HETEROTROPHIC DENITRIFICATION OF SIMULATED HIGH-STRENGTH INDUSTRIAL WASTEWATER

By

SRIKANTH KRISHNAMACHARI

Bachelor of Science (Engineering)

Regional Institute of Technology

Jamshedpur, India

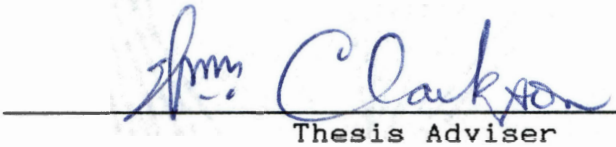
1987

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1990

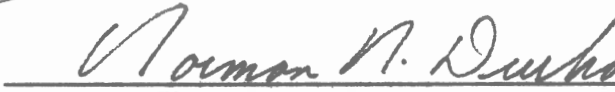
HETEROTROPHIC DENITRIFICATION OF SIMULATED

HIGH-STRENGTH INDUSTRIAL WASTEWATER

Thesis Approved:



Thesis Adviser



Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. William W. Clarkson, my principal advisor, for his support both morally and financially, and for his guidance. He has been the source of inspiration for my graduate studies here, and without his kind help and patience this thesis would not have attained this shape. Special thanks go to Drs. John N. Veenstra and Marcia H. Bates for serving on my committee, and rendering useful suggestions. I have to also thank Dr. William F. McTernan for giving innumerable assignments on critiquing articles for his coursework which helped improve my technical writing skills. I would like to thank my friend, Ben J. B. Ross for his invaluable assistance in the laboratory, and his family for their lovable company. I am grateful to the University Water Research Center for funding this project. I shall extend my sincere appreciation to my friends John Delphon and Udayakumar Matrubutam of the Microbiology Department for conducting the genus tests. I wish to thank Srihari Nandyal and Kula Ramprasad Rai for putting up with me in the apartment, and for the fun and joy we had in these two years.

I would like to take this opportunity to express my love and respect for Dr P. K. Pain, Professor of

Environmental Engineering at Regional Institute Technology (INDIA) who introduced me into this field and imbued in me the values of learning. I am also deeply indebted to Er. P. Kaliaperumal, Principal of M. K. Polytechnic, and the faculty of Civil Engineering for their encouragement during my stay there.

I dedicate this thesis to my mother and my demised father who taught me to be diligent and determined. I am also proud of my family members who gave me continued love and affection to attain this goal. Finally, I wish to thank my sister Mrs. Rajivi Narasimhan and my brother-in-law Mr. Narasimha Bhattar and their two children, Srividhya and Vivek, for, their presence in this country provides me a constant moral support and a homely environment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

1.1 Development and Scope of this Research

This investigation was undertaken to conduct a feasibility study on the microbial denitrification of simulated high-strength industrial wastewater. Though much research has already been done on the denitrification process, only a few have attempted to deal with very high concentrations of nitrogen, i.e. 1000 mg/L or more. High nitrogen concentration appears in the effluents of various industries such as fertilizer, semiconductor, and munitions. Denitrification of such effluents is necessary for preventing eutrophication of receiving water bodies such as lakes and other slow-flow water courses, by the uncontrolled growth of algae and other aquatic plants. Nitrogen in the form of ammonia (NH_3) is toxic to fish and nitrite (NO_2^-) is considered to be carcinogenic. Hence removal of nitrogen compounds from wastewaters has been receiving wide attention in recent years.

Following primary and secondary treatment processes in a typical wastewater treatment plant, biological denitrification is adopted as an advanced treatment technique as part of the nutrient removal unit.

Nitrification may precede denitrification where necessary.

1.2 Reactor Selection

As in any anaerobic treatment system, growing and sustaining a large, viable microbial biomass is an important factor for successful denitrification. While maintaining a suspended culture in a reactor may pose difficulties such as liquid-solid separation, recycling and effluent quality, an attached growth system offers solutions to these problems. The anaerobic attached film expanded bed (AFEB), one such attached growth system, was first developed by Jewell in 1971 (Clarkson, 1986) to overcome the problems stated above. Maximum biomass and surface area with the least mass transfer restrictions, and non-clogging were stated to be some of the major advantages of using such a system (Clarkson, 1986). Though the AFEB could be termed a modified version of the fluidized bed reactor, the distinction between these two systems lies in the requirement of lower flow-through velocity and smaller expansion of bed volume for the former (Jewell et al., 1981). Therefore the expanded bed reactor was an obvious choice for this feasibility study.

