# COUPLING NITRIFICATION AND DENITRIFICATION IN MIXED ATTACHED FILM AERATED EXPANDED BED SYSTEM

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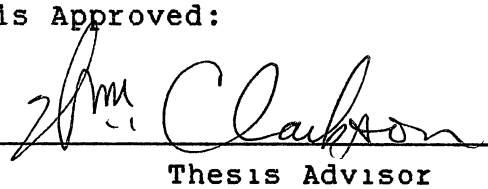

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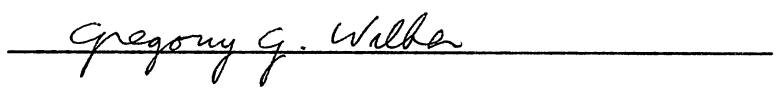

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# CHAPTER I

# INTRODUCTION

## 1.1 Development of this Research

This research was undertaken to investigate the feasibility of using a mixed attached film aerated expanded bed reactor system (MAFAEB system) to conduct biological nitrification and denitrification through a coupled reaction sequence via a shortened pathway, $NH_4^+$-N -> $NO_2$ -N -> $N_2$.

Biological nitrification and denitrification are well established treatment processes used to eliminate the nitrogenous oxygen demand and the ammonia toxicity in both municipal and industrial wastewaters and to prevent eutrophication of receiving water bodies such as lakes and other slow-flow water courses. Conventionally, nitrification and denitrification are performed separately in different biotreatment processes. The reason for this is that nitrification occurs under aerobic conditions while denitrification requires anoxic conditions.

In conventional nitrification and denitrification for highly nitrogenous wastewater treatment processes, the pathway of nitrogen removal can be simply represented by:

$$NH_4^+\text{-}N \longrightarrow NO_2^-\text{-}N \longrightarrow NO_3^-\text{-}N \longrightarrow NO_2^-\text{-}N \longrightarrow N_2$$

If a shortcut, or shortened pathway, represented by:

$NH_4^+$-N ——> $NO_2^-$-N ——> $NO_x$ —- $N_2$

could be achieved, the advantages would at least include reduction of DO and alkalinity demand during nitrification, reduction of COD demand during denitrification, and lower biomass yield. Many efforts have been made on this topic. However, until recently, few studies appear to have been undertaken to successfully prove the hypothesis and develop a process configuration that could achieve the shortcut.

The nitrifiers in wastewater treatment processes are generally autotrophs while the denitrifiers are both autotrophs and heterotrophs. In nitrification processes, the autotrophic nitrifiers use $CO_2$ as carbon source and use $NH_4^+$-N as electron donor. *Nitrosomonas* consumes 3.22mg $O_2$ for each mg of $NH_4^+$-N oxidized to $NO_2^-$-N, and 1.11 mg of $O_2$ is required for each mg of $NO_2^-$-N oxidized to $NO_3^-$-N by *Nitrobacter* according to stoichiometric relationships presented by Grady *et al.* (1980). These bacteria also consume a large amount of alkalinity [$HCO_3^-$] during the oxidation.

In denitrification processes, the heterotrophic denitrifiers use organic matter as carbon sources, and use nitrate as the electron acceptor, while the autotrophic denitrifiers use sodium thiosulfate as electron donor. They convert $NO_3^-$-N to $NO_x^-$-N first, and $NO_2^-$-N to $N_2$ last. Heterotrophic denitrification produces a certain amount of alkalinity during reduction of $NO_3^-$-N and/or $NO_2^-$-N, which normally is wasted in the treatment process effluent.

If nitrification and denitrification can be coupled to such an extent that the only task for nitrifiers is to oxidize $NH_4^+$-N to $NO_2^-$-N and for denitrifiers to reduce $NO_2^-$-N to $N_2$, a large amount of alkalinity and $O_2$ will be saved from nitrification and less organic substrate will be required by denitrification. Also some alkalinity can be supplied by heterotrophic denitrification for the nitrification process. Moreover, if two groups of organisms can play their roles in a single reactor, a great saving can be expected due to simplified process design.

Attached film biological systems have been successfully used for biological nitrification and denitrification on different scales. The aerobic expanded bed (AEB) is also a promising process for high-strength industrial wastes, with advantages such as small treatment volume and high efficiency (Jewell, 1981). Therefore, AEB was chosen for use in this research. If layered biofilms of denitrifiers and nitrifiers can be developed on the media surface to satisfy their different requirements, then the shortened pathway may be achieved.

## 1.2 Objectives of this Research

1. Develop mixed attached films including autotrophic nitrifiers and both autotrophic and heterotrophic denitrifiers;
2. Determine the difference in substrate demands with either nitrite or nitrate as denitrification electron

acceptor by mixed attached films;

3. Determine nitrification behavior of the MAFAEB system;

4. Determine denitrification behavior of the MAFAEB system;

5. Determine coupling reaction rates in response to varying loading rates, electron donors, and aeration rates and the limitations of MAFAEB treatment efficiency.

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Scope of Review

This research focuses on coupling nitrification and denitrification through a shortened pathway, primarily in a single MAFAEB reactor under low dissolved oxygen (DO) aerobic conditions. Although nitrification and denitrification in general are well-studied, information on coupling reactions through a shortened pathway is limited. Thus, this literature survey emphasizes the need to explain the micro-biological conditions of nitrifiers and denitrifiers, the stoichiometric relationships, the effects which interfere with nitrification and denitrification, and the possibility of coupling nitrification and denitrification through the shortened pathway.

## 2.2 Microbiology of Nitrification and Denitrification

Nitrification is the conversion of ammonia nitrogen $NH_4^+$-N to nitrate nitrogen $NO_3^-$-N. It may be performed by either heterotrophic or autotrophic bacteria. The major nitrifying bacteria are *Nitrosomonas* and *Nitrobacter*. They are autotrophic organisms. *Nitrosomonas* oxidizes $NH_4^+$-N to

nitrite, $NO_2^-$-N, and *Nitrobacter* oxidizes nitrite to nitrate. The energy released in these reactions is used by the nitrifying organisms in synthesizing their organic requirements from inorganic carbon sources such as carbon dioxide, bicarbonate and carbonate. (Barnes, *et al*, 1983). The above reactions can be written as follows (EPA, 1975; Painter, 1970):

$$NH_4^+ + 1.5\ O_2 \rightarrow 2\ H^+ + H_2O + NO_2^- + (58\text{-}84\ Kcal) \quad (1)$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- + (15.4\text{-}20.9\ Kcal) \quad (2)$$

The biochemistry of ammonia oxidation is rather more complex than indicated by the above equations, involving the formation of hydroxylamine and other unstable intermediates which have yet to be determined (Painter, 1970; Sharma, *et al*. 1977).

Both *Nitrosomonas* and *Nitrobacter* are obligate aerobes for growth on their respective forms of substrate nitrogen. Absence of oxygen for long periods, however, is not lethal (Painter, 1970), and in the absence of substrate the rate of decline in respiration rate is considerably slower under anaerobic than under aerobic conditions. In the absence of oxygen, *Nitrobacter* is able to reduce nitrate to nitrite in a reaction which is the reverse of Equation 2 (Sharma *et al*. 1977).

In addition to the autotrophic nitrifiers, many heterotrophic organisms are able to produce oxidized nitrogen forms from ammonia. The importance of heterotrophic nitrification is still a matter of debate(Geraats *et al*., 1990).

The specific nitrifying activity of the heterotrophs is said to be $10^{-3}$ - $10^{-4}$ times lower than that of the autotrophs, and therefore heterotrophic nitrification is often considered to be of minor ecological significance. However, this activity was measured by the accumulation of nitrite or nitrate. Since many heterotrophic nitrifiers are able to denitrify aerobically as well as anaerobically, ammonia is directly converted to nitrogen gas and nitrite or nitrate will not accumulate. When making mass balances for continuous cultures, it was found that the nitrification activity (in terms of ammonia oxidized) of the nitrifier/aerobic denitrifier, *Thiosphaera pantotropha*, is only 10-$10^{-3}$ times lower than the autotrophs(Geraats *et al.*, 1990). It seems likely that, as other bacteria of this physiological type are studied, it will be found that most nitrification rates have been underestimated because of the simultaneous nitrite reduction. Thus, in view of the fact that heterotrophs generally outnumber autotrophs in the bacteria communities found in most wastewater treatment systems, heterotrophic nitrifying organisms might well be of greater significance than previously thought (Geraats, *et al.*,1990).

Denitrification is the reduction of nitrate as it serves as the terminal hydrogen acceptor for microbial respiration in the absence of molecular oxygen. The bacteria responsible for denitrification are facultative and utilize the same basic biochemical pathway during both aerobic and anaerobic respiration(Grady *et al.*, 1980).

Denitrification can be accomplished by a large number of bacteria commonly found in wastewater treatment systems, including *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus, Proteus, Pseudomonas and Thiosphaera pantotropha.*

Under aerobic conditions organic and other materials are oxidized and oxygen acts as the effective electron acceptor. However, under conditions where the concentration of DO is low or zero, an alternative electron acceptor is needed. Inorganic anions like nitrate, phosphate, sulphate and even carbon dioxide can act as the electron acceptor.

The proportion of any microbial species present in a mixed culture will depend upon the relative abundance of appropriate electron donor material, the relative abundance of appropriate electron acceptor and the energy to be gained by using a particular electron acceptor (Barnes *et al.*, 1983). Under aerobic conditions, oxygen is the favored electron acceptor and aerobic oxidation will predominate. The next most favored reaction uses nitrate, and this is considerably more advantageous than other anaerobic pathways. Under conditions of low DO concentration, biological denitrification can be expected to occur.

The biological reaction to reduce nitrate ions to nitrite ions and subsequently to nitrogen requires that a suitable electron donor is available. When the electron donor is methanol, the reactions can be represented by the following equations:

$$NO_3^- + 1/3\ CH_3OH \longrightarrow NO_2^- + 1/3\ CO_2 + 2/3\ H_2O \quad (3)$$

$$NO_2^- + 1/2\ CH_3OH \longrightarrow N_2 + 1/2\ CO_2 + 1/2\ H_2O + OH^-. \quad (4)$$

When the electron donor is thiosulfate, the reactions can be represented by the following equations:

$$NO_3^- + 1/4\ S_2O_3^{2-} + 1/2\ HCO_3^- \longrightarrow NO_3^- + 1/2\ SO_4^{2-} + 1/2\ CO_2 + 1/4\ H_2O \quad (5)$$

$$NO_2^- + 3/8\ S_2O_3^{2-} + 1/4\ H^+ \longrightarrow 1/2\ N_2 + 3/4\ SO_4^{2} + 1/8\ H_2O. \quad (6)$$

Many organic chemicals other than methanol, for example acetic acid, citric acid and acetone, can be used as electron donors for denitrification. Methane (Rhee *et al.*, 1978) and sulphur (Batchelor *et al.*, 1978) also have been suggested.

The evidence for aerobic denitrification was obtained from a number of independent experiments (Robertson *et al.*, 1984). The maximum specific growth rate of *T. pantotropha* was higher (0.34 $h^{-1}$) in the presence of both oxygen ( > 80% air saturation ) and nitrate than in similar cultures not supplied with nitrate ( 0.27 $h^{-1}$ ) indicating that the rate of electron transport to oxygen was limiting. This was confirmed by oxygen uptake experiments which showed that although the rate of respiration on acetate was not affected by nitrate, the total oxygen uptake was reduced in its presence. The original oxygen uptake could be restored by the addition of denitrification inhibitors.

## 2.3 Stoichiometric Relationships

On the assumption that the gross composition of *Nitrosomonas* and *Nitrobacter* can be represented as $C_5H_7NO_2$, the stoichiometry of cell growth of nitrifiers has been represented as (EPA, 1975):

$$15\ CO_2 + 13\ NH_4^+ \rightarrow 10\ NO_2^- + 3\ C_5H_7NO_2\ (\textit{Nitrosomonas}) + 23\ H^+ + 4\ H_2O \quad (7)$$

$$5\ CO_2 + NH_4^+ + 10\ NO_2^- + 2\ H_2O \rightarrow 10\ NO_3^- + C_5H_7NO_2\ (\textit{Nitrobacter}) + H^+. \quad (8)$$

Although carbon dioxide is represented as the inorganic carbon source, it exists in aqueous systems in equilibrium with other species according to the equations:

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows H^+ + HCO_3^-. \quad (9)$$

Hydrogen ions produced in Equations 1, 7 and 8 react with bicarbonate according to Equation 9 which may therefore be incorporated into these three equations to give:

$$NH_4^+ + 1.5\ O_2 + 2\ HCO_3^- \rightarrow NO_2^- + 2\ H_2CO_3 + H_2O + (58\text{-}84\ Kcal) \quad (10)$$

$$13\ NH_4^+ + 23\ HCO_3^- \rightarrow 8\ H_2CO_3 + 10\ NO_2^- + 3\ C_5H_7NO_2 + 19H_2O \quad (11)$$

$$NH_4^+ + 10\ NO_2^- + 4\ H_2CO_3 + HCO_3^- \rightarrow 10\ NO_3^- + 3\ H_2O + C_5H_7NO_2. \quad (12)$$

Since the energy produced in Equation 10 is used in the cell synthesis reaction, assuming a *Nitrosomonas* cell yield of 0.15 gVSS/g $NH_4^+$-N (EPA, 1975), Equation 10 and 11 can be combined to give:

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow C_5H_7NO_2\ (\textit{Nitrosomonas}) + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3. \quad (13)$$

Similarly, Equations 2 and 12 can be combined, assume in a *Nitrobacter* cell yield of 0.02 g VSS/g$NO_2^-$-N oxidized, to give:

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + HCO_3^- + 195\ O_2 \longrightarrow C_5H_7NO_2\ (\textit{Nitrobacter}) + 3\ H_2O + 400\ NO_3^-. \quad (14)$$

The overall reaction for nitrifier synthesis and oxidation obtained by combining Equation 13 and 14 is then:

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \longrightarrow 0.021\ C_5H_7NO_2 + 1.041\ H_2O + 0.98\ NO_3^- + 1.88\ H_2CO_3. \quad (15)$$

Equation 15 reveals the very low cell yield per unit of ammonium nitrogen oxidized, the significant requirement for oxygen in nitrification, approximately 4.2 g oxygen for each g $NH_4^+$-N removed, and the requirement for alkalinity to buffer the system against hydrogen ions produced during nitrification, amounting to approximately 7 g alkalinity for each g $NH_4^+$-N oxidized.

The stoichiometric equation of heterotrophic denitrification was presented by MaCarty *et al.* (1969):

$$NO_3^- + 1.08\ CH_3OH + H^+ = 0.065\ C_5H_7NO_2 + 0.47\ N_2 + 0.76\ CO_2 + 2.44\ H_2O \quad (16)$$

The stoichiometric equation of autotrophic denitrification using thiosulfate as electron donor was calculated by Ross (1989):

$$NO_3^- + 0.79\ S_2O_3^- + 0.27\ HCO_3^- + 0.2\ H_2O = 0.05\ C_5H_7NO_2 + 0.47\ N_2 + 1.56\ SO_4^= + 0.28\ H^+ \quad (17)$$

Comparison of the stoichiometry of heterotrophic and autotrophic denitrification reveals that, whereas the heterotrophs are net alkalinity producers, autotrophic denitrifiers consume alkalinity (are net producers of acidity) in much the same way as nitrifying bacteria (Clarkson *et al.*, 1990).

## 2.4 Factors Affecting Nitrification and Denitrification

### 2.4.1 Effects of Temperature

The saturation constants for both *Nitrosomonas* and *Nitrobacter*, with respect to both inorganic nitrogen and DO, have been found to increase with increasing temperature (Painter, 1970). For *Nitrosomonas*, reported values of $K_M$ for ammonia nitrogen range from 0.54 - 1 mg/L at 20°C, 3.5 mg/L at 25°C and to 10 mg/L at 30°C (Painter, 1970).

The temperature dependence of denitrification is similar to related biological processes. The reaction occurs between 0°C and 50°C with optimum reaction rates at 35-50°C. The reaction rate increases by a factor of 1.5-2.0 / 10°C between 5°C and 15°C (EPA, 1975).

### 2.4.2 Effects of Other Substrate

Some studies indicate that high concentration of $NH_4^+$-N up to 1000 mg/L may not inhibit *Nitrosomonas*. Even at a concentration of 8000 mg/L, some oxidation can still proceed at a much reduced rate (Sharma *et al.*, 1977; Anthonisen *et*

*al.*, 1976). However, for *Nitrobacter* in pure culture, concentrations of 8 - 16 mg/L of $NH_4^+$-N reportedly increased the lag period, but only slightly decreased the growth rate (Sharma *et al.*, 1977).

Nitrite is reported in one case to have an inhibiting effect on nitrification in a laboratory-scale activated sludge plant at a concentration as low as 10 mg/L (Tomlinson *et al.*, 1966). In batch and pure culture studies with *Nitrosomonas*, however, although toxic effects were exhibited in the lag phase at 500 mg/L $NO_2^-$-N, the organisms were not susceptible in the logarithmic growth phase (Sharma *et al.*, 1977). At 1400 mg/L $NO_2^-$-N about 40% inhibition has been reported while at 2500 mg/L inhibition varied from 50% to complete. For *Nitrobacter*, 40% inhibition was reported at 1400 mg/L $NO_2^-$-N (Boon *et al.*, 1976). The effect increased with increasing concentration.

Inhibition of nitrification by free ammonia and free nitrous acid has been described by Anthonisen *et al.* (1976). Inhibition of *Nitrosomonas* by free ammonia is likely in the range 10 - 150 mg/L. Inhibition of *Nitrobacter* is likely at the much lower concentrations of 0.1 - 1.0 mg/L, leading to the possibility that in wastes containing high concentrations of $NH_4^+/NH_3$ inhibition of *Nitrobacter* may lead to the accumulation of nitrite.

### 2.4.3 Effects of Other Substances

Nitrification is subject to inhibition by a wide

variety of organic and inorganic chemicals, *Nitrosomonas* generally being more susceptible than *Nitrobacter*. Among the factors which have been found to affect the degree of inhibition by any given inhibitor are (Sharma *et al.*, 1977):

(a) the presence of microorganisms other than the nitrifiers;

(b) the concentration of the inhibitor;

(c) the concentration of the nitrifiers.

Inhibitors may act either by interfering with the general metabolism of the cell or by disrupting the primary oxidation reactions. Although many organic compounds are inhibitory to nitrifiers, especially *Nitrosomonas*, it now seems to be accepted that organic matter in general is not directly inhibitory to nitrification (Painter, 1970). Compounds such as glucose, glycerol and acetate were not found to be toxic to *Nitrosomonas* although peptone at concentrations of 1 and 10 mg/L reduced growth rate by 25% and 60%, respectively(Painter, 1970).

