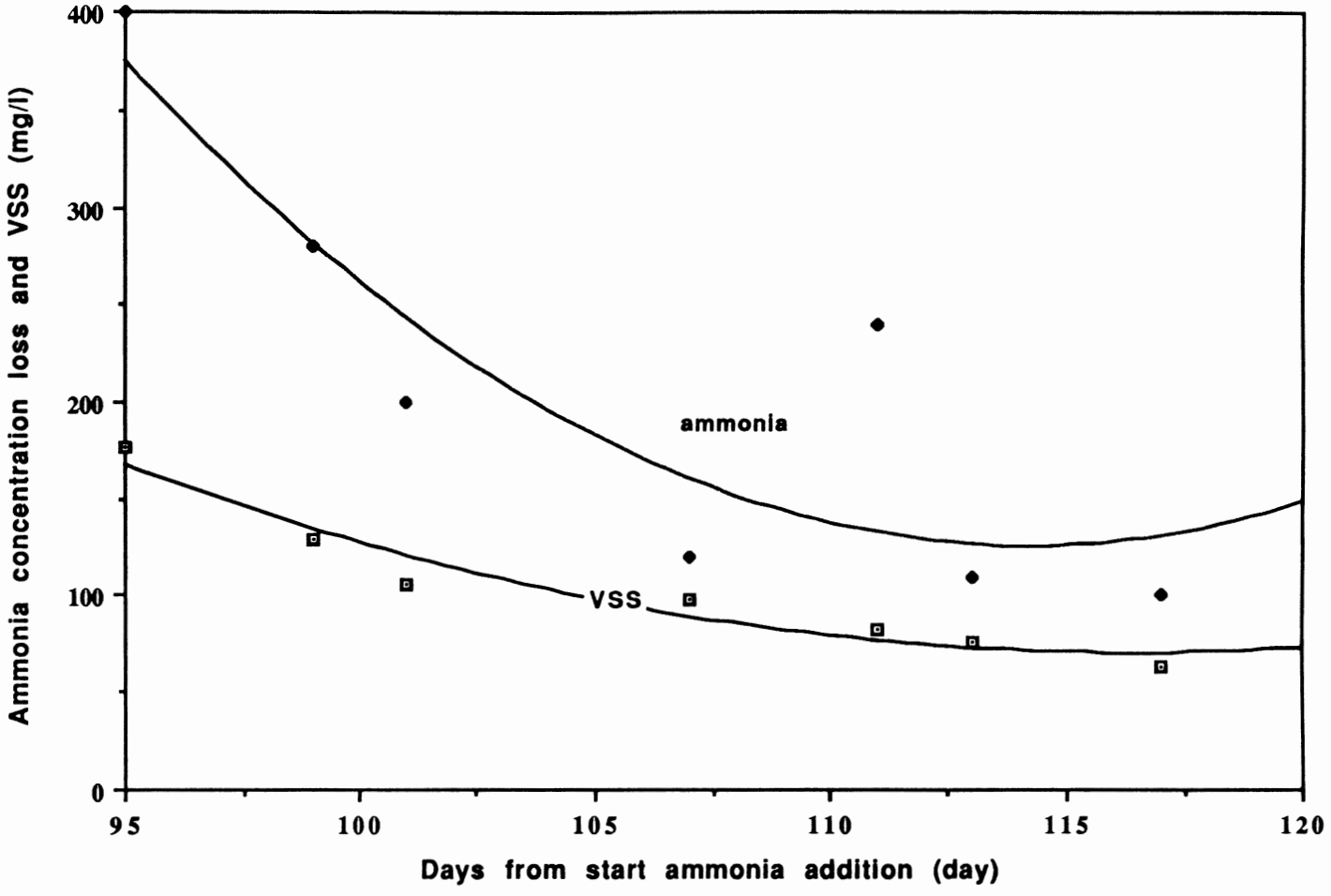

Figure 9. Ammonia Concentration Loss versus VSS

1 and 2) were higher than in the control reactor (Reactor 3), accordingly.