It was also decided to compare the denitrification treatment efficiency and other operating parameters of AFEB with those of another high-rate system. Upflow anoxic sludge blanket (UASB) reactors have been used successfully for denitrification by several researchers (Klapwijk et

al., 1979; Lettinga et al., 1980). A system in which upflow movement of the liquid occurs through a thick anaerobic sludge blanket was first developed by Coulter et al. in 1961 (Lettinga et al., 1980). Smaller reactor volume due to higher removal capacity per unit volume of reactor at high sludge concentrations, excellent settling characteristics, maintenance of a thick blanket even at high speed stirring, and minimum wash-out of flocs were considered to be some of the advantages with a UASB system (Klapwijk et al., 1979; Lettinga et al., 1980). Based on the above considerations, bench scale AFEB and UASB reactors were chosen for this research.

1.3 Objectives

The goals of this research were to establish operating parameters at the following two conditions in both the AFEB and UASB reactors:

1. The maximum reactant concentration at which nitrate removal rate and reduction efficiency would begin to significantly drop for a given hydraulic retention time (HRT).

2. The maximum loading and removal rate profiles at a fixed lower concentration.

CHAPTER II

LITERATURE REVIEW

2.1 Scope of Review

This research focuses on heterotrophic high-rate denitrification, primarily to study the reactor performance at high concentrations of NO_3^-_N to a point when failure occurs and to investigate the possible causes of such a failure. Although elimination of nitrogen compounds from water and wastewater could be accomplished by several physical/chemical or biological processes, the latter is attaining popularity because of its simplicity and economics. Physicochemical processes such as ion-exchange and ammonia stripping merely convert the nitrogen compounds from one form to the other (NH_3 --> NO_3^-) and do not completely eliminate the nitrogenous materials. A two step biological nitrification - denitrification ensures total conversion (NH_3 --> NO_3^- --> N_2) and results in complete removal, the end product being nitrogen gas.

Biological denitrification is also important from the agronomy and agriculture point of view because of fertilizer nutrient loss to the atmosphere. However, from an environmental engineering perspective, such a biological reaction is beneficial.

Although denitrification in general is well-studied, information on high-rate denitrification is scanty. Successful operation of reactors with high strength NO_3^- has been possible when appropriate denitrifiers and their required substrates are present. Maintenance of strict anoxic conditions, design of a proper reactor configuration, and control of other parameters such as pH, temperature, etc. are necessary to attain the required efficiency.

Thus the emphasis of this literature survey lies on the need to explain the microbiological conditions of denitrifying bacteria, and the comparative study of different denitrification systems and their operating and control parameters.

2.2 Microbiology of Denitrification

2.2.1 The Denitrification Mechanism

Heterotrophic microbes derive energy for the synthesis of new cells by oxidizing organic matter present in the waste. The primary mechanism is the generation of adenosine triphosphate (ATP) through substrate-level and/or oxidative phosphorylation reactions. Respiration occurs when oxidative phosphorylation produces ATP by transferring electrons to an inorganic hydrogen acceptor such as oxygen or nitrate (Grady and Lim, 1980).

Facultative bacteria utilize molecular oxygen (O_2) or other oxidized inorganic ions (e.g. nitrate, carbonate, and

sulfate) for respiration. If sufficient dissolved O_2 is available, respiration by denitrifying bacteria becomes aerobic. On the other hand, when nitrate (or any oxidized form of nitrogen) is abundant, anaerobic respiration, also known as denitrification, takes place. Of these two respiratory mechanisms, the former is preferable to bacteria because this results in maximum ATP generation. Consequently, cell production is greater in aerobic respiration. However, denitrification produces much more ATP than fermentation and is adopted by facultative microbes when availability of oxygen is restricted or when the amount of nitrate far exceeds oxygen. When no electron acceptor is readily available, microbes resort to endogenous respiration for their sustenance (Grady and Lim, 1980).

Microbial denitrification can also be accomplished by certain autotrophic bacteria, such as *Thiobacillus denitrificans*, which uses reduced sulfur compounds as electron donor (Claus and Kutzner, 1985a). Numerous species of heterotrophic denitrifiers, such as *Alcaligenes* (*Achromobacter*), *Paracoccus* (*Micrococcus*), and *Pseudomonas* can be identified by their survival on organic substrates (e.g. methanol) as carbon and energy sources. Nitrate is the primary electron acceptor and nitrogen source for the cell production of such bacteria (Knowles, 1982). In this report, the word denitrification refers to heterotrophic denitrification unless otherwise specified.