### 2.4.4 Effects of Dissolved Oxygen

Dissolved oxygen has been considered to be an absolute requirement for growth of both *Nitrosomonas* and *Nitrobacter*. There is evidence that for pure cultures of both *Nitrosomonas* and *Nitrobacter* the critical DO concentration below which nitrification does not occur is 0.2 mg/L (Schoberl *et al.*, 1964). DO concentrations higher than 1-2 mg/L are enough to keep the nitrification a zero-order reaction with

respect to nitrogen (Schoberl *et al.*, 1964; Knowles *et al.*, 1965), therefore 2 mg/L of DO has been widely suggested as a minimum for nitrification (EPA, 1975). Some observations show that higher DO concentrations of 3-4 mg/L can significantly enhance nitrification efficiency (Benefield *et al.*, 1980), but relatively little further improvement can be achieved at 5-6 mg/L of DO (Bliss *et al.*, 1981).

For denitrification, generally, strict anoxic conditions and the presence of nitrogen oxides in the medium are required for synthesis of denitrifying enzymes. However, if the amount of nitrate far exceeds the oxygen concentration, anaerobic respiration may become significant (Payne, 1981).

Strand *et al.* (1985) found that if organic matter and microbial biomass are present in sufficient excess, the $NO_3^-$-N loss rate in microbial films exposed to aerobic media can be as high as those observed in anoxic cultures. The bulk fluid dissolved oxygen concentration (0.1-14 mg/L) had a negligible effect on the microbial film's consumption rate of oxidized nitrogen. The reason for this is that dissolved oxygen does not fully penetrate microbial films with population densities greater than $0.5 \times 10^9$ cells $cm^{-2}$ (Strand *et al*, 1985).

## 2.5 Aerobic Expanded Bed

Aerobic expanded bed (AEB) reactors are submerged biofilm units using small biomass support particles with continuous recycle. The small particles provide a high

surface area to volume ratio in the reactor. After applying AEB to nitrification treatment of semiconductor wastewater, Collins *et al.* (1991) concluded that the AEB reactor, despite its physical limitations, has potential as a pre-treatment process to provide highly efficient nitrification of semiconductor wastes. Biological fluidized-bed reactors have been used successfully for BOD and nitrogen removal in many plants (Jeris *et al.*, 1977). AEB is similar to a fluidized-bed reactor. The principal difference between AEB and fluidized-bed is the bed expansion rate. Strictly speaking, the AEB reactor should not have a bed expansion rate higher than 20% (Jewell, 1981). In the research by Collins *et al.* (1991) and Zeng (1992), bed expansion rates higher than 60% were used, which are actually intermediate between expanded and fluidized bed operation.

## 2.6 Coupling Nitrification and Denitrification

### 2.6.1 Different Approaches

Many industries such as fertilizers, semiconductor, meat and milk processing and munitions production generate waste streams that contain high concentrations of nitrogenous compounds. Nitrification and denitrification of such effluents should both be employed to remove soluble nitrogen for preventing eutrophication of receiving water bodies. Conventionally, nitrification and denitrification are performed separately in different biotreatment processes.

Some efforts have been made on coupling nitrification and denitrification. Timberlake *et al.* (1988) developed a biofilm reactor, termed the permeable-support biofilm, in which oxygen was supplied to the interior of the biofilm through a permeable membrane. The reactor was tested on filtered sewage supplemented with nutrient broth. The bulk solution was anoxic and the interior of the biofilm was supplied with pure oxygen. All tests were performed on a non-steady state biofilm with a depth of 1 mm. Mass balances on total organic carbon, ammonia, organic nitrogen and nitrate showed that combined heterotrophic oxidation of organic matter, nitrification and denitrification occurred simultaneously within the biofilm.

One study conducted by Turk *et al.* (1986) investigated the feasibility of removing nitrogen from highly nitrogenous wastes by the shortened pathway. The study employed bench-scale, activated sludge cells. Free ammonia, controlled by manipulating pH, was used as inhibitor of nitrite oxidation. A multi-cell reactor system was operated in series to approximate a plug flow configuration. Nitrite build-up was achieved by intermittent contact with a higher than 5 mg/l free ammonia level at the front end of the system, which was maintained anoxic to create a high free ammonia environment. Intermediary denitrification at the point where the nitrite level was highest was used to sustain nitrite build-up level. The process configuration would normally produce an effluent devoid of nitrite, due to its oxidation to nitrate

in the remaining aerobic cells. The feasibility of initiating nitrite build-up in an activated sludge nitrogen removal system via the shortened pathway, while producing a fully nitrified effluent devoid of nitrite was confirmed (Turk *et al.*, 1987). A 40 % reduction of COD during denitrification was also claimed (Turk *et al.*, 1989).

However, nitrite build-up could not be sustained indefinitely due to acclimation of the nitrite oxidizers to free ammonia. Numerous measures have also been taken (Turk *et al.*, 1989) to prevent the eventual decline of nitrite build-up. Unfortunately, nitrite oxidizers appeared capable of tolerating ever-increasing levels of free ammonia, thus causing an irreversible decline in nitrite accumulation for most operational systems tested. They suggest if a way can be found to permanently overcome the apparent acclimation of the nitrite oxidizers to free ammonia, a cost-effective technology based on nitrite production and reduction may evolve for the removal of nitrogen from highly nitrogenous wastewaters.

One possible way to solve the problem involves heterotrophic nitrification and aerobic denitrification. It has commonly been accepted that denitrification requires completely anoxic conditions because some well-studied bacteria completely shut down their denitrifying capacity upon exposure to oxygen (Robertson *et al.*, 1984a). However, there have been periodic reports of aerobic denitrification (Marshall *et al.*, 1953; Mescher *et al.*, 1963; Krul, 1976;

Robertson et al., 1984a; Simpkin et al., 1988; Hanaki et al., 1990). The results of these experiments clearly indicate that in a number of denitrifying bacteria, aerobic denitrification does indeed occur. However, the denitrifiers convert $NO_3^-$-N at highest rates under anaerobic conditions (Robertson et al., 1984b). Many other heterotrophic nitrifiers were also found able to denitrify aerobically as well as anaerobically (Robertson et al., 1989). For wastewater treatment, this means that when nitrification is not subject to inhibition by either organic matter or any other inhibitors, simultaneous aerobic organic degradation, nitrification and denitrification can occur within a single aeration basin.

There is another possibility for coupling nitrification and denitrification, which involves aerobic nitrification and denitrification combined with methanotrophic and methylotrophic mixed cultures. Since ammonia can be oxidized by obligate methanotrophic bacteria, in any unprotected process where bacterial growth on methane occurs, the mixed culture will comprise not only obligate methane-utilizing bacteria, but also methylotrophic bacteria, specifically *Hyphomicrobium*, and a range of heterotrophic bacteria (Hamer et al., 1989). In such mixed cultures, the role of the *Hyphomicrobium* is to scavenge methanol produced from methane by the methane-utilizing species. When this same *Hyphomicrobium* was grown in pure culture at 32°C in the presence of nitrate, denitrification became evident. Although a high

level of methane inhibits ammonia oxidation, at low levels, it will stimulate nitrite formation (O'Neill et al., 1977). If methane is used as carbon source for the nitrification, methanol will be the product when ammonia is converted to nitrite. If the dissolved oxygen concentration in the solution is kept low, then denitrification may occur simultaneously.

2.6.2 MAFAEB Development

To achieve the shortened pathway, the main problem comes from the fact that one of the reactions is favored aerobically while another is favored under anoxic conditions. The other problems include: 1. avoiding inhibition of autotrophic nitrifiers by organic matter used by some denitrifiers; 2. stopping nitrification at the $NO_2^-$-N stage with efficient $NO_2^-$-N utilization by denitrifiers.

Hanaki et al. (1990) conducted a lab-scale nitrification study in a mixed flow reactor with DO control at 25°C using substrate containing 80 mg/L of $NH_4^+$-N. At 0.5 mg/L DO, ammonia oxidation was not affected. However, $NO_2^-$-N oxidation was strongly inhibited by 0.5 mg/L of DO, and 60 mg/L of $NO_2^-$-N accumulated. The maximum specific growth rate $\mu_m$ for $NH_4^+$-N oxidation was not significantly changed by low DO because of elevated growth yield. When Jones et al. (1990) were investigating a process incorporating sequencing batch reactors for organic removal and denitrification and a fixed-film device for nitrification, they

found a small amount of $NO_2^-$-N in the denitrification feed stream had resulted in a robust population of organisms capable of reducing $NO_2^-$-N faster than $NO_3^-$-N, resulting in a 30% increase in the denitrification rate over systems fed only $NO_3^-$-N as an electron acceptor.

Consequently, if a proper condition can be created, the symbiosis of two groups of organisms is possible. Since both organisms share the same pH range, the goal ought to be possible.

Collins et al. (1991) and Zeng (1992) successfully conducted nitrification of high strength industry wastewater with AEB, and Clarkson et al. (1990) successfully conducted denitrification of high strength industry wastewater with attached film expanded bed (AFEB). They used diatomaceous earth as inert support to attach microorganisms. These reactor configurations can be combined to meet the requirements for the coupled nitrification and denitrification.

To take advantage of their differences in growth requirements, nitrifiers and denitrifiers should be acclimated separately prior to seeding the coupled biofilm reactor. This may be done by feeding the nitrifiers with $NH_4^+$-N under low DO to acclimate mainly *Nitrosomonas* and feeding both $NO_2^-$-N and $NO_3^-$-N as electron acceptors for denitrifiers.

Inert support media should be supplied for both groups of organisms separately to develop attached biofilms or be supplied to denitrifiers to develop the first layer of

combined biofilm. Then, nitrifiers should be attached to develop double films with the nitrifiers exposed to liquid phase DO. In this way, two groups of organisms could be put in a single reactor and fed with an influent containing $NH_4^+$-N with low organic electron donor (only sufficient for those heterotrophic denitrifiers to convert $NO_2^-$-N to $N_2$) under low DO conditions.

The key problems are understanding how to supply $O_2$ for nitrifiers and how the system works. The reactor should be a combination of AFEB, AEB and fluidized-bed reactors. Influent enters the reactor from the bottom. Compressed air should be introduced into the reactor from the aeration bottle through recycle tubing connected to the bottom of the reactor. The amount of air should be controlled to maintain a low DO in the reactor and offer a mild mixing. Since at low DO conditions oxygen supply may become critical, a large recycle may be necessary, especially for high strength influents. The recycle rate can be altered according to the organic and $NH_4^+$-N concentration of the original influent and allowable loading of the system.

To summarize, acclimating nitrifying and denitrifying organisms separately may induce their biodegradation specificity to particular substrates; attaching the two groups of organisms together may develop aerobic and anaerobic zones within the biofilms, which may keep nitrifiers and denitrifiers always active in their favorable local environment; mild mixing may improve diffusion between

liquid phase and solid phase; alkalinity produced by heterotrophic denitrifiers may be utilized by autotrophic denitrifiers and nitrifiers; large recycle rate may supply sufficient oxygen to the reactor and maintain a lower inlet $NH_4^+$-N and organic concentration; low DO (about 0.5-2.0 mg/L) may not only avoid unnecessary oxidation from $NO_2^-$-N to $NO_3^-$-N but also avoid the suppression of denitrification.

# CHAPTER III

# MATERIALS AND METHODS

## 3.1 Experimental Apparatus

The mixed attached films aerobic expanded bed (MAFAEB) system is shown in Figure 1. The MAFAEB reactors consisted of an Imhoff cone, which was 1 L in volume. The effluent from the top of the expanded bed reactor was introduced to a 500-mL aeration bottle in which the effluent was aerated by compressed air. Effluent recycling was carried out to increase dissolved oxygen and expand the bed in the reactor. A positive displacement pump (7553-50, Cole-Parmer) was used to recycle the aerated effluent to the bottom of the reactors. The pump was fitted with two model 7015-20 pump heads (Cole-Parmer Instrument Co.). Treated water left the system through an overflow opening located at the upper part of the aeration bottle. The feeding solution from 25-L or 4-L containers was pumped to the bottom of the reactor by a Cole-Parmer model 7553-50 pump fitted with positive displacement pump head (model 7016-13). The feed and the recycled effluent joined together in a plastic tubing leading to the bottom of the reactor. The biofilm support media in the reactor consisted of diatomaceous earth particles (diameter 1-3 mm), which provide a high surface area to

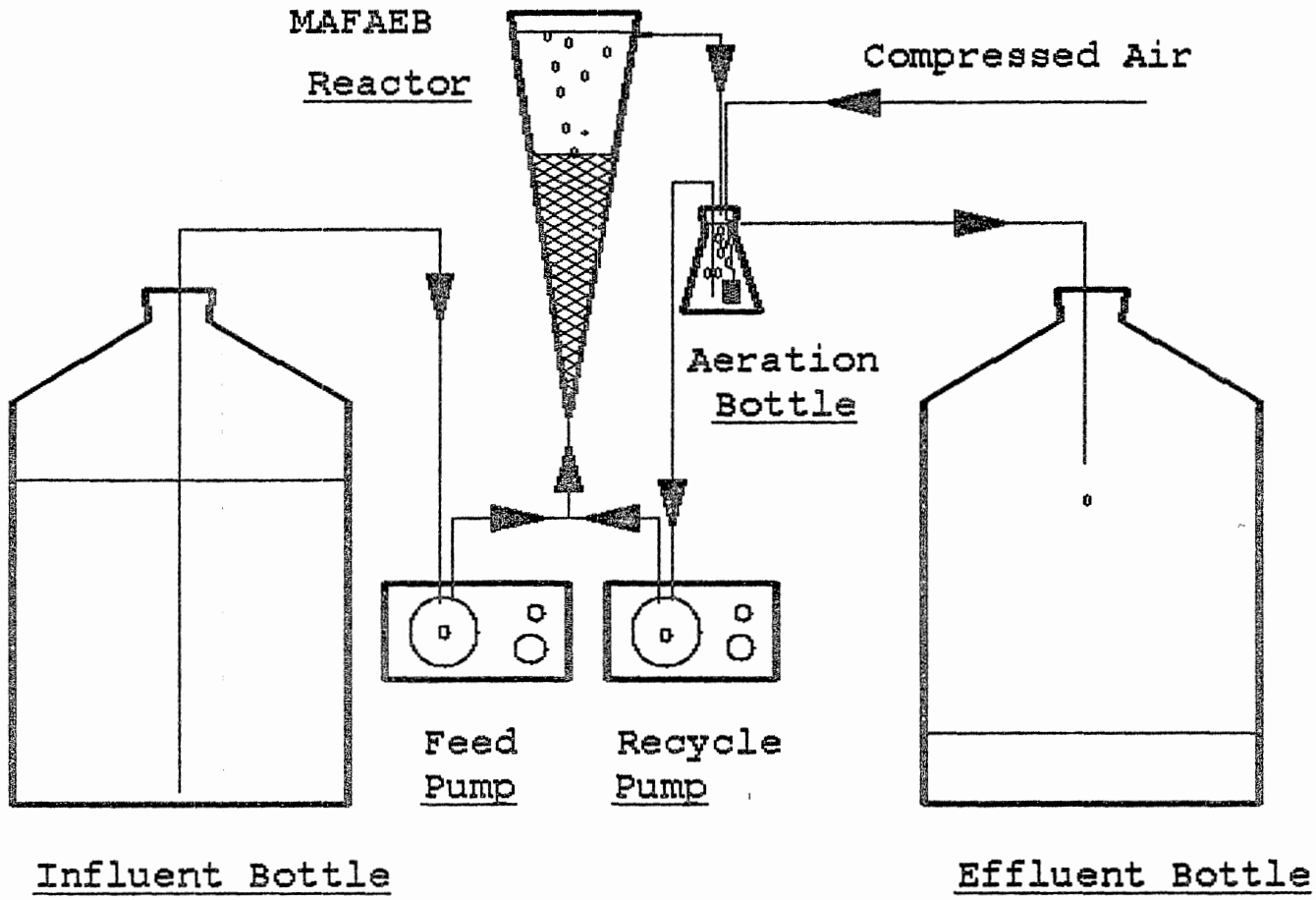


Figure 1. Schematic Diagram of MAFAEB System

volume ratio and have a bulk density of 0.40 g/cm$^3$. The support media bed was expanded by the mixture of the feed and the recycled effluent, and the expansion rate was adjusted through changing the recycle rate. The bed was expanded over a range of approximately 20-100 percent at various times during the study by the recycle flow. The experiment was carried out at room temperature.

## 3.2 Feed Solution

Synthetic substrate was used in this research to simulate industrial or municipal wastewater. The composition of the feed solution for nitrification tests was determined mainly based on the stoichiometric equations described in Chapter II with respect to the biomass growth requirements for trace nutrients. The composition of 250 mg $NH_4^+$-N/L feed solution for example, is given in Table I. All chemicals were dissolved separately in tap water and then mixed well in 25-L or 4-L containers; pH ranged from 7.7-8.0 in all the feed solutions, except denitrification influent, used in this experiment.

Methanol or sodium acetate were added as energy sources for heterotrophic denitrifiers. Sodium thiosulfate was added as energy source for autotrophic denitrifiers. Some ferrous sulfate was also added as trace nutrient. A typical composition of 500 mg $NO_3^-$-N/L feed solution is given in Table II and a typical composition of 500 mg ($NO_3^-$-N + $NO_2^-$-N)/L feed solution in Table III.

TABLE I

COMPOSITION OF 250 mg $NH_4^+$-N/L FEED SOLUTION

| Ingredients | Concentration, g/L |
|---|---|
| Ammonium Sulfate | 1.2 |
| Ammonium Chloride | 1.0 |
| Sodium Bicarbonate | 6.0 |
| Potassium Dihydrogen Phosphate | 0.2 |
| Magnesium Sulfate | 0.2 |

TABLE II

COMPOSITION OF 500 mg $NO_3^-$-N/L FEED SOLUTION

| Ingredients | Concentration |
|---|---|
| Potassium Nitrate | 3.6 g/L |
| Methanol | 1.9 ml/L |
| Potassium Dihydrogen Phosphate | 0.1 g/L |
| Magnesium Sulfate | 0.01 g/L |
| Ferrous Sulfate | 0.002 g/L |

TABLE III

COMPOSITION OF 500 mg $NO_3^-$-N + $NO_2^-$-N/L FEED SOLUTION

| Ingredients | Concentration |
|---|---|
| Potassium Nitrate | 1.8 g/L |
| Sodium Nitrite | 1.2 g/L |
| Methanol | 1.9 ml/L |
| Potassium Dihydrogen Phosphate | 0.1 g/L |
| Magnesium Sulfate | 0.01 g/L |
| Ferrous Sulfate | 0.002 g/L |

The feed solutions for coupling reactions consist of combined nitrification and denitrification feedstock ingredients with ammonium in place of nitrate or nitrite. Typical feed solutions are listed in Tables IV, V and VI.