TABLE X

EFFLUENT AMMONIA FROM THREE REACTORS IN SIDE EXPERIMENT

reactor	1	2	3
ammonia nitrogen (mg/l)	1960	1520	20
	1920	1480	24

Biomass Concentration, TSS and VSS

The attached biomass concentrations decreased in all three reactors, but in different degrees for the whole ammonia addition phase (Figure 10). Reactor 1 showed the biggest decrease while the other two were almost the same. The lowest biomass concentration was about 30 mg/l, which was close to the results of previous research (Ross, 1989). We should be able to exclude interference from the ammonia addition, since this phenomenon also happened in the control reactor at almost the same time. Though the nitrate nitrogen loading was increased in the latter stage (750 and 1000 mg/l), the attached biomass concentrations did not increase. To explain this phenomenon may require further focused research. No such observation could be

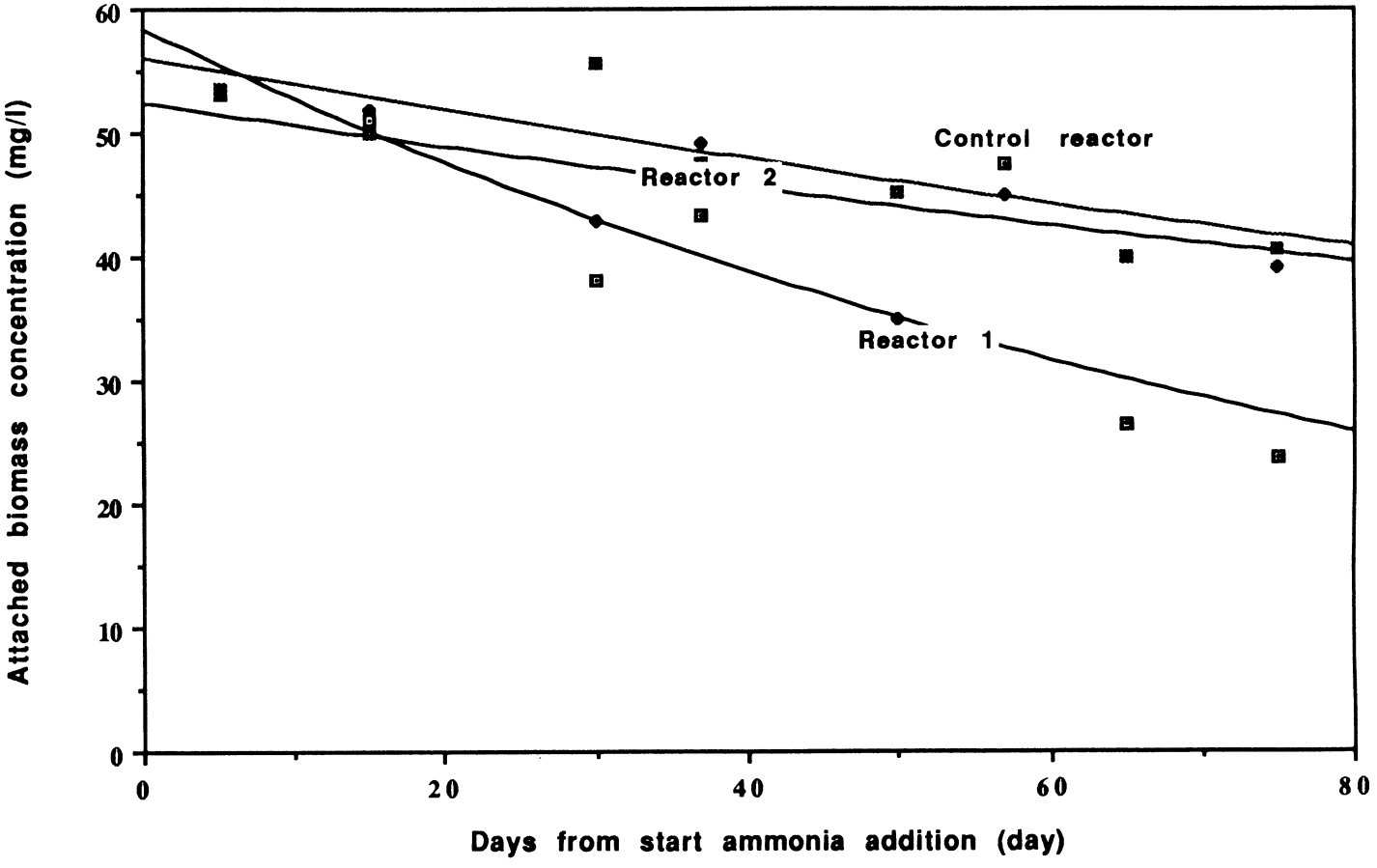

Figure 10. Attached Biomass Concentration from Start Ammonia Addition

found in the literature. Along with the decrease in attached biomass concentration, the total and volatile suspended solids increased.

During the two higher nitrate nitrogen loading rates, the attached biomass concentration and bed volume did not change significantly, unlike in the duplication phase in which the biomass concentration slightly decreased and bed volume increased with nitrate loading increase. The concentration stabilized in the range of about 25-35 mg/l after having decreased during the last phase. Then total and volatile suspended solids began to decrease while the biomass concentration stabilized.

The total suspended solids and volatile suspended solids varied during the ammonia addition phase in all three reactors. The VSS and TSS concentrations in all three reactors increased, and then decreased during the ammonia investigation phase as shown in Figure 11 and 12. It was difficult to find the relationship between the ammonia addition and TSS and VSS, since the control reactor behaved in the same way even without ammonia addition. But ammonia addition might have accelerated the changes, since the higher the ammonia addition, the bigger the changes of biomass loss, and TSS and VSS (Figure 10, 11 and 12). In the latter experiments, the difference between TSS and VSS was high, mainly due to the high concentration of entrapped solids within the expanded bed.