Nitrate reduction may take place via two processes, resulting in different end products. Assimilatory nitrate reduction yields ammonia by producing nitrite and then hydroxylamine [NH_2OH] as intermediary compounds, as shown below. Some photosynthetic bacteria, algae, and certain fungi assimilatively reduce nitrate to ammonia (Payne, 1981).

$$NO_3^- \text{-----} > NO_2^- \text{-----} > [NH_2OH] \text{-----} > NH_3 \qquad (1)$$

In dissimilatory nitrate reduction, generally accepted as true denitrification, nitrate is reduced to dinitrogen (N_2) via the reduction of nitrite, nitric oxide (NO), and nitrous oxide (N_2O) in the following general sequence.

$$NO_3^- \text{---} > NO_2^- \text{---} > NO \text{---} > N_2O \text{---} > N_2 \qquad (2)$$

Intermediary compounds

2.2.2 The Characteristics of Denitrifiers

All those bacteria which are known to be true denitrifiers need not follow all the steps mentioned in the dissimilatory nitrate reduction sequence. In other words, formation of one or more of these intermediary compounds can be preferentially bypassed, e.g. *Bacillus licheniformis* exhibits nitrate and nitric oxide reductases but is devoid of nitrite and nitrous oxide reductases (Jeter and Ingraham, 1981). All of these intermediary compounds besides nitrate can serve as terminal electron acceptors, and the choice of a particular oxide of nitrogen depends on the growth conditions of the concerned organism. Some

bacteria reduce nitrate to nitrite only by dissimilative respiration and are not considered as true denitrifiers, e.g. *Escherichia coli* (Payne, 1981; Jeter and Ingraham, 1981).

Although denitrifiers are ubiquitous in nature, their morphological characteristics vary greatly. Most denitrifiers are gram-negative and facultative, such as the rod shaped *Pseudomonas* and *Alcaligenes*, and the coccoid *Kingella*, *Neisseria*, and *Paracoccus*. The only obligate anaerobic denitrifier, *Thiomicrospira denitrificans*, is autotrophic. Some gram-positive denitrifiers belong to the genera *Bacillus* (endospore forming), and *Corynebacterium* and *Propionibacterium* (nonspore forming) (Payne, 1981; Jeter and Ingraham, 1981).

Complexity in understanding the mechanisms arises due to the organisms' ability to switch functions corresponding to their needs and prevailing conditions of conducive environments. Anomalies are noted because of the lack of definitive stepwise reduction processes. For example, some strains of *Alcaligenes faecalis* (formerly classified under *Achromobacter*), *Neisseria* sp., and *Flavobacterium* sp., can reduce only nitrite and not nitrate. Certain bacteria denitrify only to N_2O (Jeter and Ingraham, 1981). It is also curious to know that certain bacteria are biased in their selection of carbon compounds when complex organics are present. *Pseudomonas fluorescens* and *Hyphomicrobium* sp. denitrified more vigorously with single carbon

compounds, such as methanol, than with others, such as urea (Sperl and Hoare, 1971; Blaszczyk et al., 1980). *Pseudomonas mendocina* denitrified well with ethanol (Blaszczyk et al., 1980).

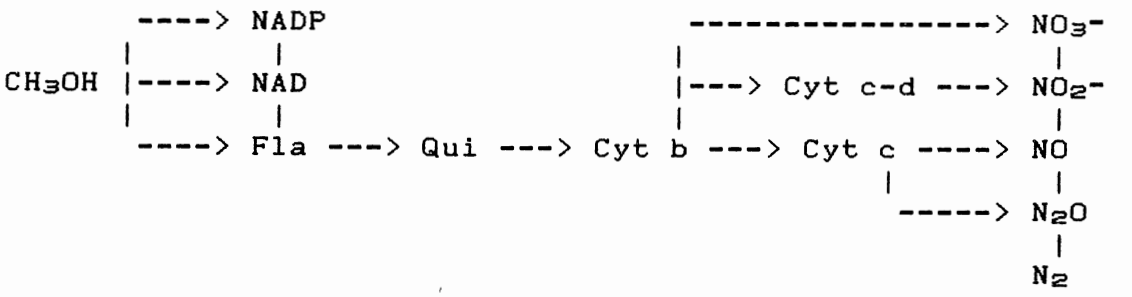
2.3 Electron Transfer Pathway

Although a number of organic compounds can be used as electron donors for denitrification, methanol (CH_3OH) was chosen for this study for the following reasons:

1. Low cost and ease of availability.
2. High solubility in water.
3. Ease of biodegradation.
4. Lower vapor pressure in water than compounds like acetone (McCarty et al., 1969).
5. Low microbial cell yield (Jeris and Owens, 1975).
6. Widely used in full-scale denitrification processes.

When methanol is the electron donor, the end products of denitrification are carbon dioxide (CO_2), water (H_2O), and cell mass ($C_5H_7O_2N$) besides N_2. The stoichiometric relationship of these end products and the reactants are described in the following section.

The electron transport chain for denitrification as adapted by Payne (1981) is given below. As depicted in this pathway, each catalyst induces the reductases of nitrate, nitrite, and nitric and nitrous oxides as the situation demands.