TABLE IV

COMPOSITION OF COUPLING REACTION FEED SOLUTION (1)

| Ingredients | Concentration |
|---|---|
| Ammonium Chloride | 0.50 g/L |
| Methanol | 0.25 mL/L |
| Sodium Thiosulfate | 1.00 g/L |
| Sodium Bicarbonate | 2.00 g/L |
| Potassium Dihydrogen Phosphate | 0.05 g/l |
| Magnesium Sulfate | 0.05 g/L |
| Ferrous Sulfate | 0.002 g/L |

TABLE V

COMPOSITION OF COUPLING REACTION FEED SOLUTION (2)

| Ingredients | Concentration, g/L |
|---|---|
| Ammonium Sulphate | 0.53 |
| Ammonium Chloride | 0.44 |
| Sodium Acetate | 0.25 |
| Sodium Thiosulfate | 1.13 |
| Sodium Bicarbonate | 1.50 |
| Potassium Dihydrogen Phosphate | 0.05 |
| Magnesium Sulfate | 0.05 |
| Ferrous Sulfate | 0.002 |

TABLE VI

COMPOSITION OF COUPLING REACTION FEED SOLUTION(3)

| Ingredients | Concentration, g/L |
|---|---|
| Ammonium Sulphate | 0.53 |
| Ammonium Chloride | 0.44 |
| Sodium Acetate | 0.75 |
| Sodium Bicarbonate | 1.50 |
| Potassium Dihydrogen Phosphate | 0.05 |
| Magnesium Sulfate | 0.05 |
| Ferrous Sulfate | 0.002 |

## 3.3 Start-up Procedure

The same seed, which was collected from an activated sludge aeration tank of the sewage treatment plant of Ponca City, Oklahoma, was used for acclimation of all the organisms used in this experiment. The seed for autotrophic nitrification was acclimated in a 25-L plastic bottle. The bottle was aerated by a cylindric air distributor with compressed air. The supernatant was drained every day and replenished with 10 L feed solution containing 250 mg $NH_4^+$-N/L. The seed for both autotrophic and heterotrophic denitrification was acclimated in a 25-L plastic barrel. The content in the barrel was mixed with a magnetic stirrer. The barrel was kept covered to exclude oxygen. The supernatant was also drained every day and replenished with 10 L feed solution containing 125 mg $NO_3^-$-N/L and 125 mg $NO_2^-$-N/L.

Prior to placing support medium into the reactor, the inert particles were washed well to eliminate very fine

particles. The reactor was then filled with 150 mL of these particles and expanded to about 20% above its static volume by recycling the supernatant through the bottom of the reactor.

The acclimated denitrifiers were introduced first in the MAFAEB reactor, and ($NO_3^-$-N + $NO_2^-$-N) feed solution was fed continuously with a hydraulic retention time (HRT) of about 6 hours to begin establishing the biofilm. As washout of biomass occurred during the initial start-up period, small amounts of fresh inoculum from the seed bottle were added to replace the loss. Both autotrophic and heterotrophic denitrifiers were successfully attached on the diatomaceous earth particles. The static bed volume grew from 150 mL to 300 mL in 11 weeks.

Then, the aeration bottle was connected into the system and was aerated with compressed air. ($NO_3^-$-N + $NO_2^-$-N) feed solution was replaced with $NH_4^+$-N feed solution and acclimated nitrifiers were inoculated in the same way as denitrifiers. The MAFAEB showed steady nitrification ability within about three weeks. When the coupling reaction feed solution was fed, it was evident that nitrification and denitrification occurred simultaneously.

## 3.4 Analytical Techniques

### 3.4.1 Ammonia Nitrogen

Concentration of ammonia nitrogen was measured according to the methods described in Standard Methods (APHA et

al., 1985), Section 417 C. The distillation method was used, and its validity was checked by distillation with known concentrations of pure reagent.

### 3.4.2 Nitrite and Nitrate

The techniques used for determination of concentrations of nitrite and nitrate were given in Standard Methods (APHA et al., 1985) Section 429. A Dionex ion chromatograph, series 2000i/sp, was used for the measurements. Standard solutions were prepared for each analysis.

### 3.4.3 Chemical Oxygen Demand

Chemical oxygen demand (COD) was measured with Reactor Digestion Method described in HACH WATER ANALYSIS HANDBOOK (HACH Company, 1992).

### 3.4.4 Total Suspended Solids

Total suspended solids were measured according to the methods described in Standard Methods (APHA et al., 1985), Section 209 C. Filtered solids were dried at 103-105°C.

### 3.4.5 Volatile Suspended Solids

The procedures described in Standard Methods (APHA et al., 1985), Section 209 D, were followed for determination of volatile suspended solids. The residue from total suspended solids determination was used for the determination of volatile suspended solids.

### 3.4.6 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 samples. 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 suspended solids procedure described in Section 3.4.3. Blanks consisted of biomass-free diatomaceous earth particles prepared along with those 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 sample was taken. The samples were tamped well before the final volume of the rehydrated sample was recorded.

### 3.4.7 pH

pH values of samples were measured with a model 900 Accumet pH meter (Fisher Scientific Co.). This meter was calibrated with standard solution each time when used on every set of samples.

### 3.4.8 Alkalinity

Alkalinity was measured according to the procedures described in Standard Methods (APHA *et al.*, 1985), Section 403. Sulfuric acid of 0.02*N* was used for titration. The end point of pH 4.3 of titration was determined with a model 900 Accumet pH meter (Fisher Scientific Co.).

### 3.4.9 Dissolved Oxygen

Dissolved Oxygen (DO) was measured with a model 97-08-00 $O_2$ electrode (Orion Research Co.). Procedures described in Standard Methods (APHA *et al.*, 1985), Section 421 C, were followed to check the results measured with the $O_2$ electrode once a week. The difference between the results from these two methods was always smaller than 0.2 mg/L of DO.

## 3.5 Sampling and Implementation Timeline

Zeng (1992) found that nitrification in AEB reactor with HRT at about 0.75 h could reach a new steady state well within two days after operational conditions had been changed, which was identical with what happened in most of the reaction conditions in this study. For this reason, most experimental conditions were maintained for at least two to three days in this experiment.

Influent samples were taken when it was freshly made while effluent samples were taken from the top of the reactor or from the aeration bottle. The pH and DO were measured by inserting probes into the top layer of liquid phase in the reactor. The readings were taken after stirring the liquid phase with the probes until a steady reading was reached. The analyses for the influent and effluent were conducted daily. Usually, the last day's results were reported.

Since there was some instability of the pump feeding rate, the flow rate of influent was measured daily by measuring the influent consumed within 24 hours. The recycle rate was measured weekly by measuring the recycle flow from the reactor to aeration bottle, then subtracting the influent flow.

All analyses were conducted immediately after sampling. No sample storage was involved. Since this experiment is only a feasibility study, the water loss by evaporation and splash were overlooked in this experiment.

The experiment lasted for approximately eight months. The sequence of operations is shown in the implementation timeline (Figure 2).

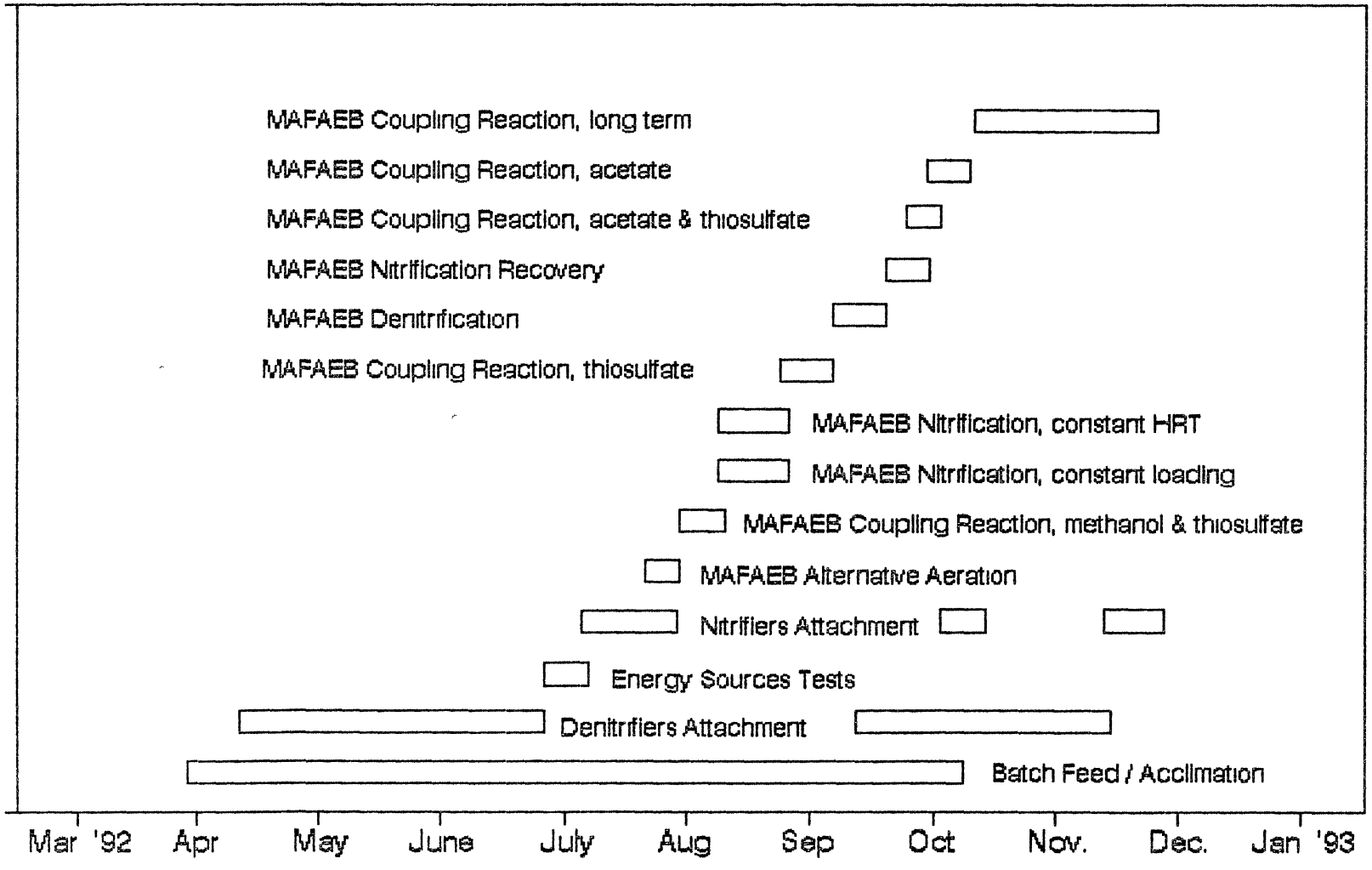


Figure 2. Implementation Timeline for the Sequence of Operations

# CHAPTER IV

# EXPERIMENTAL APPROACHES AND RESULTS

## 4.1 Development of Autotrophic and Heterotrophic Denitrifiers in Mixed Attached Films

Since both methanol and thiosulfate inhibit nitrification (Beccari, 1980; Hooper *et al.*, 1973), no accessory energy sources (electron donors) more than that required by denitrifiers during the coupling reaction should be added. Unit nitrate or nitrite conversion rates with methanol and sodium thiosulfate as energy sources were tested under electron-donor-limitation in the presence of excess electron acceptors (nitrate or nitrite). Methanol and sodium thiosulfate were added in varying amounts to account for any possible interference between autotrophic and heterotrophic denitrification activities. The test conditions and results are listed in Table VII.

According to the data in Table VII, the unit energy source conversion rates may be obtained by solving the following equation groups:

Nitrate as electron donor:

$$\begin{cases} 3.0\ C_{Thio\ 1} + 1.5\ C_{Methanol\ 1} = 8 \times 86, \\ 1.5\ C_{Thio\ 1} + 3.0\ C_{Methanol\ 1} = 8 \times 152; \end{cases}$$

and nitrite as electron donor:

$$\begin{cases} 3.0\ C_{Thio} + 1.5\ C_{Methanol} = 8 \times 152, \\ 1.5\ C_{Thio} + 3.0\ C_{Methanol} = 8 \times 213; \end{cases}$$

where,

$C_{Thio}$: Conversion rate of $NO_x^-$ vs. thiosulfate;

$C_{Methanol}$: Conversion rate of $NO_x^-$ vs. methanol.

The results are:

nitrate as electron acceptor,

35.6 mg $NO_x^-$-N/g $Na_2S_2O_3$ and 388 mg $NO_x^-$-N/mL $CH_4O$;

nitrite as electron acceptor,

163 mg $NO_x^-$-N/g $Na_2S_2O_3$ and 487 mg $NO_x^-$-N/mL $CH_4O$.

TABLE VII

TEST CONDITIONS AND RESULTS OF ENERGY SOURCES FOR MIXED DENITRIFICATION

| Electron Acceptor | Nitrite | | Nitrate | |
|---|---|---|---|---|
| Electron Donor | $Na_2S_2O_3$: 3.0 g/8 L | | Methanol: 1.5 mL/8 L | |
| Loading Rate | 7.68 g/L-D* | | 8.93 g/L-D* | |
| | Influent | Effluent | Influent | Effluent |
| $NO_3^-$-N, mg/L | 1.3 | 0 | 268 | 182 |
| $NO_2^-$-N, mg/L | 229 | 77.9 | 0 | 0 |
| N-Removed, mg/L | 152 | | 86 | |
| Electron Donor | $Na_2S_2O_3$: 1.5 g/8 L | | Methanol: 3.0 mL/8 L | |
| | Influent | Effluent | Influent | Effluent |
| $NO_3^-$-N, mg/L | 1.3 | 0 | 268 | 116 |
| $NO_2^-$-N, mg/L | 229 | 17.4 | 0 | 0 |
| N-Removed, mg/L | 213 | | 152 | |

* g/L-D: g/per liter static volume per day.

It was also found that part of the alkalinity produced

by heterotrophic denitrification was consumed in the autotrophic denitrification. Total alkalinity produced from the simultaneous growth was much less than when only heterotrophic denitrifiers were grown.

## 4.2 Nitrification with MAFAEB System

After attached biofilms had shown steady state nitrification ability, nitrification was carried out in two MAFAEB reactors. The nitrification ability of the mixed attached films was tested first under a constant loading rate over a range of different hydraulic retention times (HRT) then under constant HRT with different loading rate conditions. The results are presented in Tables VIII and IX, and Figures 3 and 4. All the nitrogen forms in the figures have been converted to nitrogen bases.

TABLE VIII

NITRIFICATION WITH MAFAEB SYSTEM - CONSTANT LOADING RATE

| Items | | HRT, Hours 0.25 | 0.38 | 0.50 | 0.75 | 1.50 | 3.0 |
|---|---|---|---|---|---|---|---|
| Influent Ammonium, | mg/L | 42 | 63.9 | 85.2 | 128 | 251 | 500 |
| Effluent Ammonium, | mg/L | 7.3 | 10.8 | 10.2 | 14.8 | 21.2 | 29.7 |
| Effluent Nitrate, | mg/L | 17.6 | 11.7 | 22.2 | 25.0 | 46.2 | 112 |
| Effluent Nitrite, | mg/L | 14.5 | 31.7 | 44.5 | 74.0 | 157 | 287 |
| Influent DO, | mg/L | 5.9 | 6.2 | 5.8 | 5.7 | 5.7 | 5.5 |
| Effluent DO, | mg/L | 3.2 | 3.2 | 3.2 | 2.8 | 2.6 | 2.2 |
| influent pH | | 7.8 | 7.8 | 7.8 | 7.85 | 7.85 | 7.85 |
| Effluent pH | | 7.4 | 7.5 | 7.4 | 7.4 | 7.5 | 7.5 |
| Loading Rate, | g/L-D* | 4.12 | 4.17 | 4.09 | 4.1 | 4.27 | 4.00 |
| Nitrification, | % | 82.6 | 83.1 | 88.0 | 88.4 | 91.6 | 94.1 |

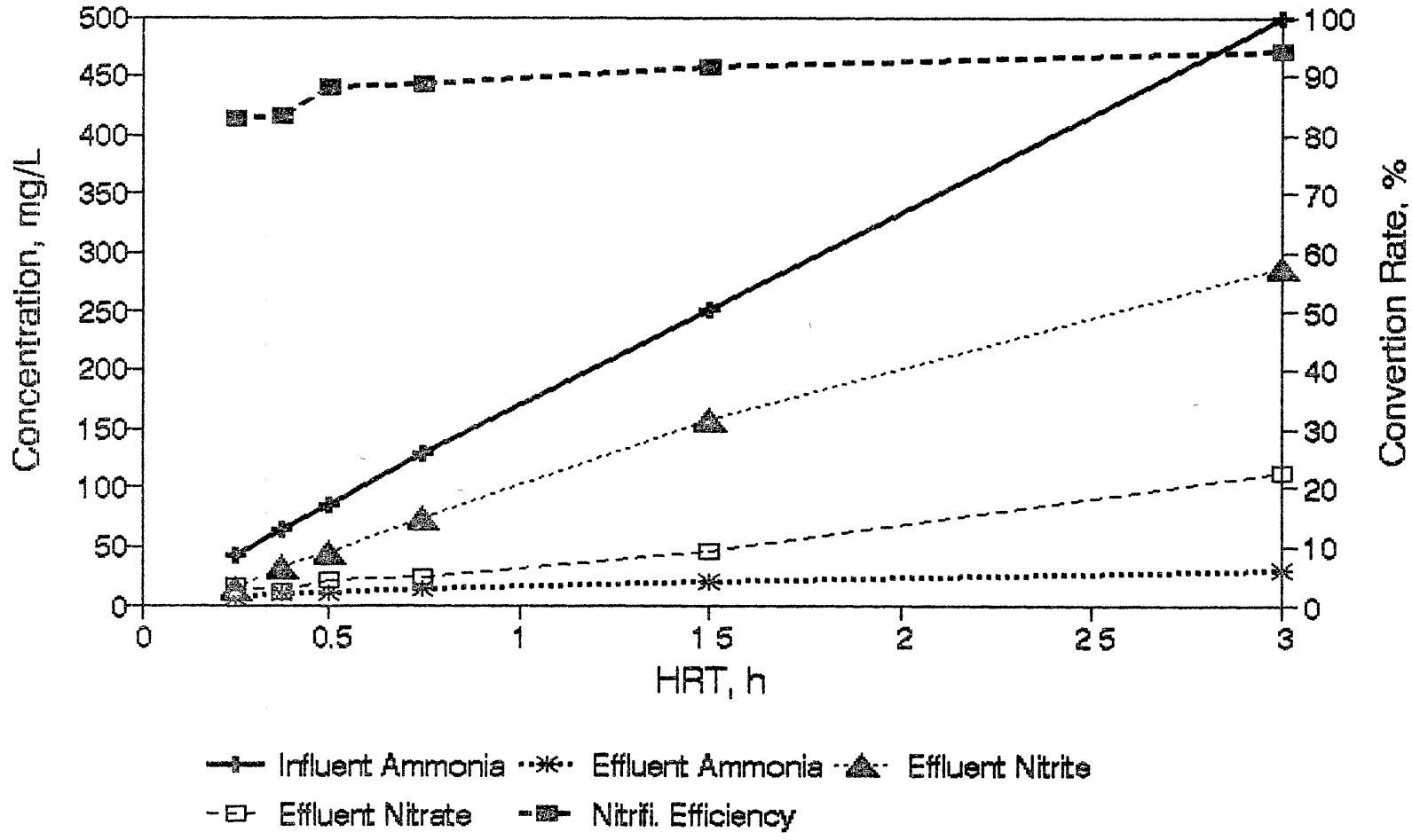


Figure 3 NITRIFICATION WITH MAFAEB SYSTEM

(Constant loading rate, varying HRT)