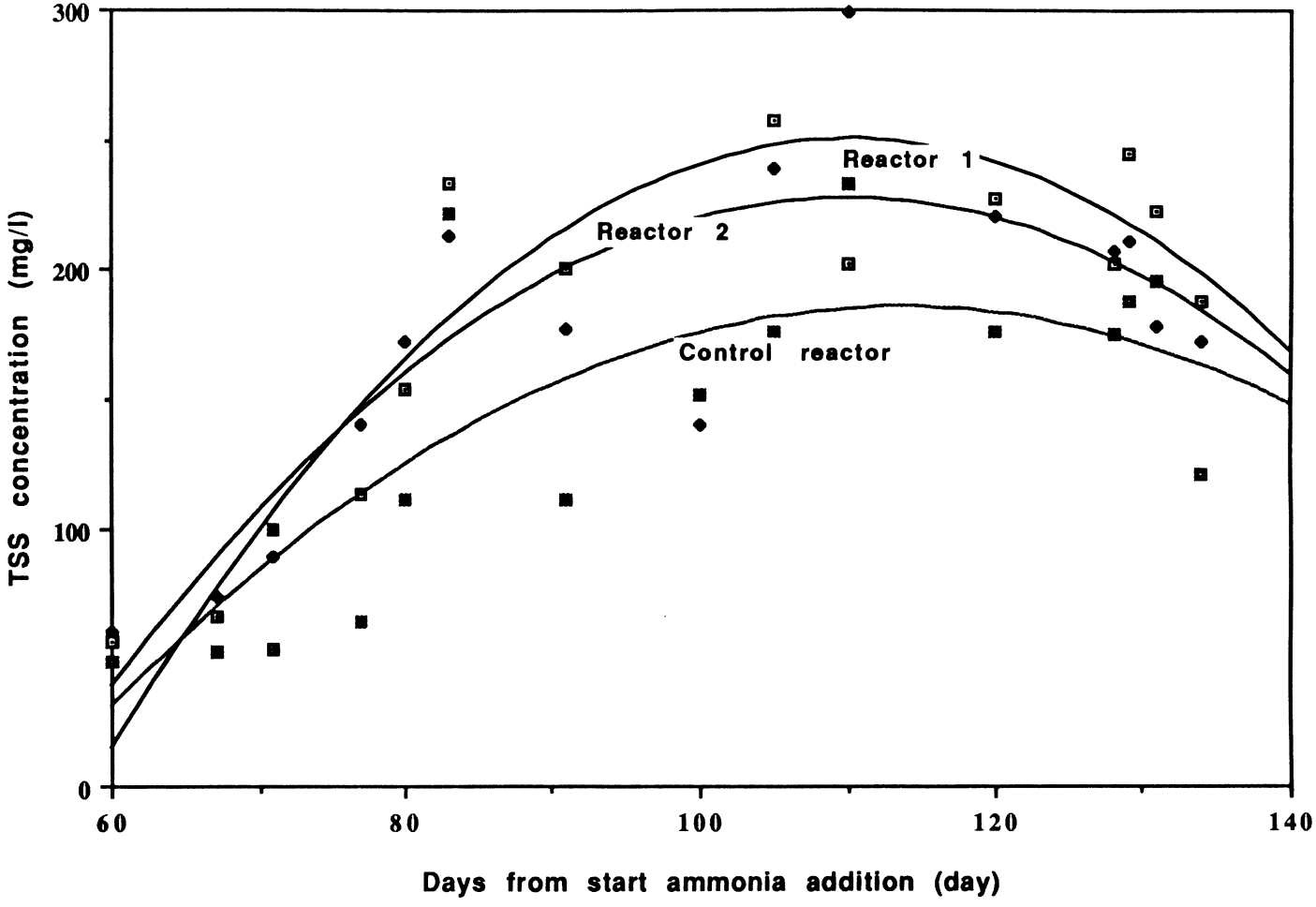

Figure 11. Total Suspended Solids in Three Reactors

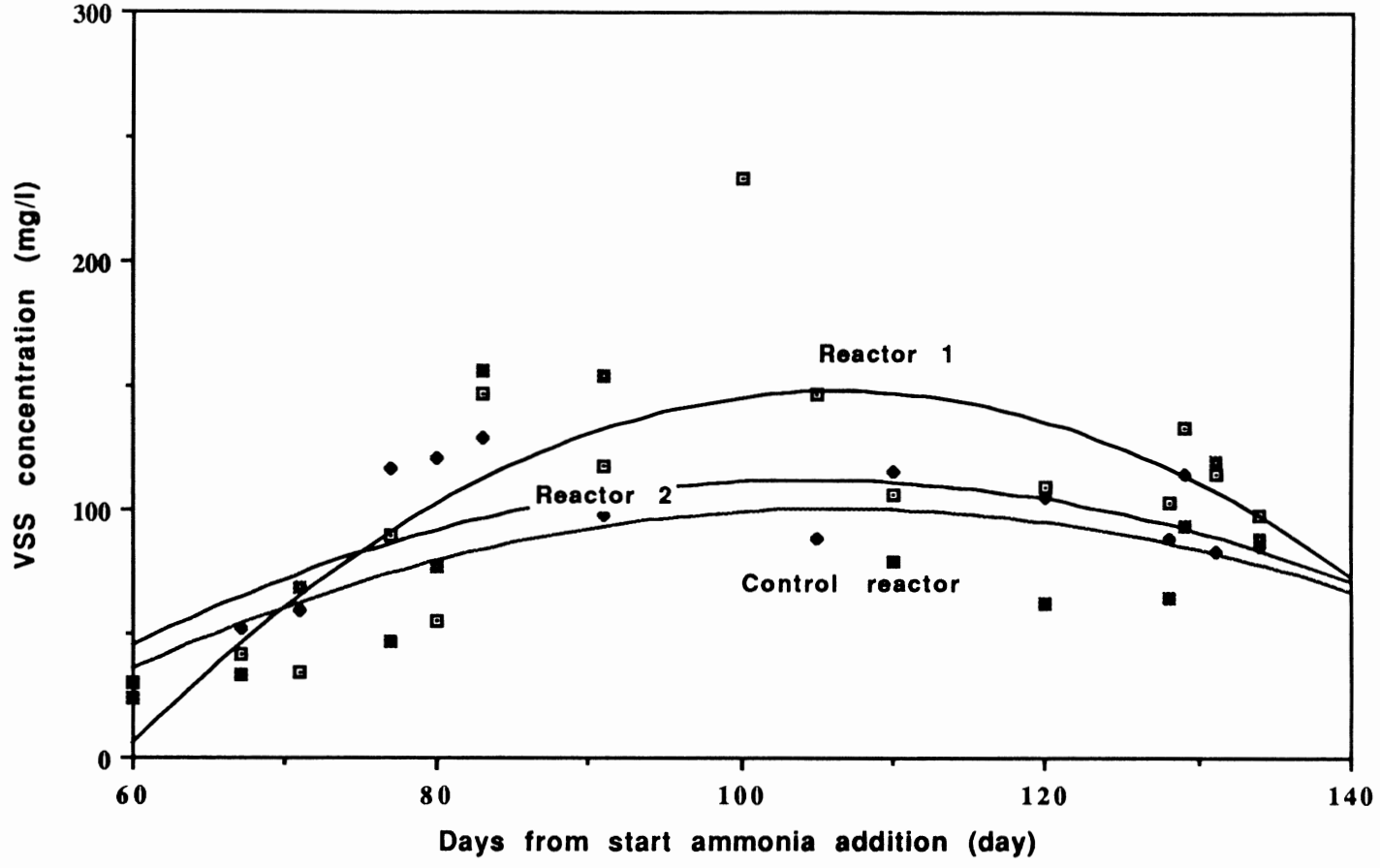

Figure 12. Volatile Suspended Solids in Three Reactors

CHAPTER V

ENGINEERING SIGNIFICANCE

At the ammonia to nitrate nitrogen ratio of 6:1, the industrial wastewater with both high nitrate and ammonia loading can be treated with this autotrophic denitrification process to remove the nitrate. If used as a pre-treatment, autotrophic denitrifying bacteria have good capacity to tolerate the ammonia interferences. Under the presence of a great amount of ammonia, denitrification rates can still be maintained at a high level.

With the hydraulic retention time of about 3 hours, nitrate nitrogen concentration of 500 mg/l, and ammonia nitrogen concentration of 3000 mg/l, complete