(3)

2.4 Stoichiometric Relationship

With methanol as the electron donor, McCarty et al. (1969) showed that nitrate could be considered to be reduced to dinitrogen in a two step process via nitrite. The overall reduction reaction, without considering cell synthesis, is

$$NO_3^- + 5/6\ CH_3OH \longrightarrow 1/2\ N_2 + 5/6\ CO_2 + 7/6\ H_2O + OH^- \quad (4)$$

As can be seen, the reduction of one mole of nitrate results in the production of half a mole of N_2 gas, and one mole of hydroxyl ion (OH^-), thus indicating that alkalinity is being produced in heterotrophic denitrification. This should be compared with autotrophic denitrification in which H^+ ions are produced thus repressing alkalinity. The methanol requirement would be more than that shown in the above equation because some carbon and little nitrogen would also be used for cell synthesis.

Making use of the half reaction technique developed by McCarty (1975) for stoichiometric functions, Grady and Lim (1980) presented the following equation relating the

electron donor and acceptor, and cell synthesis stoichiometrically:

$$R = Rd - feRa - fsRc \quad (5)$$

where, fe and fs are the fractions of the electron donor used for maintenance energy and cell synthesis respectively, and R represents the overall stoichiometric reaction. The half reactions for methanol as electron donor (Rd), nitrate as electron acceptor (Ra), and for cell synthesis with nitrate as nitrogen source (Rc) are given below.

$$Rd: \quad 1/6\ CH_3OH + 1/6\ H_2O = 1/6\ CO_2 + H^+ + e^- \quad (6)$$

$$Ra: \quad 1/5\ NO_3^- + 6/5\ H^+ + e^- = 1/10\ N_2 + 3/5\ H_2O \quad (7)$$

$$Rc: \quad 1/28\ NO_3^- + 5/28\ CO_2 + 29/28\ H^+ + e^- = 1/28\ C_5H_7O_2N + 11/28\ H_2O \quad (8)$$

The values of fs and fe would be 0.28 and 0.72 respectively, to obtain the stoichiometric equation as presented by McCarty et al. (1969):

$$NO_3^- + 1.08\ CH_3OH + H^+ = 0.065\ C_5H_7O_2N + 0.47\ N_2 + 0.76\ CO_2 + 2.44\ H_2O \quad (9)$$

From equation (9), the stoichiometric methanol requirement for reduction of 1 mole of nitrate can be evaluated. On a mass basis, this value would be 2.47 mg methanol per mg nitrate-nitrogen. The amount of new cells synthesized would be (0.065) x (113) = 7.35 mg cells

(volatile solids) from (1.08) x (32) = 34.56 mg of methanol (51.84 mg expressed as COD). Therefore, cell yield will be (7.35)/(51.84) = 0.14 mg VS/mg COD removed. Assuming cells are 85 % volatile, cell yield expressed in terms of total suspended solids (SS) will be 0.16 mg SS per mg COD removed. Stensel et al. (1973) found the cell yield in their experiments to be 0.183 mg SS/mg COD at 20° C and 0.195 mg SS/mg COD at 30° C with methanol as substrate.

For autotrophic denitrification using thiosulfate as electron donor and bicarbonate as carbon source, the following stoichiometric relationship was calculated by Ross (1989):

$$NO_3^- + 0.79\ S_2O_3^- + 0.27\ HCO_3^- + 0.2\ H_2O = 0.05\ C_5H_7O_2N + 0.47\ N_2 + 1.56\ SO_4^{-2} + 0.28\ H^+ \quad (10)$$

Comparing equations (9) and (10), cell production in heterotrophic denitrification is seen to be ~22 % higher than that by autotrophic denitrification. However, heterotrophic denitrification yields end products that are in harmless gaseous form other than H_2O and cell mass, whereas autotrophic denitrification produces sulfate (1.56 moles per mole nitrate reduced). This may warrant another treatment unit for sulfate removal and hence costs may be more for autotrophic denitrification.

2.5 Denitrification Treatment Processes

Although the conversion mechanisms in the denitrifi-

cation process are dependent upon the type and physiology of the microorganism, the nature and conditions at which these reactions take place would also greatly affect process efficiency. In other words, the type of reactor and growth conditions within the reactor play an important role in microbial nitrate reduction. Suspended and attached growth processes for denitrification are considered in the following sections.

2.5.1 Suspended Growth Reactors

As the name implies, microorganisms are held in suspension within a reactor, without supporting media for microbial attachment. Suspended growth reactors are of various types such as completely mixed stirred tank reactors (CSTR), also known as activated sludge reactors (ASR), wash-out reactors [i.e. CSTRs without clarifiers (WOR)], and upflow sludge blanket reactors (UASB). UASB reactor, in which wastewater is forced in an upward direction through a thick anaerobic sludge mass, was used in this feasibility study.