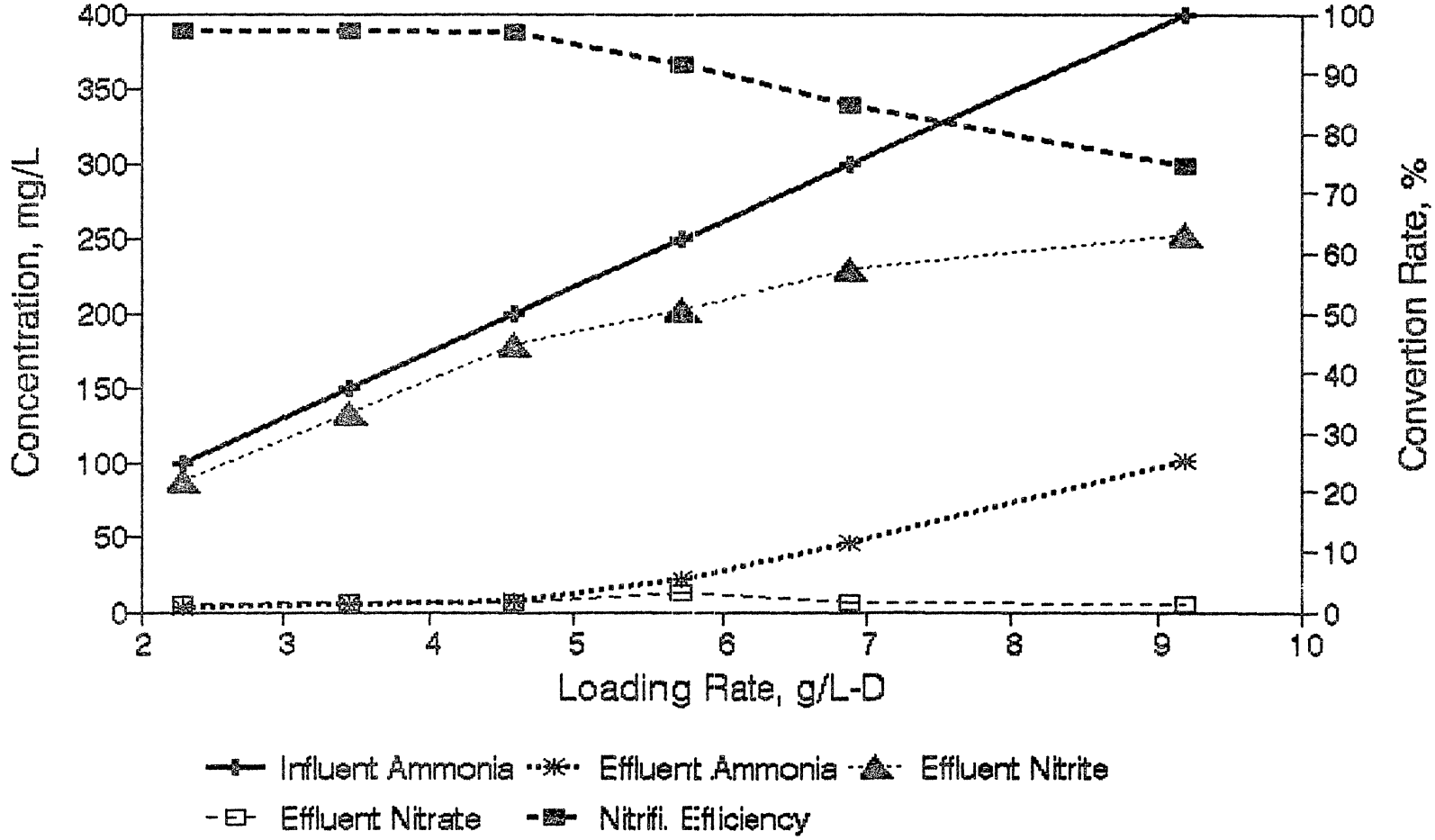


Figure 4 NITRIFICATION WITH MAFAEB SYSTEM
(Constant HRT, varying loading rate)

TABLE IX

NITRIFICATION WITH MAFAEB SYSTEM - CONSTANT HRT

| Items | Loading Rate, g/L-D 2.29 | 3.44 | 4.59 | 5.73 | 6.88 | 9.17 |
|---|---|---|---|---|---|---|
| Influent Ammonium, mg/L | 100 | 150 | 200 | 250 | 300 | 400 |
| Effluent Ammonium, mg/L | 2.8 | 4.4 | 6.5 | 21.3 | 45.8 | 102 |
| Effluent Nitrate, mg/L | 4.9 | 5.1 | 5.3 | 12.4 | 5.3 | 5.0 |
| Effluent Nitrite, mg/L | 87.0 | 133 | 178 | 203 | 229 | 253 |
| Influent DO, mg/L | 7.7 | 7.5 | 6.7 | 6.4 | 6.0 | 5.4 |
| Effluent DO, mg/L | 4.6 | 3.2 | 2.7 | 1.7 | 1.4 | 0.7 |
| influent pH | 7.85 | 7.85 | 7.85 | 7.85 | 7.95 | 7.95 |
| Effluent pH | 7.5 | 7.5 | 7.4 | 7.5 | 7.8 | 7.85 |
| Nitrification, % | 97.2 | 97.0 | 96.8 | 91.5 | 84.7 | 74.5 |

## 4.3 Denitrification with Mixed Attached Films

After more than one month of nitrification tests, one of the MAFAEB reactors was turned to anoxic conditions. Within hours, denitrification activity was noted in the MAFAEB without aeration. Denitrification was tested at constant HRT with different loading rates when steady state had been reached in the MAFAEB reactor. No obvious decreases in denitrification efficiency occurred at loadings up to about 14 g/L-D.

Sodium acetate was used as electron donor in this test. It was found that no adaptation time was needed for sodium acetate to replace methanol and/or sodium thiosulfate as electron donor, and the system showed tremendous potential

for denitrification.

The results of denitrification in the MAFAEB system are shown in Table X and Figure 5.

TABLE X

DENITRIFICATION WITH MAFAEB SYSTEM - CONSTANT HRT

| Items | | Loading Rate, g/L-D 2.87 | 42.8 | 5.65 | 6.89 | 8.93 | 14.1 |
|---|---|---|---|---|---|---|---|
| Influent Nitrate, | mg/L | 101 | 157 | 215 | 262 | 346 | 544 |
| Effluent Nitrate, | mg/L | 0 | 0.4 | 0.8 | 0.9 | 0.6 | 2.2 |
| Effluent Nitrite, | mg/L | 0 | 0 | 0 | 0 | 0 | 0 |
| HRT, | Hours | 0.85 | 0.88 | 0.91 | 0.91 | 0.93 | 0.93 |
| Effluent pH | | 7.85 | 7.8 | 7.6 | 7.6 | 7.6 | 7.6 |
| Denitrification, | % | 100 | 99.8 | 99.6 | 99.6 | 99.8 | 99.6 |

## 4.4 Coupling Reaction with MAFAEB System

### 4.4.1 Coupling Reaction with Methanol and Sodium Thiosulfate as Electron Donors

After an attached biofilm had been well established in the MAFAEB reactor, coupling reaction feed solution (1) was fed. The aeration rate was controlled that the DO in the reactor was close to 2 mg/L since it was much more difficult to control the DO to below 2 mg/L. The recycle ratio was set at 200 - 400% to supply obligatory oxygen for the nitrification. Some air bubbles were also introduced into the reactor directly through the recycling tubing to supply additional oxygen and mild agitation.

During the first two days, loading rate was kept at

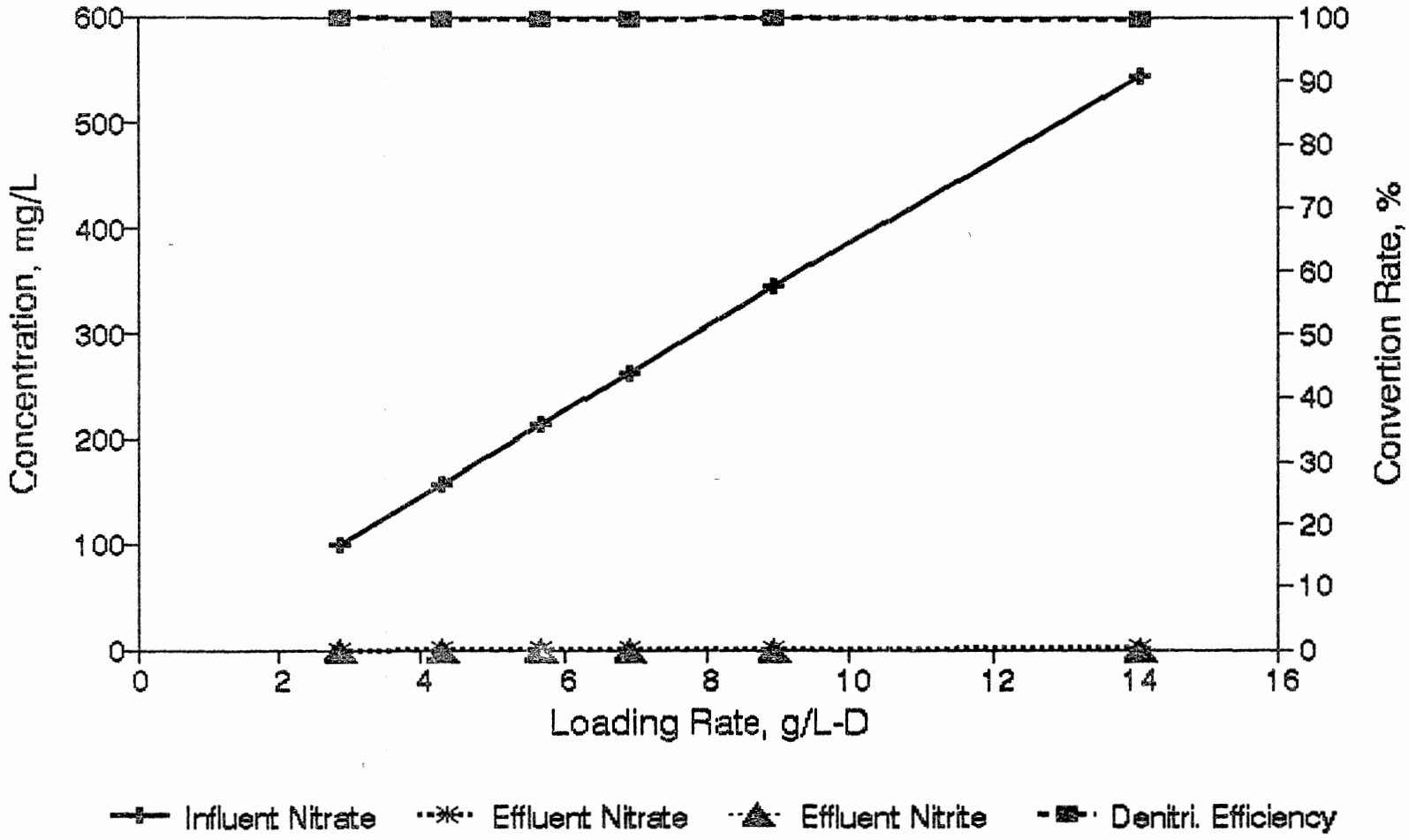


Figure 5. DENITRIFICATION WITH MAFAEB SYSTEM

(Constant HRT, without Aeration)

lower than 1 g/L-D. Loading rate was increased to 1.84 g/L-D on the third day. On the fourth day, the nitrogen removal rate reached 74.4%, and no nitrite or nitrate accumulated in the effluent, which means the nitrogen removal rate was equal to the nitrification rate. On the fifth day, nitrogen removal rate was sustained at 74.0%, however, there were 21.2 mg/L $NO_2^-$-N and 1.0 mg/L $NO_3^-$-N remaining in the effluent. The nitrification rate was as high as 91.2%. The possibility of coupling nitrification and denitrification in a single reactor was clearly proved.

However, between the fifth and eighth days, the nitrogen removal rate dropped to 42.6% and the nitrification rate dropped to 69.2%. Tremendous flocs formed in the MAFAEB system. Both nitrification and denitrification were inhibited at the same time. Since all the electron donors added for denitrification were consumed while a significant amount of nitrite and nitrate remained in the solution, this may suggest that part of the energy sources added must have been biodegraded through another pathway. The first eight days results of this experiment are presented in Table XI.

### 4.4.2 Alternative Aerobic and Anaerobic Coupling Reaction

Since nitrification and denitrification require totally different conditions, alternating aerobic and anaerobic reactor operation was conducted to test the influence on the coupling reaction. Considering that there will be a certain

amount of oxygen to be consumed after aeration is stopped, the non-aeration time should be longer than the aeration time. At first, aeration time was set for 5 min and non-aeration for 15 min. The nitrification rate was 71.7% and the nitrogen removal rate was 46.8% over a period of 8 hours. The denitrification rate lagged behind the nitrification rate. In the second test, aeration time was set for 20 min and non-aeration time for 40 min. The comparison between the alternating aeration (sampling immediately after stopping alternating test) and low DO aeration pattern (sampling under steady state condition just before alternating aeration test) is shown in Table XII and Figure 6.

TABLE XI

COUPLING REACTION WITH METHANOL AND THIOSULFATE AS ELECTRON DONORS

| Day | Influent | Effluent | | | Nitrification | Nitrogen Removal |
|---|---|---|---|---|---|---|
| | Ammonium mg/L | Ammonium mg/L | Nitrate mg/L | Nitrite mg/L | % | % |
| 1 | 56.0 | | | | | |
| 2 | 66.6 | | | | | |
| 3* | 127.5 | 10 | 0 | 2.4 | 92.2 | 90.3 |
| 4 | 129 | 33 | 0 | 0 | 74.4 | 74.4 |
| 5 | 129 | 11.3 | 21.2 | 1.0 | 91.2 | 74.0 |
| 6 | 123 | 9.3 | 25.7 | 13.6 | 92.4 | 60.5 |
| 7* | 240 | 105 | 19.3 | 1.6 | 56.3 | 47.5 |
| 8* | 129 | 39.7 | 6.3 | 28.1 | 69.2 | 42.6 |

* Unbalanced results because of altering influent concentration.

TABLE XII

COMPARISON OF ALTERNATING AERATION WITH

LOW DO COUPLING REACTION PATTERN

| Items | Alternative Aeration | Low DO |
|---|---|---|
| Influent Ammonium, mg/L | 129 | 129 |
| Effluent Ammonium, mg/L | 66.2 | 58.7 |
| Effluent Nitrate, mg/L | 0.4 | 0.6 |
| Effluent Nitrite, mg/L | 18.2 | 17.8 |
| Nitrification, % | 48.7 | 54.5 |
| Nitrogen Removal, % | 34.3 | 42.3 |

4.4.3 Coupling Reaction with Acetate and Thiosulfate as Electron Donors

After conducting the coupling reaction described above, the floc problem was so serious that it was necessary to remove flocculant biomass before undertaking any more tests. The system was fed with dilute $NH_4^+$-N feed solution at very low HRT to wash out the flocs and resume biofilm nitrification ability.

It was apparent that the flocs formed in the MAFAEB system have the ability to oxidize methanol and thiosulfate in low DO conditions. According to Kohno (1988), a filamentous organism known to cause sewage sludge bulking utilized thiosulfate as an energy source but failed to oxidize the compound when acetic acid was available. So a small amount of acetate was added along with thiosulfate

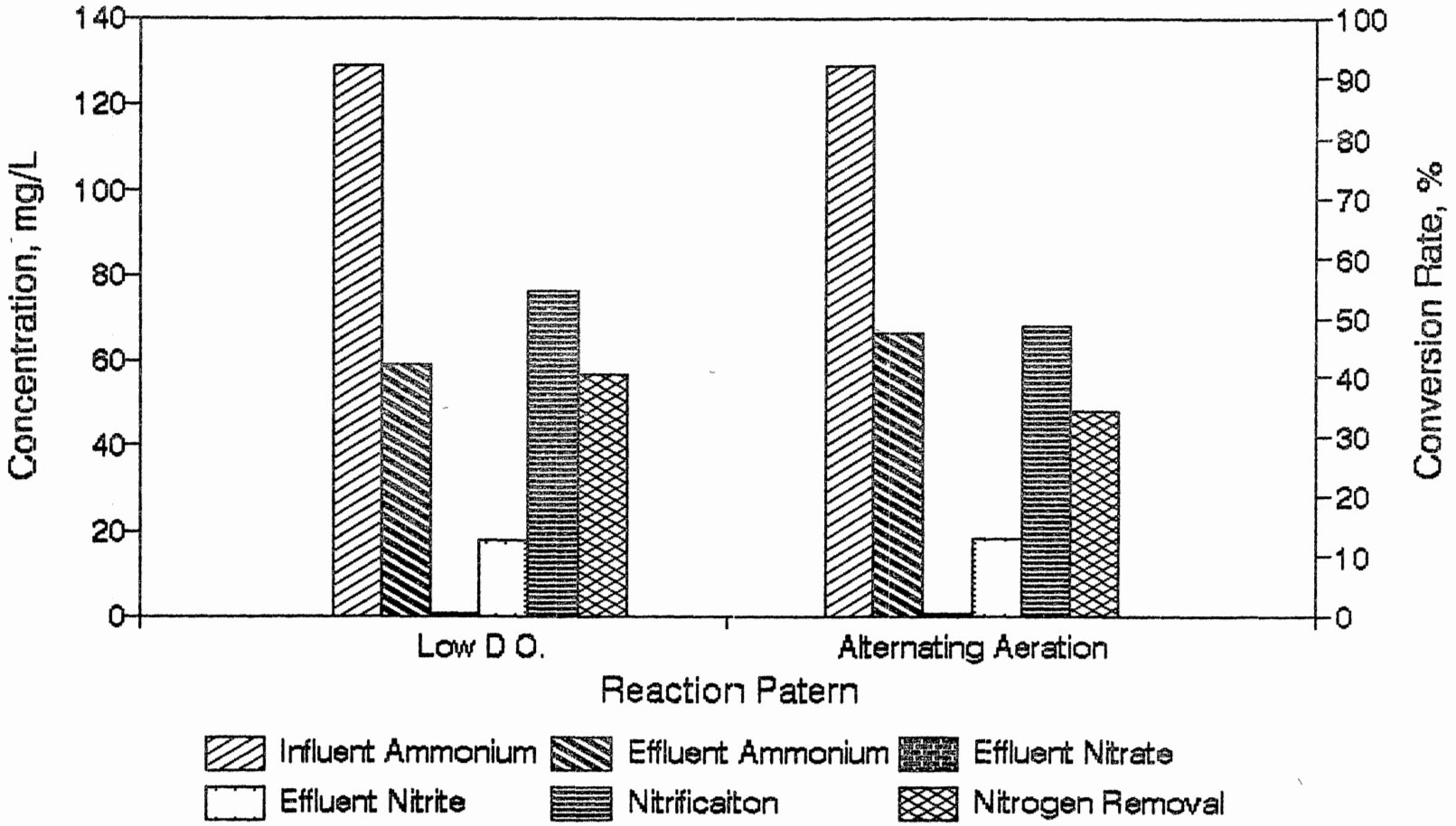


Figure 6. COMPARISON OF REACTION PATTERNS
(low DO and alternating aeration)

(Table VI) and used as electron donors in the next series of coupling reaction experiments. The results are listed in Table XIII, and Figures 7 and 8.