conversion of nitrate can be achieved. While nitrate nitrogen concentration was 750 and 1000 mg/l, and ammonia nitrogen was 4500 and 6000 mg/l, nitrate removal efficiency above 80 % can still be achieved. Operated under the above conditions, system performance is stable in term of nitrate removal efficiency and other important process parameters.

Based on the results of this research, a pilot scale or full scale application should be feasible. The experimental results indicate that autotrophic denitrification is applicable to reduce the nitrate strength or toxicity of the related industrial wastewaters, when used as a pretreatment step, and is able to handle the

high loading rates, when as a final denitrification step. Results of this study may be used to determine the dilution of raw industrial wastewater when necessary to achieve a given effluent quality.

Biomass control will be very important in process design. Unexpected loss of biomass can cause operational problems and high concentrations of TSS and VSS in effluent. For instance, in these experiments, TSS and VSS had been high in effluent during the latter period. High additions of ammonia did not show a direct, predictable relationship to the loss of biomass in this research, but did seem to accelerate the biomass loss. Odor problems from dissolved sulfide and ammonium bicarbonate can become troublesome, although they were noted only in effluent containers and not in the reactor itself during these experiments. Care must be taken to eliminate these problems. Ammonia desorption from media surfaces can occasionally produce extra ammonia in the process effluent.