2.5.1.1 Upflow Anoxic Sludge Blanket Reactors.

In 1961, Coulter et al. designed a reactor configuration containing a thick anaerobic sludge blanket for the upflow movement of wastewater (Lettinga et al., 1980). The precursor for this UASB system was the "biolytic" tank designed by Wilson and Phelps in 1910, which was very

similar to UASB in operation but apparently did not function well (Jewell, 1985).

UASB reactors were used for denitrification by two different research groups (Klapwijk et al. in the Netherlands, and Miyaji and Kato in Japan) at two different places around the same time in 1975 (Klapwijk et al., 1981). UASB process was used by Klapwijk et al. (1981) for denitrification with sodium acetate, alcoholic wastewater and domestic sewage as carbon sources. Lettinga et al. (1980) have shown that high removal capacity of the reactor is possible due to small reactor volume requirement, and recycling would not be necessary due to low linear velocity for fluidization of sludge particles. Some other advantages were also cited in favor of the UASB process, such as good settling characteristics of the sludge, saving capital and operating costs due to the absence of carrier particles and dilution/recycling needs. Besides, washout of biomass could be avoided because of less linear velocity compared with fluidized beds. However, slow or intermittent stirring in a UASB reactor would be necessary for the dispersion of sludge in the liquid phase.

2.5.2 Attached Growth Reactors

Attached growth systems were developed to solve some of the problems encountered in the suspended growth systems, such as relative instability, liquid-solid phase separation, and poor effluent quality. In an attached

growth system, as the name implies, biomass attaches itself to inert carrier particles, and performs biological operations with the wastewater that passes through. Such attached growth reactors usually have an upward flow of the liquid. The biofilm support media consist of such inert particles as ceramic, sand, plastic, activated carbon, coal or anthracite, glass, gravel, and diatomaceous earth. Carbon and diatomaceous earth are the most preferred for expanded bed reactors because they are less dense and hence would require lower energy for bed expansion than others.

2.5.2.1 Attached Film Expanded Bed Reactors. In 1971, Jewell proposed the concept of expansion of inert support media due only to attachment of biomass rather than expansion by fluidization. Unlike the fluidized bed which requires higher linear velocity for the fluidization of the bed at more than 100 % expansion, AFEB requires less than 25 % fluid expansion. The rest of the expansion in an AFEB is achieved through biofilm growth on the carrier media, with little entrapment of solids within the media. Therefore, for treating the same strength of waste matter, a fluidized bed would require more dilution to cause higher fluidization than an AFEB, and this might increase the operating costs. Besides, fluidization in a FBR is limited by washout of bioparticles at high upflow velocities. On the other hand, AFEB offers more surface area per unit volume and hence more biofilm growth. Another distinctive feature with the AFEB is the ability to handle solids while

largely eliminating clogging problems usually associated with anaerobic systems (Jewell et al., 1981; Clarkson, 1986). Also, an AFEB system provides for long sludge retention times with low HRTs (Kelly and Switzenbaum, 1984). This feature of AFEB makes it the only anaerobic process comparable to aerobic processes with the same HRTs for domestic sewage treatment (Jewell, 1985).

Four distinct phases exist within an AFEB reactor: the inert support media, the attached biofilm, the entrapped solids, and the clear supernatant liquid. A well established AFEB reactor would have the entrapped biomass actively engaged in hydrolyzing the particulate matter, if any, present in the wastewater, and the attached biofilm rapidly utilizing the solubilized substrate. The existence of such a symbiotic functioning between the entrapped solids and the attached biofilm was established by Morris and Jewell (1981) in their study on organic particulate removal with the AFEB.

Although information about heterotrophic denitrification in the AFEB is not available at this time, the process parameters for denitrification obtained from a number of fluidized bed reactors are available. Anaerobic process descriptions may be obtained from the work of Jewell and associates on the AFEB. A major goal of this research was to adapt the high-rate AFEB process to denitrification and compare results with other process configurations.

2.5.3 Other Treatment Processes

Some of the miscellaneous treatment processes employed for denitrification include anaerobic submerged (or flooded) filters (Bailey and Thomas, 1975) and algal columns or rotating disks (for nitrification) combined with packed bed reactors (Przytocka-Jusiak et al., 1984a, 1984b). The influent NO_3^-_N concentration in such studies did not exceed 500 mg/L and were primarily focused on defining the microbiology and kinetics of denitrification, and the feasibility of nitrification - denitrification as a two stage process, rather than attempting to achieve high-rate denitrification. However, Jewell and Cummings (1975) compared the performance of a CSTR with a submerged filter column (SFC) using NO_3^-_N concentrations of up to 4000 mg/L and showed that a nitrate removal rate of > 5.6 kg NO_3^-_N/ cu.m-day was possible with SFC.