TABLE XIII

COUPLING REACTION WITH THIOSULFATE

AND ACETATE AS ELECTRON DONORS

| Item | Day 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Influent Ammonium, mg/L | 212 | 212 | 219 | 219 | 219 | 234 | 234 |
| Effluent Ammonium, mg/L | 14.4 | 22.2 | 31.5 | 57.3 | 63.8 | 70.5 | 80.8 |
| Effluent Nitrate, mg/L | 0 | 0.6 | 0.9 | 1.2 | 1.7 | 1.4 | 1.5 |
| Effluent Nitrite, mg/L | 82.8 | 80.9 | 83.7 | 63.8 | 72.0 | 66.8 | 65.2 |
| Nitrification, % | 93.2 | 89.5 | 85.1 | 73.8 | 70.9 | 69.9 | 65.5 |
| Nitrogen Removal, % | 54.2 | 51.1 | 45.2 | 44.2 | 37.2 | 40.7 | 37.0 |
| Denitr. of Available $NO_2^-$-N & $NO_3^-$-N, % | 58.1 | 57.1 | 53.1 | 59.8 | 52.5 | 58.3 | 56.5 |
| Loading Rate, g/L-D | 3.08 | 3.08 | 2.97 | 3.75 | 3.56 | 3.44 | 3.44 |
| N-Removal Rate, g/L-D | 1.67 | 1.57 | 1.32 | 1.67 | 1.33 | 1.40 | 1.27 |

When thiosulfate was removed from the feed solution after the above test, nitrification efficiency recovered, and nitrogen removal rate dropped. After reaching a new steady state level, a comparison between coupling reactions with or without adding thiosulfate can be seen, as shown in Figure 9. Figure 9 shows that nitrification efficiency was somewhat greater in the absence of thiosulfate. At the same time, thiosulfate also was utilized as electron donor in the denitrification process of the coupling reaction.

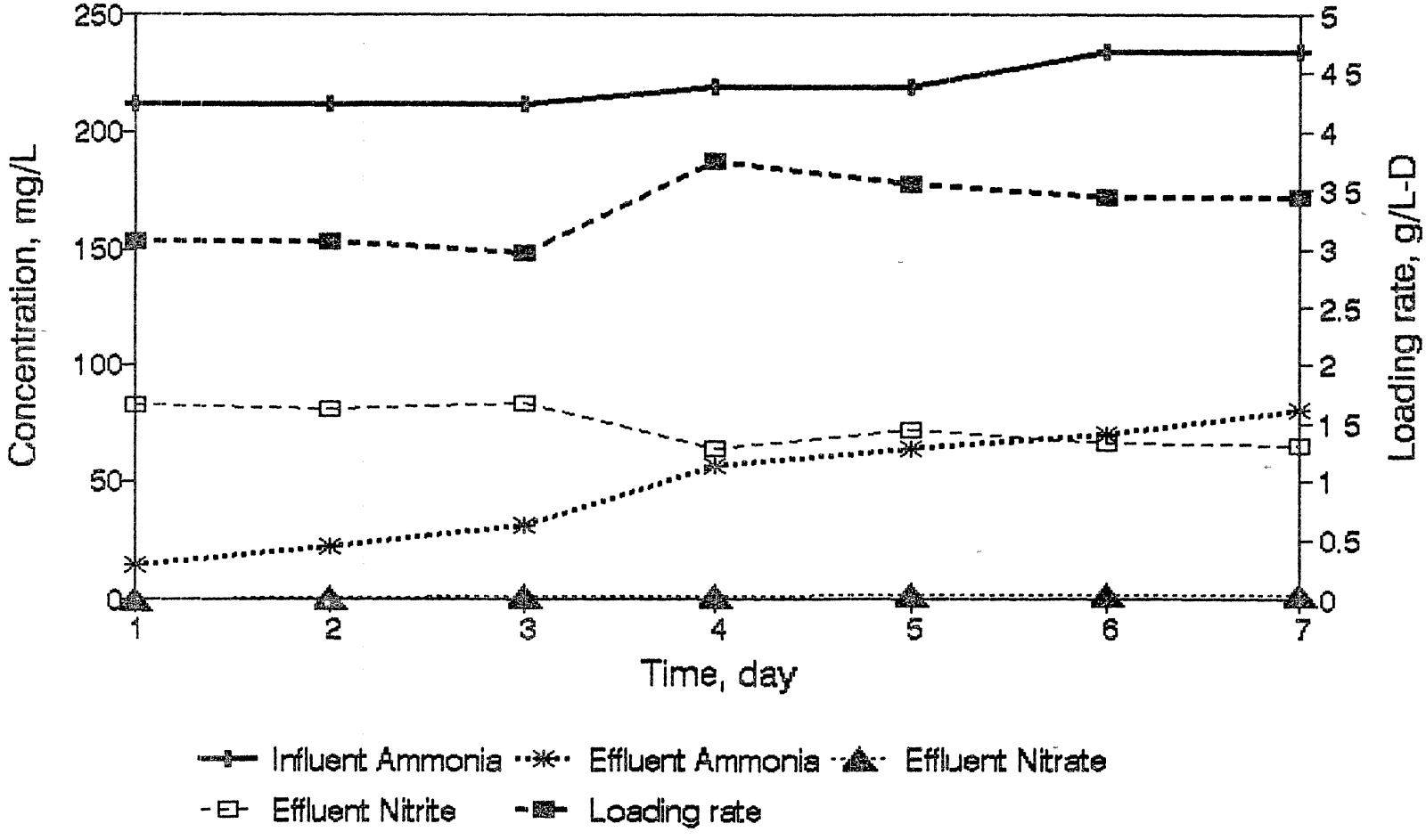


Figure 7 COUPLING REACTION WITH MAFAEB SYSTEM

(thiosulfate and acetate as electron donors [1])

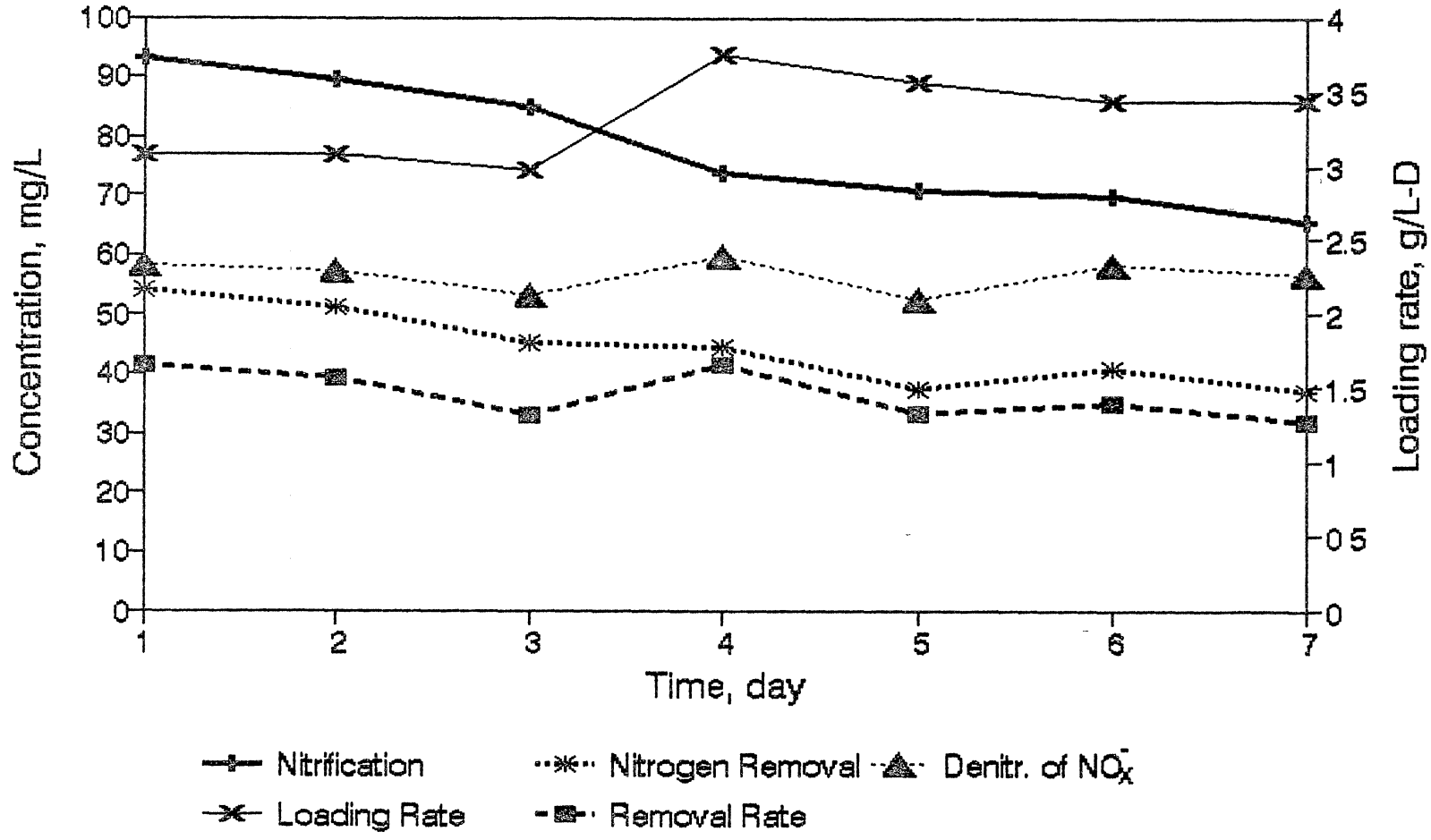


Figure 8 COUPLING REACTION WITH MAFAEB SYSTEM

(thiosulfate & acetate as electron donors [2])

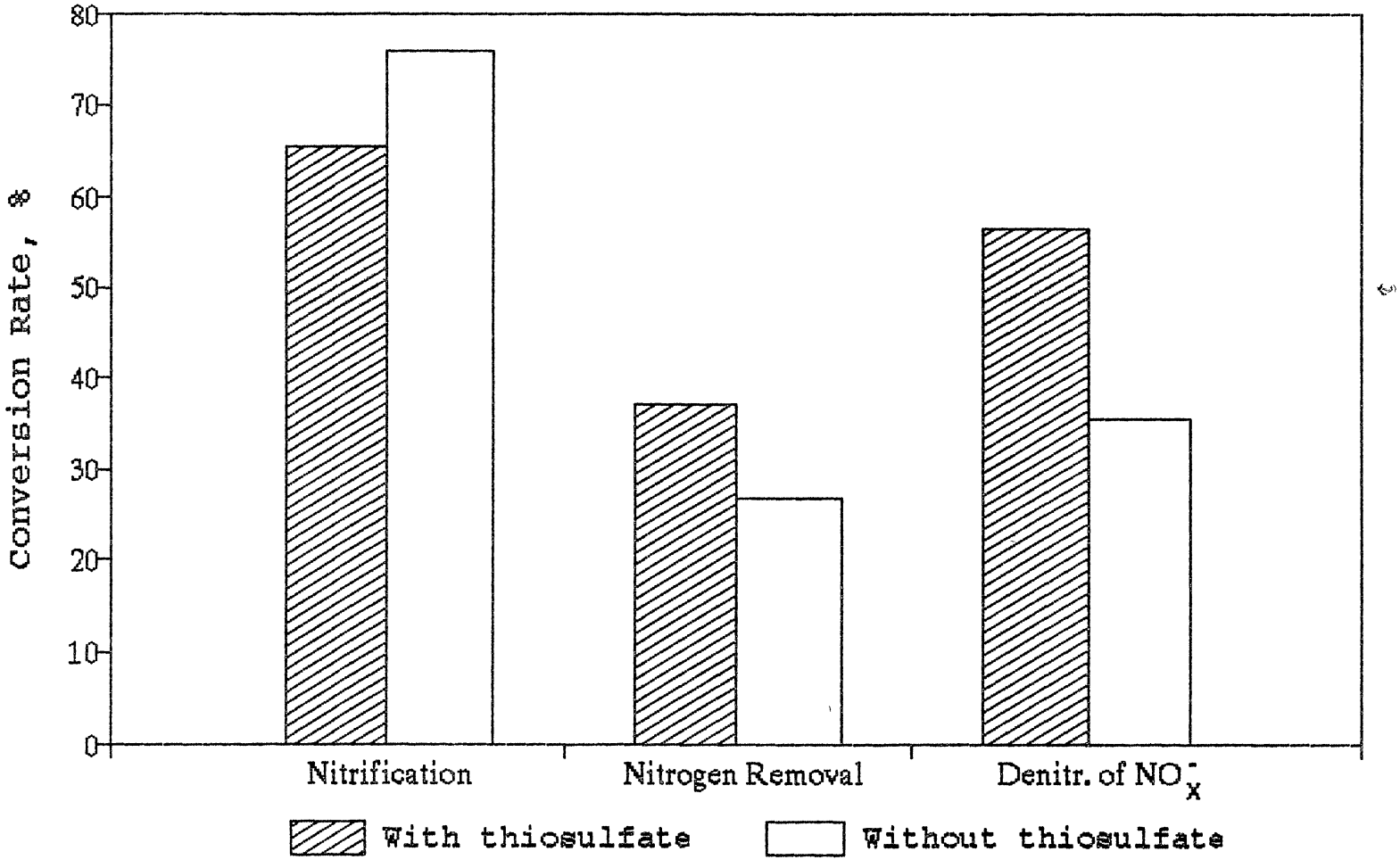


Figure 9. COMPARISON OF COUPLING REACTIONS
(with or without adding sodium thiosulfate)

### 4.4.4 Coupling Reaction with Thiosulfate as Electron Donor

Subsequent experiments were performed using only sodium thiosulfate as electron donor for the coupling reaction. The thiosulfate addition was from 273 - 1275 mg/L. The results are shown in Table XIV, and Figures 10 and 11.

TABLE XIV

COUPLING REACTION WITH SODIUM THIOSULFATE AS ELECTRON DONOR

| Thiosulfate, mg/L | 273 | 563 | 850 | 1275 |
|---|---|---|---|---|
| Influent Ammonium, mg/L | 50 | 100 | 150 | 151 |
| Effluent Ammonium, mg/L | 9.5 | 39.4 | 48.3 | 72.5 |
| Effluent Nitrate, mg/L | 10.9 | 15.6 | 23.2 | 14.8 |
| Effluent Nitrite, mg/L | 17.7 | 22.6 | 48.4 | 31.7 |
| Nitrification, % | 81.0 | 60.6 | 67.8 | 51.7 |
| Nitrogen Removal, % | 23.8 | 22.4 | 20.1 | 20.7 |
| Denitr. of Available $NO_2^-$-N & $NO_3^-$-N, % | 29.4 | 37.0 | 29.6 | 40.0 |
| Loading Rate, g/L-D | 2.02 | 2.84 | 4.23 | 4.23 |
| N-Removal Rate, g/L-D | 0.48 | 0.64 | 0.85 | 0.87 |

### 4.4.5 Coupling Reaction with Acetate as Electron Donor

In the next experimental series, the effect of using only sodium acetate as electron donor for the coupling reaction was tested. The acetate addition was from 375 mg/L