CHAPTER VI

CONCLUSIONS

This research is a continuation of bench-scale experiments on high rate autotrophic denitrification in AAFEB. High rate autotrophic denitrification experimental results were duplicated first, then ammonia interference on this process was tested. The research results verified again that an autotrophic denitrification process has good potential to treat highly loaded nitrate wastewater, either directly for influent wastewater itself or as a final stage for complete nitrate removal of nitrified wastewater. Major conclusions obtained from this research are as follows:

1. In this experiment, the AAFEB maintained very high nitrate removal efficiency at nitrate nitrogen concentrations of 750 and 1000 mg/l and HRT of 3.3, 2.8 and 1.4 hours, corresponding to nitrate nitrogen loading rates of 5.5, 8.5 and 17 kg/m^3 d, respectively. These values agree with the values of Ross (1989). Used as a final denitrification step, autotrophic denitrification process can remove nitrate at very high loading rates.
2. Under those nitrate loading rates, autotrophic denitrifying bacteria can tolerate high concentrations of ammonia. The denitrification rates remain high

under the presence of high concentrations of ammonia. Therefore, used as a pretreatment step, autotrophic denitrification can work for the reduction of nitrate toxicity or strength for the latter processes.

3. In this research, complete conversion of nitrate was achieved while 500 mg/l of nitrate nitrogen and 3000 mg/l of ammonia nitrogen were present. When increasing either nitrate nitrogen to 750 and 1000 mg/l, or ammonia nitrogen to 4500 and 6000 mg/l, the denitrification rates dropped within a range of 82.5-93 %. But system performance was still stable.
4. Based on these results, autotrophic denitrification can be placed at the beginning of a process as pre-treatment for a wastewater in which high concentrations of both nitrate and ammonia coexist. But it may be necessary to dilute wastewater into a proper range, according to the results of study. The proper range mainly depends upon the desired nitrate removal efficiency of pre-denitrification step and the ability of nitrifying bacteria to tolerate nitrates.
5. A portion of ammonia will be utilized by bacteria for synthesis. A great amount can be adsorbed onto the media surface. The amount sorbed on the surface could not be determined in research. Only some relative data were shown. The sorbed ammonia may then be desorbed along with biomass sloughing from the media overtime. A small amount may also be stripped into atmosphere at the pH range of these experiments.
6. The biomass stability of a reactor is very important. In this research any direct relationship between

ammonia presence and biomass concentration change was not found. However, high ammonia levels did accelerate biomass sloughing and produced higher TSS and VSS in effluent.

7. During the start-up phase, bacterial growth was intensively promoted with high nitrate and other necessary chemical additions, which produced much higher attached biomass concentration in this research (about 55 mg/l) than that of previous research (20-27 mg/l). The attached biomass concentration decreased when these biomass attached media were used for both duplication and ammonia investigation experiments later on.
8. Compared with the previous research, both thiosulfate and bicarbonate were added in lesser amounts per unit of nitrate removal. No negative results were observed because of the deductions. Nitrates were the limiting factor in the system.
9. Ion chromatography can be used for thiosulfate analysis. The corresponding retention time and peak is distinctive.

CHAPTER VII

RESEARCH NEEDS

This is the first research conducted to investigate ammonia effects on high rate autotrophic denitrification. Many questions were raised during this research and may require further study:

1. Determination of the true upper limit of ammonia concentration which causes an unacceptable drop of denitrification rate under certain nitrate ranges.
2. The effects of other process control parameters on the nitrate-ammonia system, such as hydraulic retention time, pH, temperature, etc.
3. The effects of other present components on denitrification.
4. Denitrifying biomass stability in AAFEB reactors under changing conditions of nitrate loading and other chemical components.
5. Mechanism of ammonia utilization during denitrification and the fate of ammonia.
6. Mechanism of ammonia effects on biomass stability in AAFEB.

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