2.6 Summary of Process Parameters

The operating and kinetic parameters for denitrification obtained from studies with several types of reactors are presented in Table I. For the sake of convenience and conformity, the term Ne representing 'nitrogen equivalent' will be used in this report in presenting the values. Ne is the total amount of oxidized nitrogen present as NO_3^- and NO_2^-. Such a term is also used here because of the presence of some nitrite in the influent and effluent.

The primary operating parameters of interest in this feasibility study were the influent concentration of Ne, HRT, volumetric loading rates, removal rates and removal efficiency of Ne and COD, the ratio of methanol consumption [expressed as COD of soluble organics (CODs)] to Ne removed, COD to volatile suspended solids (VSS) ratio of effluent particulate matter [(CODp/VSS), which represents biomass loss], and total alkalinity produced to Ne removed. The pertinent values are presented in Table I.

2.7 Inhibitions and Interferences

2.7.1 Nitrite Build-up

Both high-rate denitrification processes (Bode et al., 1987; Jeris et al., 1974) and systems which were used for removal of lower nitrate concentrations (Beccari et al., 1983; Huang et al., 1984; Strand et al., 1985; Harada et al., 1987; Wilderer et al., 1987) have reported measurable effluent nitrite concentrations. However, many denitrification systems produced negligible nitrite (< 5 mg NO_2^-_N/L) in effluent (Miyaji and Kato, 1973; Jeris and Owens, 1975; Jewell and Cummings, 1975; Bosman et al., 1978; Klapwijk et al., 1979; Bridle et al., 1980; Ramadori et al., 1987; Walker et al., 1989). Nitrite formation seems to be a function of operating kinetics or environmental conditions rather than reactor type. It is interesting to note that nitrite was found to accumulate in certain batch studies also (Monteith et al., 1980;

TABLE I

SUMMARY OF OPERATING PARAMETERS FOR DENITRIFICATION

Infl. Conc. mg Ne/L	HRT hours	Loading Rate kg Ne / cu.m-d	Removal Rate kg Ne / cu.m-d	Ne Rem. %	COD / Ne	COD Rem. %	Reactor Type	Organic Substrate	Remarks	Reference
180					7.25	70	UASB	Domes. Sewage		Klapwijk et al. (1979)
500			12.0		3.66	90	UASB	Fusel oil		Klapwijk et al. (1981)
858	3.2			89		98	UASB	Alc waste		Tettinga et al. (1980)
497	0.8	14.3	13.72	96.2			UASB		Autotrophic	Ross (1989)
900		7.5	7.20	98.6	3.00	91	UASB	Methanol		Miyaji and Kato (1975)
220	14 5			99	4.20		CSTR	Methanol		Aitken (1984)
50-100					4.10		CSTR	Methanol	Carbon limit.	Beccari et al (1983)
1220		14.7	14.7	100	7.00	62	CSTR	Methanol	Temp. 32°C	Bode et al (1987)
2100 10000	16 96			100 100	3.2 3.0	75 76	CSTR	Tetra Hydro Furfuryl Alc.	Pilot plant Full scale	Ramadori et al (1987)
1450			10.56 38.40		4.95		FBR	Molasses	Temp. 20°C Temp. 38°C	Bosman et al (1978)
30.2	0.1		6.80		3.60		FBR	Methanol		Jeris et al. (1974)
25.2	0.03	13.5 (Mean)	5.45 (Max.20.7)	95	4.20		FBR	Methanol		Jeris and Owens (1975)
5800			30-40				2 FBRs in series	Methanol	Temp. not reported	Walker et al. (1989)
17-3500	0.24 - 4.80	8.0	5.6	70	3.20		Submer. filter	Methanol		Jewell and Cummings (1975)
1000	2.40	12.14	12.14	100			PBR	Methanol		Blaszczyk et al. (1985)
773	1.10	17.46	17.23	98.7			AFEB		Autotrophic	Ross (1989)

Jaworowska-Deptuch et al., 1985).

Betlach and Tiedje (1981) offered a kinetic explanation for the accumulation of nitrite by studying species belonging to the genera *Alcaligenes*, *Pseudomonas*, and *Flavobacterium*. They suggested two possibilities, one being nitrate inhibition, and the other a lag between nitrate and nitrite reduction rates. The latter theory was supported from decreased nitrite reduction (and eventually accumulation of nitrite) by *Alcaligenes* sp. and *Pseudomonas fluoroscens*. Such a hypothesis was also used in developing a mathematical model and demonstrated later by lab experiments by Wilderer et al. (1987).