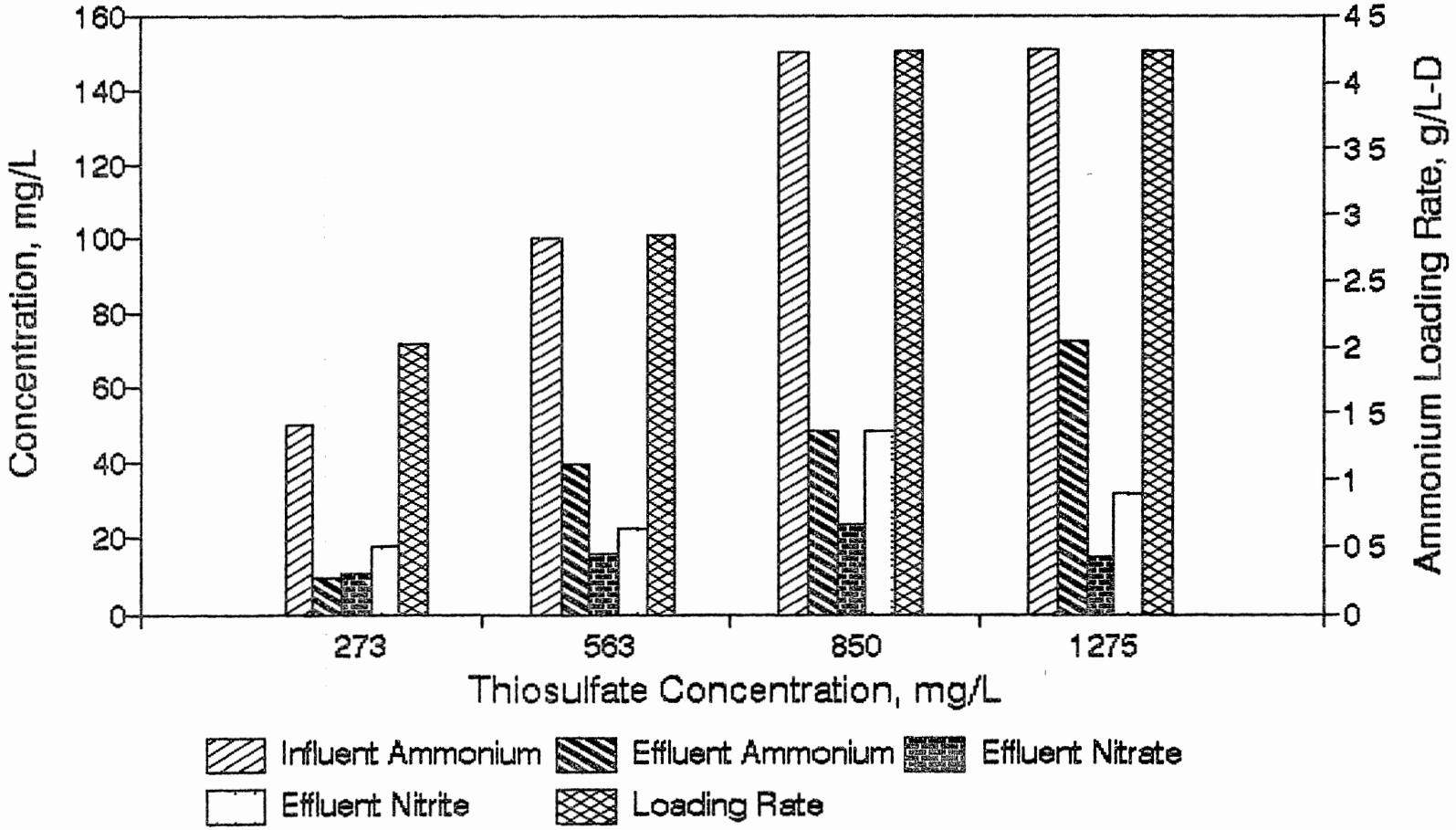


Figure 10. COUPLING REACTION WITH MAFAEB SYSTEM

(thiosulfate as electron donor, [1])

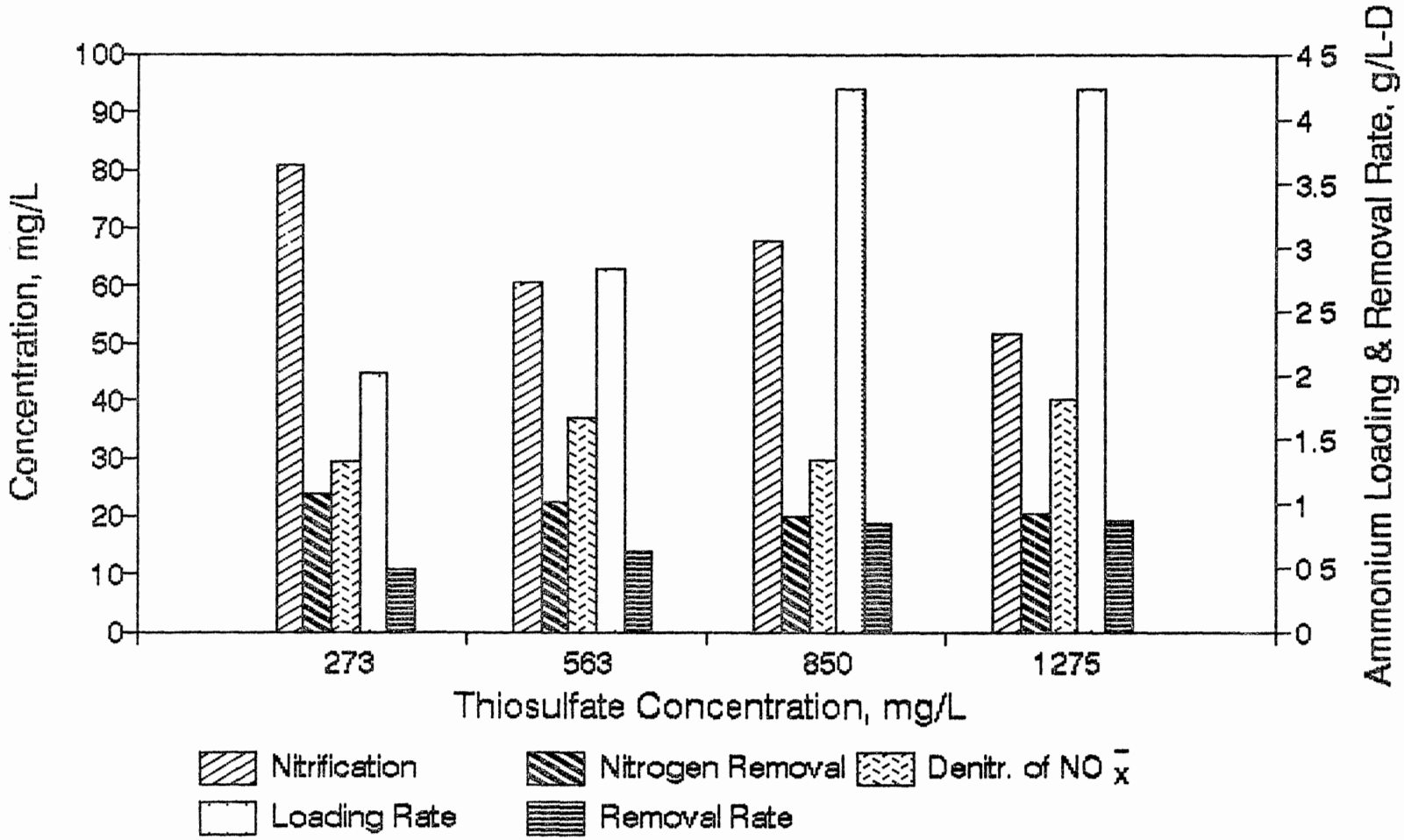


Figure 11 COUPLING REACTION WITH MAFAEB

(thiosulfate as electron donor [2])

to 1875 mg/L. The results are found in Table XV, and Figures 12 and 13.

TABLE XV

COUPLING REACTION WITH SODIUM ACETATE AS ELECTRON DONOR

| Acetate, mg/L | 375 | 750 | 1125 | 1500 | 1875 |
|---|---|---|---|---|---|
| Influent Ammonium, mg/L | 221 | 226 | 222 | 222 | 222 |
| Effluent Ammonium, mg/L | 27.1 | 36.6 | 59.2 | 117 | 136 |
| Effluent Nitrate, mg/L | 4.3 | 2.1 | 2.8 | 0.7 | 0.3 |
| Effluent Nitrite, mg/L | 139 | 93.2 | 91.4 | 47.9 | 12.3 |
| Nitrification, % | 87.7 | 83.8 | 73.3 | 47.3 | 38.7 |
| Nitrogen Removal, % | 22.9 | 41.6 | 30.9 | 25.4 | 33.1 |
| Denitr. of Available $NO_2^-$-N & $NO_3^-$-N, % | 26.1 | 49.7 | 42.1 | 53.7 | 85.3 |
| Loading Rate, g/L-D | 3.16 | 3.23 | 3.18 | 3.18 | 3.18 |
| N-Removal Rate, g/L-D | 0.73 | 1.35 | 0.98 | 0.81 | 1.05 |

Comparing Tables XIV and XV, acetate is a more efficient electron donor for the denitrification in the coupling reaction. Figure 13 and Table XV show that there is a maximum nitrogen removal at sodium acetate concentration of 750 mg/L without seriously decreasing the nitrification rate, so this condition was selected to run a long term test, which lasted for 40 days. The results of this 40-day MAFAEB trial are given in Table XVI, and Figures 14 and 15.

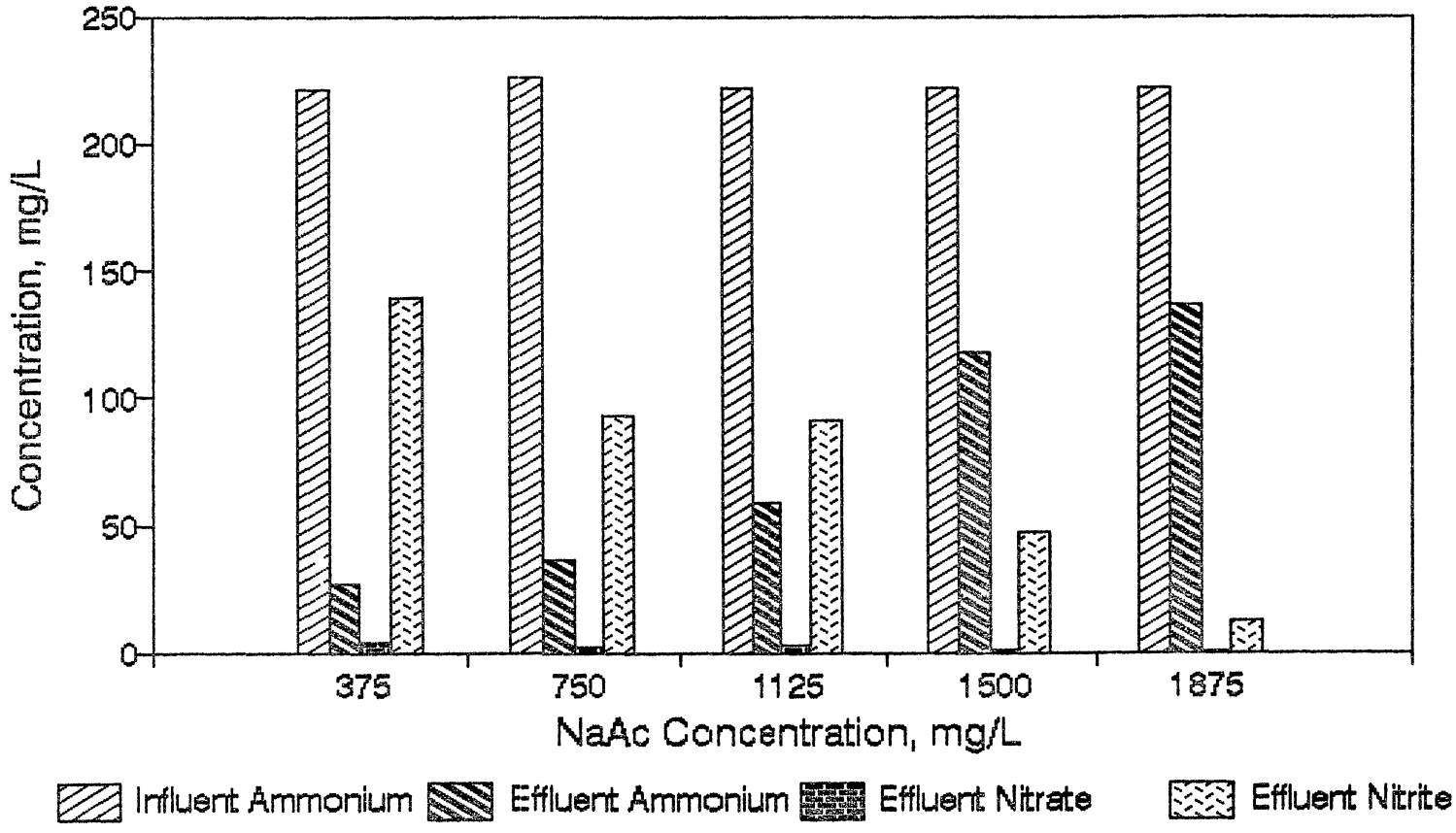


Figure 12 COUPLING REACTION WITH MAFAEB

(sodium acetate as electron donor, [1])

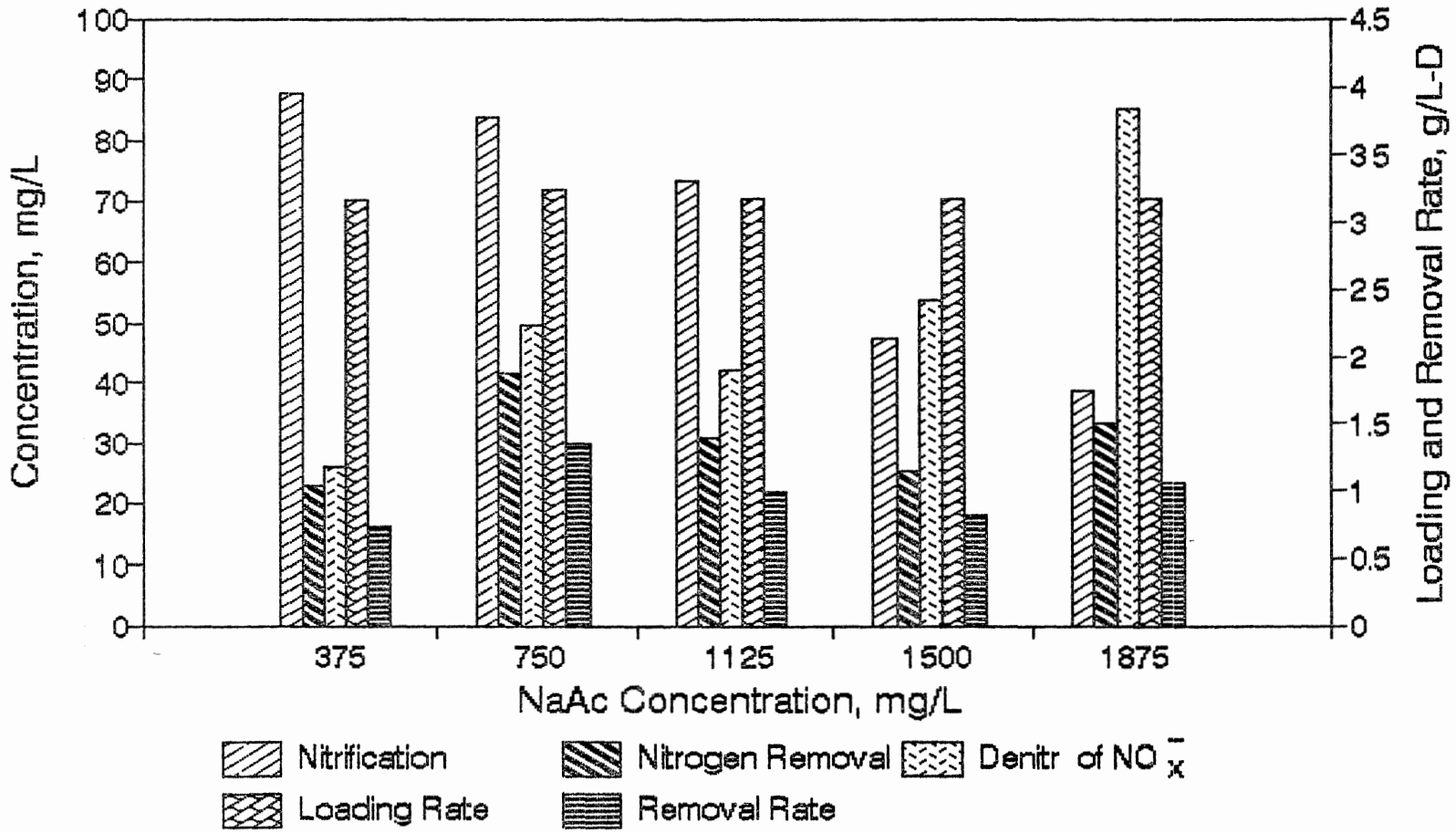


Figure 13. COUPLING REACTION WITH MAFAEB

(sodium acetate as electron donor [2])

TABLE XVI

MAFAEB COUPLING REACTION GENERAL CONDITIONS AND RESULTS

(VALUES CALCULATED FROM FOURTH DAY

THROUGH THE END OF THE TEST)

| Item | | Data |
|---|---|---|
| Static Bed Volume, | mL | 250-255 |
| Bed Expansion Rate, | % | 100 |
| Recycle Rate, | L/D | 1350 |
| Substrate Flow Rate, | L/D | 3.43 ± 0.05 |
| HRT, | Hrs | 1.77 ± 0.03 |
| Influent $NH_4^+$-N, | mg/L | 222.0 ± 0.8 |
| Effluent $NH_4^+$-N, | mg/L | 61.5 ± 6.5 |
| Influent $NO_3^-$-N, | mg/L | undetectable |
| Effluent $NO_3^-$-N, | mg/L | 6.9 ± 1.3 |
| Influent $NO_2^-$-N, | mg/L | undetectable |
| Effluent $NO_2^-$-N, | mg/L | 83.3 ± 6.4 |
| Nitrification Efficiency, | % | 72.3 ± 3.0 |
| Nitrogen Removal Efficiency, | % | 31.7 ± 1.8 |
| Denitrification Efficiency of Available $NO_2^-$-N & $NO_3^-$-N, | % | 44.2 ± 2.7 |
| Loading Rate, | g $NH_4^+$-N/L-D | 3.00 ± 0.04 |
| Removal Rate, | g $NH_4^+$-N/L-D | 0.95 ± 0.06 |
| Influent pH, | | 7.75 |
| Effluent pH, | | 7.3 |
| Influent DO, | mg/L | no control |
| Effluent DO, | mg/L | 2.06 ± 0.19 |
| Influent COD, | mg/L | 390 |
| Effluent COD, | mg/L | 155 * ** |
| Influent Alkalinity, mg as $CaCO_3$/L | | 1164 |
| Effluent Alkalinity, mg as $CaCO_3$/L | | 252 * |
| Effluent TSS, | mg/L | 61 * |
| Effluent VSS, | mg/L | 51 * |
| Attached VS, g VS/L static bed | | 27.8 |
| Entrapped VS, g VS/L static bed | | 4.21 |

* Five days accumulated sample.