Bock et al. (1983) noted that the nitrite oxidase of *Nitrobacter* sp. may sometimes catalyze the reverse reaction and contribute to nitrite accumulation under anoxic conditions (Wilderer et al., 1987). Nitrite may also build up when microbes such as *Enterobacteriaceae*, *Bacilli*, and *Clostridia* that reduce nitrate assimilatively to nitrite are present in the mixture of bacterial population (Knowles, 1982).

Another study by Waki et al. (1980) revealed an initial accumulation of nitrite when the reactor environment was switched from aerobic to anaerobic. When glucose or any other organic substrate that can be utilized by fermentative bacteria serves as the carbon source for denitrification, nitrite accumulated in the medium (Jaworowska-Deptuch et al., 1985; Wilderer et al., 1985).

Blaszczyk et al. (1985) observed an accumulation of nitrite (~650 mg NO_2^-_N/L) when the influent concentration reached ~3000 mg NO_3^-_N/L and attributed this nitrite formation to the high-strength influent.

2.7.2 Oxygen Inhibition

Generally, strict anoxic conditions and the presence of nitrogen oxides in the medium are required for synthesis of denitrifying enzymes. As explained in Section 2.2.1, when molecular oxygen is available, the bacteria would respire aerobically and produce more ATP even if nitrogen oxides are present. However, if the amount of nitrate far exceeds the oxygen concentration, anaerobic respiration may become significant (Payne, 1981).

A study by Strand et al. (1985) showed that when cell counts were less than 0.5 x 10^9 cells/sq.cm in a fixed growth reactor, the presence of dissolved oxygen had depressed nitrate reduction. However, when the anaerobic biofilm was thick enough, (maximum 2 x 10^9 cells/sq.cm), dissolved oxygen could not penetrate the film and hence had no effect on denitrification.

Waki et al. (1980) observed a lag in the synthesis of reductases of nitrogen oxides when anaerobic conditions were imposed. Knowles (1982) also reported that reductases of NO_2^-, NO, and N_2O were more sensitive to O_2 than that of NO_3^-. A *Bacillus* sp. retained 30-40 % of its capacity to respire aerobically even while growing as a denitrifier

(Payne, 1981). Lam and Nicholas (1968) reported that while nitrate reductase activity was not hindered by O_2, nitrite reductase activity was strongly affected in *Micrococcus* (now *Paracoccus*) *denitrificans*. The threshold value on the minimum amount of O_2 required to repress denitrification cannot be ascertained because this value varies with organisms (Payne, 1981).

2.7.3 Other Interferences

Grady and Lim (1980) have reported that methanol could inhibit denitrification at concentrations higher than 3000 mg/L. However, such a high concentration of methanol would not be needed in a denitrification system and so is not of concern here. Besides, systems with recycling and completely mixed systems may to some extent dilute the influent methanol substrate concentrations to values much less than 3000 mg/L.

Due to increasing alkalinity during denitrification within a reactor, increased calcium carbonate ($CaCO_3$) precipitation may occur if the influent waste stream contains sufficient calcium. Such a deposition of $CaCO_3$ reduced nitrate removal efficiency in a FBR used for high-rate denitrification by Walker et al. (1989). Adjustments of pH within the reactor may alleviate this problem.

CHAPTER III

METHODS AND PROCEDURES

3.1 Scope of Study

In heterotrophic denitrification, microbes respire anaerobically by transferring electrons from an organic substrate such as methanol, reducing nitrate to nitrogen gas. Harmless end products - N_2, CO_2, and H_2O - are produced besides cell mass ($C_5H_7O_2N$); hence biological denitrification has been considered beneficial for wastewater treatment. Nutrients can be removed more economically by exploitation of a natural biological phenomenon than by physicochemical processes.

Several studies conducted on denitrification have been focused on defining the kinetics rather than attempting to achieve the highest possible loading and removal rates. Therefore, this study was conducted to demonstrate the feasibility of denitrification at high loading rates and high concentrations of nitrate-nitrogen. Suitable reactors should exhibit stability at high concentrations and high loading rates, minimal operational difficulties, suitability for microbial growth, and adaptability to continued operation. Based on these considerations, bench scale anaerobic attached film expanded bed and upflow

anoxic sludge blanket reactors were chosen for this study.

Planning, scheduling, design and development of experimental apparatus, establishment of biofilm attachment and sludge blanket, and conduct of the experiments occupied approximately 16 months. The sequence of activities is shown in the time diagram (Figure 1).

3.2 Materials and Methods

3.2.1 Experimental Apparatus

3.2.1.1 Upflow Anoxic Sludge Blanket Reactor. The UASB reactor was made of glass in a cylindrical shape with the bottom curved as a hemisphere. There was an outer cylinder fused with an inner one at 5.0 cm below the top of the latter, and the outlet was connected to the outer cylinder. The inner cylinder had a diameter of 10.0 cm and the outer cylinder, 14.0 cm. The volume of the inner cylinder, which was also the total volume of the reactor, was 2.5 L.