** Include Nitrite COD 89.7 mg/L.

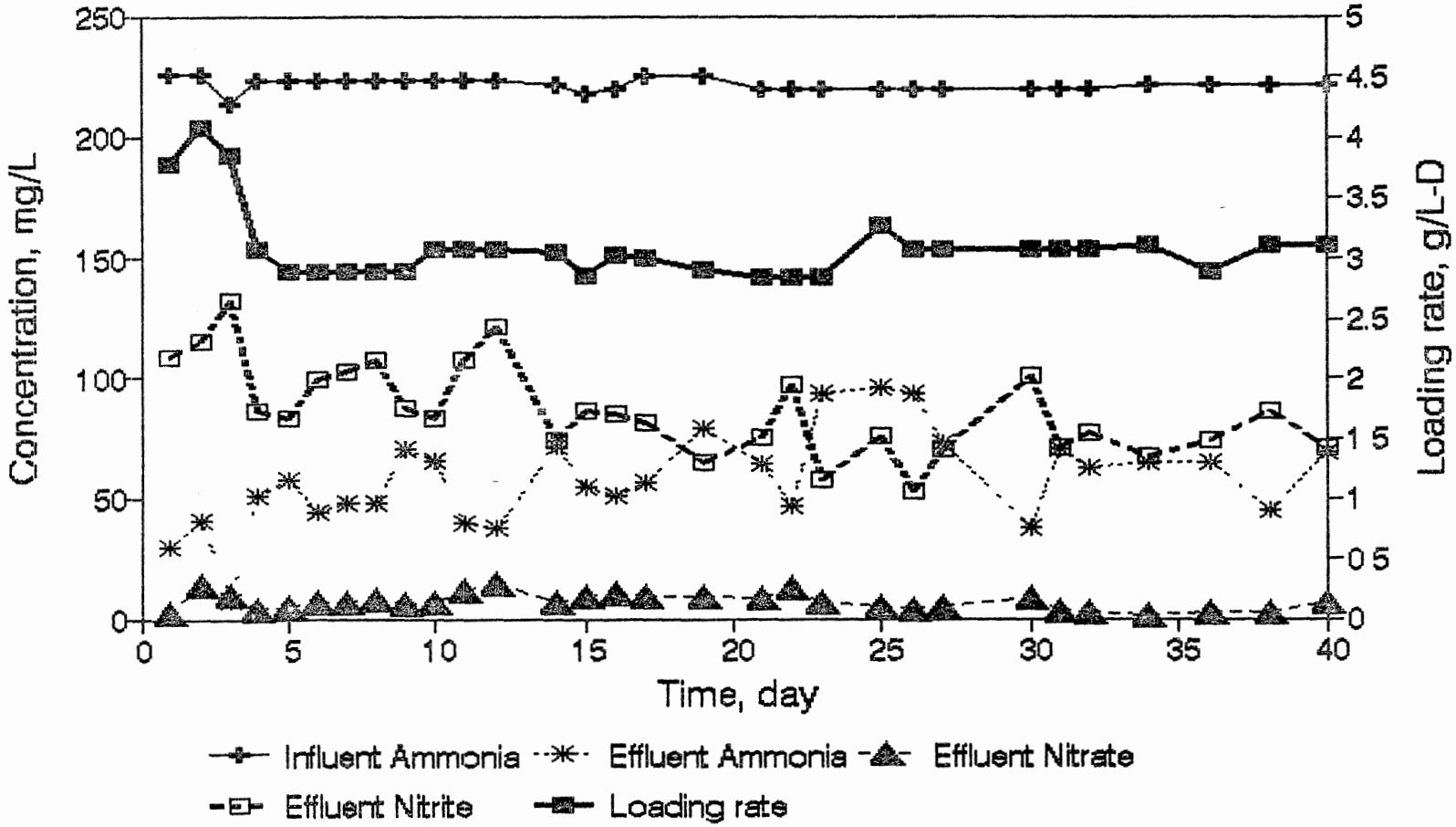


Figure 14 COUPLING REACTION WITH MAFAEB SYSTEM

(with acetate as electron donor, long term [1])

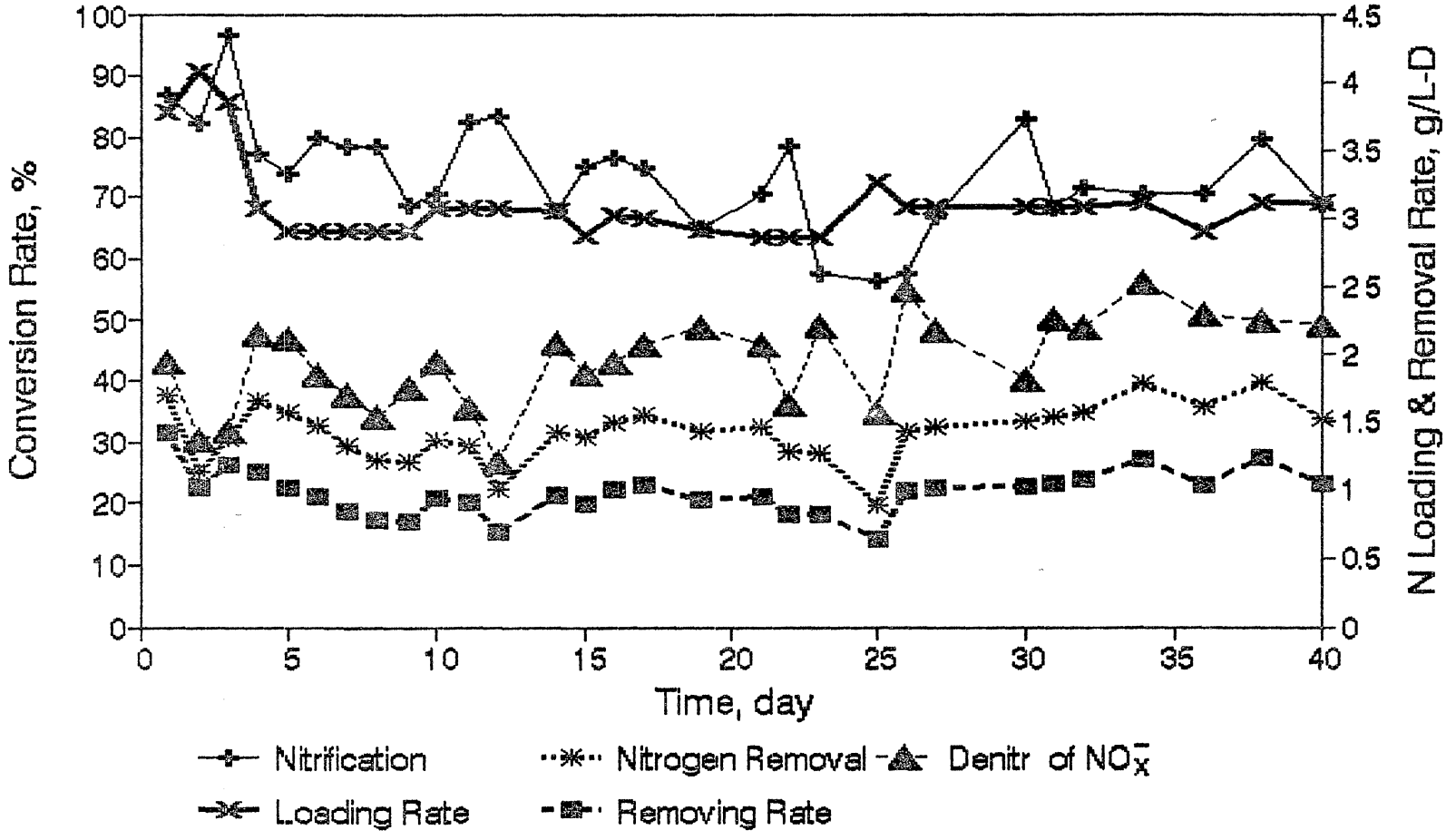


Figure 15. COUPLING REACTION WITH MAFAEB SYSTEM
(with sodium acetate as electron donor, long term [2])

The flow rate, hydraulic retention time, concentrations of all the nitrogen forms, conversion ratios, loading and removing rates and effluent DO were calculated at 95% of confidence intervals. Effluent COD, alkalinity, total suspended solids and volatile suspended solids were measured from a 5-day accumulated sample. Influent concentrations were measured with samples freshly made.

During the long term test, the pH of the influent and effluent was very steady. DO was controlled by adjusting the aeration rate to the aeration bottle and adjusting the air bubbles introduced into the reactor.

The alkalinity consumption of coupling reaction and nitrification were measured and listed in Table XVII. The COD consumptions of coupling reaction with sodium acetate as electron donor and denitrification with methanol as electron donor were also measured, and the results listed in Table XVIII.

TABLE XVII

ALKALINITY CONSUMPTION FOR COUPLING

REACTION AND NITRIFICATION

| | | Nitrification | Coupling Reaction |
|---|---|---|---|
| Alkalinity mg as $CaCO_3$/L | Influent | 3780 | 1164 |
| | Effluent | 407 | 252 |
| $NH_4^+$-N mg/L | Influent | 542 | 220 |
| | Effluent | 48.5 | 51.5 |
| mg $HCO_3^-$ /mg $NH_4^+$-N | | 6.83 | 5.40 |

TABLE XVIII

COD CONSUMPTION FOR DENITRIFICATION

AND COUPLING REACTION

| | | Denitri-fication | Coupling Reaction |
|---|---|---|---|
| COD mg/L | Influent | 5300 | 390 |
| | Effluent | 285 | 155 |
| $NO_3^-$-N and/or $NO_2^-$-N mg/L | Influent | 1509 | 168.3 |
| | Effluent | 0 | 90.8 |
| mg COD/mg $NO_2^-$-N or $NO_3^-$-N | | 3.32 | 4.19* |

*After correction for each mg of $NO_2^-$-N consuming 1.1 mg COD.

DO had a very subtle influence on the coupling reactions. Since DO could not be strictly controlled during this experiment, the DO values used for analysis are only rough estimations from several readings taken during a day. The results shown in Table XIX were calculated at 95% confidence interval. The whole DO range in the last 36 days of the long term test was divided to three categories (high: 2.8 - 2.2, middle: 2.1 - 1.8 and low: 1.7 - 1.4 mg/L). Two population T-test and F test were used to test if there were significant differences of the means of the nitrification efficiency and the nitrogen removal rate between high DO and middle DO, and between middle DO and low DO (Appendix). These results should be interpreted as an indication of reaction behavior only. More strict DO control means should be adopted in further studies of this factor.

## 4.5 Nitrification Recovery in MAFAEB System

After conducting coupling reaction or denitrification experiments, the system was purged of excess flocs as described previously. During the recovery process, the nitrification rate was tested. Table XX and Figure 16 show that full nitrification ability was closely approached within 5 days.

TABLE XIX

COMPARISON OF INFLUENCES BY DIFFERENT DO

| Item | DO, mg/L 2.59±0.20 | 1.94 ±0.06 | 1.57±0.09 |
|---|---|---|---|
| Influent Ammonium, mg/L | 222.9±1.4 | 221.9±2.0 | 221.1±0.8 |
| Effluent Ammonium, mg/L | 52.5±10.6 | 59.1±5.4 | 73.6±12.4 |
| Effluent Nitrate, mg/L | 8.9±2.2 | 7.1±2.1 | 4.3±1.4 |
| Effluent Nitrite, mg/L | 96.1±11.7 | 81.5±4.2 | 70.7±7.3 |
| Nitrification, % | 76.4±4.7 | 73.4±2.5 | 66.7±5.7 |
| Nitrogen Removal, % | 29.3±2.3 | 33.4±1.6 | 32.8±4.5 |
| Loading Rate, g/L-D | 2.97±0.06 | 2.98±0.07 | 3.06±0.09 |
| N-Removing Rate, g/L-D | 0.87±0.07 | 1.00±0.06 | 1.00±0.14 |

TABLE XX

NITRIFICATION RECOVERY IN MAFAEB SYSTEM

| Item | Time, day 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Influent Ammonium, mg/L | 151 | 146 | 146 | 146 | 151 |
| Effluent Ammonium, mg/L | 73.5 | 46.5 | 28.4 | 24.3 | 18.4 |
| Effluent Nitrate, mg/L | 6.3 | 8.1 | 8.8 | 7.1 | 10.7 |
| Effluent Nitrite, mg/L | 60.9 | 75.8 | 94.9 | 98.8 | 110.1 |
| Loading Rate, g/L-D | 4.34 | 4.34 | 4.34 | 4.34 | 4.34 |
| Nitrification, % | 51.3 | 68.2 | 80.6 | 83.4 | 87.8 |

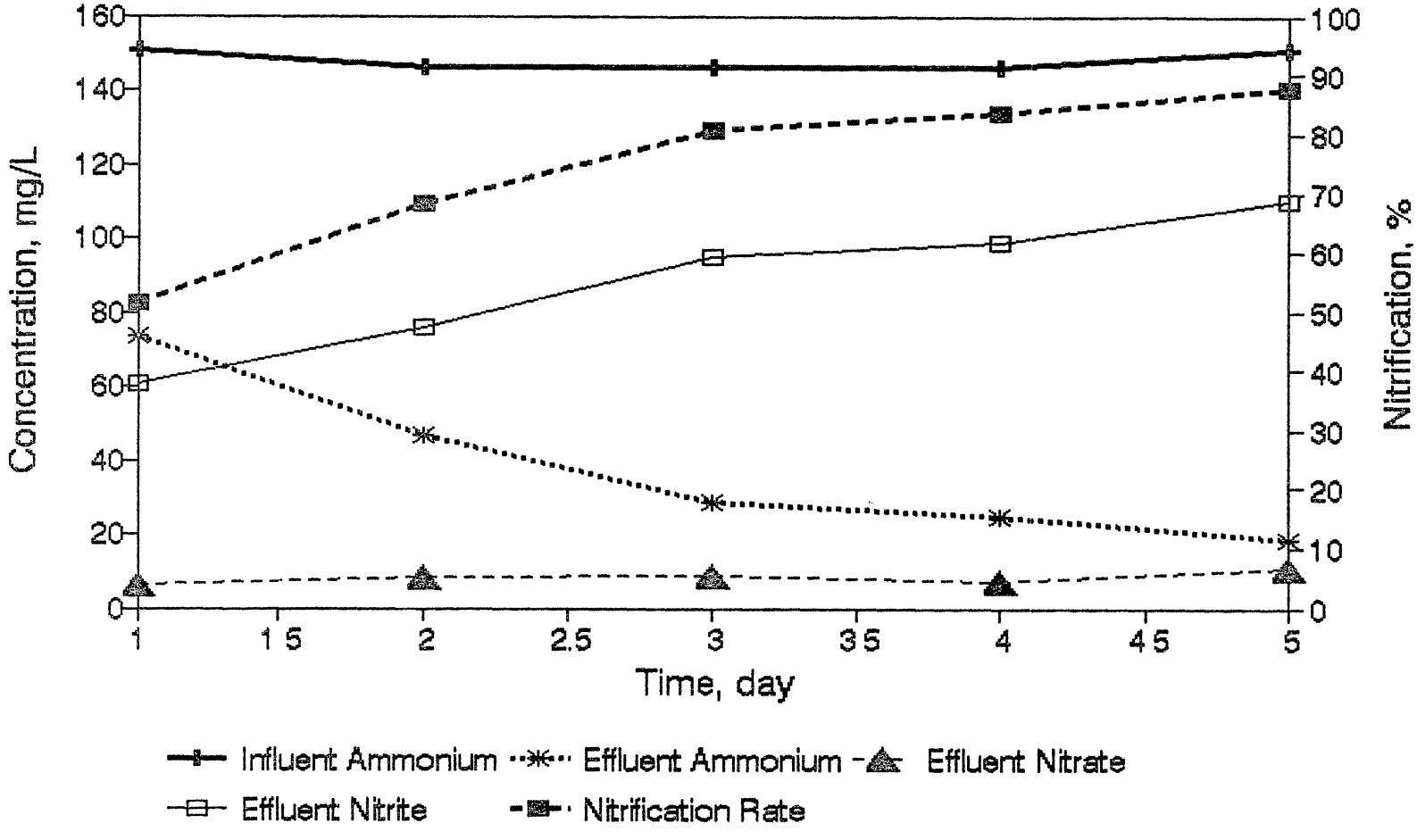


Figure 16 MAFAEB NITRIFICATION RECOVERY

(at loading rate of 4.34 g/D-L)

# CHAPTER V

# DISCUSSION AND CONCLUSIONS

## 5.1 Simultaneous Growth of Autotrophic and Heterotrophic Denitrifiers

Both autotrophic and heterotrophic denitrifiers developed active biofilm populations when they were acclimated together in this research. However, in the presence of different electron acceptors, their contributions to the denitrification were different. Table VII shows that when nitrite was used as electron acceptor, both thiosulfate and methanol were utilized more effectively than when nitrate was used as electron acceptor. With nitrate, the contribution of thiosulfate to denitrification was very limited, and the reaction consumed more electron donors with nitrate as electron acceptor than with nitrite. This may support the suggestion that the shortened pathway of nitrite reduction will save energy sources.

## 5.2 Nitrification with MAFAEB System

After only 24 days of acclimation for the nitrifiers added to the denitrifying attached films, the system demonstrated steady state nitrification ability. A comparison of the nitrification results of this experiment with Collins

et al.(1991) and Zeng (1992) is shown in Table XXI. The nitrification efficiency in this experiment was slightly lower than that obtained by Collins et al. (1991) at higher loading rates, but comparable to that of Zeng (1992). This nitrification capacity is significant in light of operational factors such as larger size of support media used in this research and lower density of nitrifiers in the attached films.

TABLE XXI

COMPARISON OF NITRIFICATION RESULTS

| Item | Collins et al.(1991) | Zeng (1992) | This Experiment |
|---|---|---|---|
| Influent $NH_4^+$-N, mg/L | 199 | 220 | 200 |
| Conversion Rate, % | 98 | 94.0 | 96.8 |
| Loading Rate, g $NH_4^+$-N/L-D | 11.52 | 7.5 | 4.59 |
| HRT, hours | 0.41 | 0.77 | 1.05 |
| Reactor Type | AEB | AEB | MAFAEB |
| Media Particle Size, mm | 0.4-0.6 | 0.2-0.6 | 1-3 |
| Attached VS, g VS/L Bed | 42.5 | 46.7 | 27.8* |
| Bed expansion % | 62 | 60 | 100 |

* Includes nitrifiers and denitrifiers.

## 5.3 Denitrification with MAFAEB System

After aeration was stopped and feeding with nitrate resumed, the MAFAEB system restored steady state denitrification in a few days. The data from Table X show that the denitrification efficiency of this system was extremely high.

Table XXII shows a comparison of experimental results

with other heterotrophic denitrification results. Since the main objective of this research is not to determine maximum denitrification rates, the results of this experiment listed in the table are only representative reasonable rather than maximum loading rates. From Table X and Figure 5 we can anticipate the maximum loading rate may be even higher.

TABLE XXII

COMPARISON OF DENITRIFICATION RESULTS

| Item | Jeris et al. (1975) | Miyaji et al. (1975) | Clarkson et al. (1992) | This Experiment |
|---|---|---|---|---|
| Influent $NO_3^-$-N, mg/L | 21.5 | 900 | 934 | 544 |
| HRT, Hrs | 0.11 | 3.8 | 3.4 | 1.86 |
| Loading Rate, $gNO_3^-$-N/L-D* | 5.42 | 6.5 | 6.54 | 7.15 |
| Removal Rate, $gNO_3^-$-N/L-D* | 5.37 | 6.38 | 6.16 | 6.99 |
| Conversion Rate, % | 99.0 | 98.6 | 94.2 | 99.6 |
| Reactor Type | FLUIDIZED | UASB | AFEB | MAFAEB |
| Media Particle Size, mm | < 0.6 | -- | 0.2-0.6 | 1-3 |
| Organic Substrate | METHANOL | WASTE | METHANOL | ACETATE |
| Attached VS, gVS/L Bed | 30-40 | -- | 82 | 27.8 |
| Bed Expansion, % | 100 | -- | 15-20 | 100 |

* g $NO_3^-$-N/per liter of expanded bed per day.

It was found that when returning the system from nitrification or coupling reaction to denitrification, some attached film particles floated on the liquid surface and tended to be washed out. Sludge particles floated due to entrapped gas, indicating that denitrification occurred in the inner layer of the particles. This phenomenon dis-

appeared after a couple of weeks, but the previously smooth attached biofilm surface became spiky or fuzzy at this time. Although these phenomena occurred during operation as a denitrification reactor, it may suggest that during the coupling reaction, the denitrifiers not only attach and grow in the inner layer of the particles, but also attach with nitrifiers on the outer layer, to form thoroughly mixed rather than layered attached films only.

## 5.4 Alternating Aeration

Table XII shows that alternating aeration for coupling reaction is not as efficient as the system operated under constant low DO conditions. Although much of the operating cycle was devoted to denitrification, its conversion rate still lagged behind that of nitrification. This may suggest that denitrification recovery from aerobic conditions is not as fast as nitrification from anoxic conditions.

## 5.5 Inhibition Effects

Methanol and thiosulfate were reported to have inhibitory effects on nitrification (Beccari, 1980; Hooper *et al.*, 1973). Throughout this experiment both nitrification and denitrification seemed to be inhibited. Acetate was fed to the system due to its lack of inhibition effect on nitrification. However, its effect on the coupled reaction rates was very similar to that of methanol and thiosulfate. Some other mechanisms must have been in action.

All the electron donors added to the reactor were favored by denitrifiers at anoxic conditions. If only nitrification were inhibited, when a large amount of electron donor was added to the coupling reaction, there should have been no nitrate or nitrite left in the effluent. However, Tables XIV and XV show that when concentrations of electron donors were increased, both nitrification and denitrification were slowed. At the same time, tremendous flocs accumulated in the reactor and the aeration bottle. Considering the large recycle rate utilized, the effects of inhibition should not be so large. All of this suggests that the nitrification rate was likely not affected by inhibitors, but by low oxygen content, which was caused by co-oxidation of the electron donors added for denitrification. Oxidation of those substrates competed for oxygen with ammonia oxidation. At the same time, the availability of electron donors to the denitrifiers was also depleted.

## 5.6 DO Effects

This system lacked means to strictly control DO. The DO in this experiment was controlled by adjusting the aeration rate and adjusting the amount of air bubbles introduced into the reactor through recycle tubing.

It was found that when DO in the upper end of the reactor was much higher than about 2.0 mg/L, nitrification improved, but denitrification was slowed, resulting in an overall reduction of nitrogen removal. Conversely, when DO

was set too low, nitrification was seriously inhibited and the availability of oxidized nitrogen forms was limited. When DO was controlled around 2.0 mg/L, there was a compensation between nitrification and denitrification (Table XIX). The results of statistical analyses results (Appendix) support the above observations.

After running the experiment under this condition with 750 mg/L sodium acetate as an energy source for 40 days, the system outputs remained reasonably steady (Figure 14, 15 and Table XVI).

## 5.7 Contamination effects

The MAFAEB is an open system filled with mixed attached organisms. When the system favored aerobic heterotrophic conditions or sulfur-oxidizing conditions, they became prominent in the reactor. At the beginning stage of the coupling reaction experiments with newly acclimated, mixed, attached films, the coupling reaction tended to be completely balanced between nitrification and denitrification at a low loading rate (Table XI, day 4). However, both nitrification and denitrification conversion rates dropped briskly as flocs accumulated in the reactor and the aeration bottle. This suggests that aerobic organisms oxidizing methanol and thiosulfate predominated in the system. After returning to only a nitrification feed solution at small HRT, the flocs were washed out and coupling reaction ability was resumed. However, in only a few days, contamination

again predominated. To address this situation, the concentration of energy sources were greatly reduced (much less than stoichiometric needs of denitrifiers) in the rest of the experiments.

By comparing the results in Tables XIII, XIV, and XV, it is clear that acetate in the feed solution obstructed thiosulfate oxidation. However, when the concentration of thiosulfate was too high, inhibition to nitrification become serious. At the same time, it was found that acetate could be used as electron donor for denitrification in the coupling reaction system, and there was a maximum nitrogen removal rate at the acetate concentration of 750 mg/L.

The long term coupling reaction experiment (Figure 14 and 15) was conducted to demonstrate that the contamination or co-oxidation problems could be controlled in the coupling reaction. Although difficulties existed for exactly controlling flow rate and DO, the pH outcomes of the system were extremely steady and no significant flocs were accumulated in the reactor. This indicate that as long as the concentration of energy sources was kept low, a steady state reaction could be reached and maintained with a somewhat limited nitrogen removal rate.

## 5.8 Alkalinity and COD Consumption

Nitrification consumes large amounts of alkalinity, while heterotrophic denitrification produces alkalinity. Table XVII shows that alkalinity consumed per unit ammonium

conversion is 6.83 mg $HCO_3^-$/mg $NH_4^+$-N. This figure is much lower than the theoretical value of 8.64 (Grady et al., 1980), because the main product of nitrification in this experiment was nitrite. During coupling reactions, the alkalinity consumptions were even lower; only 5.40 mg $HCO_3^-$/mg $NH_4^+$-N was consumed. The coupling reaction with sodium acetate as electron donor and with 34.4% nitrogen removal rate can save alkalinity by 20.9% compare with nitrification.

The COD consumption for denitrification in the coupling reaction should be lower because one reduction step is saved in nitrogen removal. However, Table XVIII shows that total COD consumption was higher instead of lower than that of heterotrophic denitrification. This also supports the conclusion that part of the electron donor supply was oxidized through aerobic competition.

# CHAPTER VI

## CONCLUSIONS

1. This research has shown that autotrophic and heterotrophic denitrifiers can be attached together on support media, and simultaneous growth can be achieved in both batch acclimation and mixed attached growth. However, their contributions to denitrification depend on what kind of electron acceptor is available. When nitrite was used as electron acceptor, both thiosulfate and methanol were utilized more effectively than with nitrate as electron acceptor. When nitrate was used as electron acceptor, the contribution from thiosulfate to denitrification was very limited.
2. For denitrification, the electron donor requirement can be lowered with nitrite instead of nitrate as electron acceptor. In the coupling reaction system, however, COD consumption is higher than in denitrification, apparently due to co-oxidation.
3. Nitrifiers can be easily attached onto an existing attached denitrifying film layer. The mixed attached films demonstrated both nitrification and denitrification abilities.

4. The MAFAEB system can be used for either nitrification or denitrification purposes. The capacity for denitrification of the system is much higher than that for nitrification. At a loading rate of 4.59 g/L-D (static volume), the nitrification efficiency was found to be 96.8%, while at a denitrification efficiency of 99.6%, the loading rate was ≥ 13.98 g/L-D (static volume).
5. Coupled nitrification and denitrification reactions can occur in MAFAEB system. The DO should be maintained at about 2 mg/L. Higher DO will sacrifice denitrification with improvement of nitrification but reduction of total nitrogen removal, while lower DO will sacrifice nitrification without improving nitrogen removal.
6. Methanol, sodium thiosulfate, and sodium acetate can be used as electron donors for operation in the coupling reaction mode. Acetate affects thiosulfate oxidation. At high concentrations, all can be oxidized by competing bacteria. When this occurs, nitrification will be limited by a shortage of oxygen, while denitrification will be limited by a shortage of electron donors.
7. Compared to pure nitrification or denitrification operation, coupling reactions with sodium acetate as electron donor (34.4% nitrogen removal rate) can save alkalinity by 20.9%. The total COD consumption per unit nitrogen removal in the coupling reaction is higher

than that in heterotrophic denitrification due to some electron donors being oxidized through competing aerobic reactions.

8. Contamination or co-oxidation problems are not destructive to coupling reaction. Steady state reaction can be maintained at low electron donor concentrations.

# CHAPTER VII

## SIGNIFICANCE OF THE STUDY

This is the first effort of which the author is aware to couple nitrification and denitrification through a shortened pathway in a single mixed attached film aerated expanded bed reactor. This investigation also included simultaneous growth of autotrophic and heterotrophic denitrifiers and utilization of the MAFAEB system in either nitrification and denitrification mode.

This study demonstrated that coupling reactions do occur in a single MAFAEB reactor, and a steady state reaction can be reached and maintained as long as the electron donor concentration is relatively low.

One possible application of the results from this study is attached film expanded bed denitrification with mixed autotrophic and heterotrophic denitrifiers. In this way, both organic and inorganic electron donors can be utilized. If controlled well, no alkalinity adjustment will be necessary.

Another possible usage is to develop attached nitrifiers through first attaching denitrifiers on the support media, then attaching nitrifiers on the denitrifying bacteria layer. In this way, much time and chemicals can be

saved.

Since this system can be used as both a nitrification and denitrification system, it may be used as an intermediate stage between nitrification and denitrification facilities where nitrogen must be totally removed. This system can be used as a buffer to compensate the capacity deficiency between the two facilities when waste characteristics or operating conditions vary.

Where nitrification is mandatory while oxidized nitrogen forms are not strictly regulated, and also some COD is available in the wastewater, this system can be directly used to perform nitrification and partial removal of oxidized nitrogen forms and COD. Thus such a process could have a role in industrial pretreatment (particularly for oxygen demand reduction).

# CHAPTER VIII

# FURTHER RESEARCH NEEDS

## 8.1 Coupling Reaction in a Strictly Controlled low DO MAFAEB System

The Results from this experiment demonstrated that coupling nitrification and denitrification through a shortened nitrite pathway is possible. However, there was no means to strictly control DO throughout this research period so that the optimized DO conditions and maximum loading rate for coupling reaction could not be assessed. If DO could be effectively controlled at exact values around or lower than 2 mg/L all the time, and sufficient oxygen could be supplied for nitrification, the control and extent of the coupling reaction should be largely improved.

## 8.2 Coupling Reaction at Elevated Temperatures

This experiment was carried out at room temperature. During this period, the room temperature was 17 - 22°C. Due to the heat released by nitrification and denitrification, the temperature in the reactors was always 2 - 5°C higher than the room temperature.

Since both nitrification and denitrification are

temperature dependent, reaction rates will be increased at higher temperatures. The effects of temperature on coupling reactions may deserve further investigation.

## 8.3 The Maximum Loading Rate for Denitrification with MAFAEB without Aeration

When the MAFAEB was used without aeration to conduct denitrification in this experiment, there was little reduction of denitrification efficiency when the loading rate reached 14 g $NO_3^-$-N/L-D static volume. The attached biofilm particles took on an irregular surface configuration. The reasons for the extremely high denitrification capacity and the deformation of the biofilm particles deserve further investigations.

## 8.4 Nitrification with MAFAEB System

Difficulties in developing attached films for nitrifiers were encountered throughout this experiment. One possible solution is to attach other organisms, for example, heterotrophic denitrifiers which tend to be easier to attach on support media, before acclimating nitrifiers onto the same media.

The nitrification efficiency of the nitrifiers developed in this experiment is lower than that of Collins et al. (1991) and Zeng (1992) obtained from AEB reactors. However, this lower efficiency was obtained from a short

term acclimation and short period of experiment. Long term acclimation and nitrification experiment may be needed to verify the maximum loading rate under reasonably high nitrification efficiency in a mature system.

## 8.5 Other Possible Usages of the MAFAEB System

Since nitrifiers are much easier to attach on the denitrifying biofilm layer than on the bare media surface itself, other organisms may also have this property. The versatility of this system will allow reactions to occur under aerobic or anaerobic conditions at different energy levels by changing electron acceptors. After specialized acclimation, other aerobic, anaerobic, or facultative organisms may be developed on this system. If energy and nutrient conditions favor biodegradation of some particular substances, for example, TCE, pesticides or herbicides, those reactions may also be conducted in this type of system.

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# APPENDIX

## TWO POPULATION T-TEST FOR NITRIFICATION EFFICIENCY AND NITROGEN REMOVAL RATE AT DIFFERENT DO CONCENTRATIONS

```
TITLE 'TWO POPULATION T-TEST FOR NITRIFICATION EFFICIENCY AT
HIGH DO AND MIDDLE DO';
OPTIONS PS=60;
DATA TTEST;
INPUT POPULATION$ NITRIFICATION EFFICIENCY;
CARDS;
HIGH 80.00
HIGH 78.35
HIGH 78.57
HIGH 68.62
HIGH 82.50
HIGH 83.35
HIGH 64.84
HIGH 78.50
HIGH 66.82
HIGH 82.82
MIDDLE 77.14
MIDDLE 74.11
MIDDLE 74.95
MIDDLE 76.59
MIDDLE 74.80
MIDDLE 70.68
MIDDLE 70.45
MIDDLE 68.09
;
PROC TTEST;
    CLASS POPULATION;
    VAR NITRIFICATION EFFICIENCY;
RUN;
```

TTEST PROCEDURE

Variable: NITRIFICATION EFFIEIENCY

| POPULATION | N | Mean | Std Dev | Std Error |
|---|---|---|---|---|
| HIGH | 10 | 76.43700000 | 6.98040917 | 2.20739920 |
| MIDDLE | 8 | 73.35125000 | 3.23614161 | 1.14414884 |

| Variances | T | DF | Prob>\|T\| |
|---|---|---|---|
| Unequal | 1.2411 | 13.3 | 0.2361 |
| Equal | 1.1502 | 16.0 | 0.2670 --> Fail to reject, no significant difference. |

For H0: Variances are equal, F' = 4.65 DF = (9,7)

Prob>F' = 0.0550 --> Fail to reject, no significant difference.

```
TITLE 'TWO POPULATION T-TEST FOR NITRIFICATION EFFICIENCY AT
MIDDLE AND LOW DO';
OPTIONS PS=60;
DATA TTEST;
INPUT POPULATION$ NITRIFICATION EFFICIENCY;
CARDS;
MIDDLE 77.14
MIDDLE 74.11
MIDDLE 74.95
MIDDLE 76.59
MIDDLE 74.80
MIDDLE 70.68
MIDDLE 70.45
MIDDLE 68.09
LOW 67.84
LOW 57.50
LOW 56.27
LOW 57.00
LOW 71.45
LOW 70.59
LOW 70.53
LOW 79.73
LOW 68.74
;
PROC TTEST;
    CLASS POPULATION;
    VAR NITRIFICATION EFFICIENCY;
RUN;
```

TTEST PROCEDURE

Variable: NITRIFICATION EFFICIENCY

| POPULATION | N | Mean | Std Dev | Std Error |
|---|---|---|---|---|
| LOW | 9 | 66.62777778 | 8.02397622 | 2.67465874 |
| MIDDLE | 8 | 73.35125000 | 3.23614161 | 1.14414884 |

| Variances | T | DF | Prob>\|T\| | |
|---|---|---|---|---|
| Unequal | -2.3112 | 10.8 | 0.0417 | --> Reject, significantly different. |
| Equal | -2.2093 | 15.0 | 0.0431 | |

For H0: Variances are equal, F' = 6.15 DF = (8,7)

Prob>F' = 0.0270 --> Reject, significantly different.

```
TITLE 'TWO POPULATION T-TEST FOR NITROGEN REMOVAL RATE AT
HIGH AND MIDDLE DO';
OPTIONS PS=60;
DATA TTEST;
INPUT POPULATION$ NITROGEN REMOVAL RATE;
CARDS;
HIGH 0.95
HIGH 0.85
HIGH 0.78
HIGH 0.77
HIGH 0.90
HIGH 0.69
HIGH 0.92
HIGH 0.83
HIGH 1.01
HIGH 1.03
MIDDLE 1.13
MIDDLE 1.01
MIDDLE 0.89
MIDDLE 1.00
MIDDLE 1.03
MIDDLE 0.94
MIDDLE 0.94
MIDDLE 1.05
;
PROC TTEST;
    CLASS POPULATION;
    VAR NITROGEN REMOVAL RATE;
RUN;
```

TTEST PROCEDURE

Variable: NITROGEN REMOVAL RATE

| POPULATION | N | Mean | Std Dev | Std Error |
|---|---|---|---|---|
| HIGH | 10 | 0.87300000 | 0.10924488 | 0.03454627 |
| MIDDLE | 8 | 0.99875000 | 0.07529703 | 0.02662152 |

| Variances | T | DF | Prob>\|T\| | |
|---|---|---|---|---|
| Unequal | -2.8833 | 15.7 | 0.0110 | |
| Equal | -2.7649 | 16.0 | 0.0138 | --> Reject, significantly different. |

For H0: Variances are equal, F' = 2.10 DF = (9,7)

Prob>F' = 0.3388 --> Fail to reject, no significant difference.

```
TITLE 'TWO POPULATION T-TEST FOR NITROGEN REMOVAL RATE AT
MIDDLE AND LOW DO';
OPTIONS PS=60;
DATA TTEST;
INPUT POPULATION$ NITROGEN REMOVAL RATE;
CARDS;
MIDDLE 1.13
MIDDLE 1.01
MIDDLE 0.89
MIDDLE 1.00
MIDDLE 1.03
MIDDLE 0.94
MIDDLE 0.94
MIDDLE 1.05
LOW 0.95
LOW 0.82
LOW 0.64
LOW 0.99
LOW 1.08
LOW 1.23
LOW 1.04
LOW 1.24
LOW 1.05
;
PROC TTEST;
    CLASS POPULATION;
    VAR NITROGEN REMOVAL RATE;
RUN;
```

TTEST PROCEDURE

Variable: NITROGEN REMOVAL RATE

| POPULATION | N | Mean | Std Dev | Std Error |
|---|---|---|---|---|
| LOW | 9 | 1.00444444 | 0.18888562 | 0.06296187 |
| MIDDLE | 8 | 0.99875000 | 0.07529703 | 0.02662152 |

| Variances | T | DF | Prob>\|T\| | |
|---|---|---|---|---|
| Unequal | 0.0833 | 10.7 | 0.9351 | --> Fail to reject, no significant difference. |
| Equal | 0.0796 | 15.0 | 0.9376 | |

For H0: Variances are equal, F' = 6.29  DF = (8,7)

Prob>F' = 0.0253 --> Reject, significantly different.

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