The feed solution to the reactor was pumped continuously from a 25 L glass bottle. The reactor contents were mixed continuously by a 20 x 20 cm Sargent-Welch magnetic stirrer. A caged stir bar (Fisher Scientific) was placed inside the reactor. The rate of stirring was set by observation prior to starting the actual experiment.

Feed solution containing the substrates and the necessary nutrients for microbial growth was pumped from

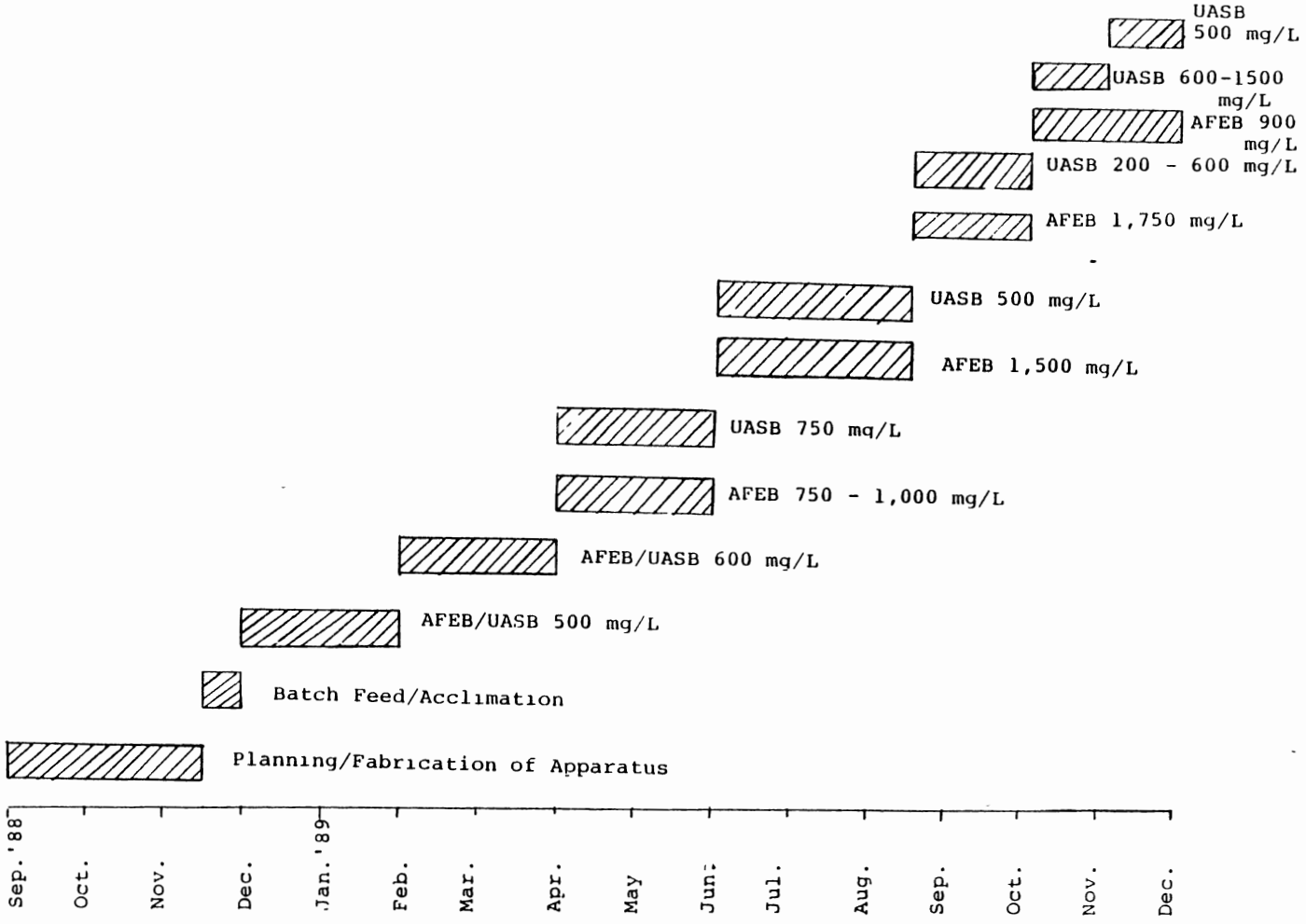


Figure 1. Time Diagram for the Sequence of Activities

the feed bottle through a positive displacement Masterflex pump (model No. 7553-60, Cole-Parmer). This feed pump contained a standard pump head (model No. 7016-20). Masterflex tubing was used in all the pump drive heads. The feed entered into the reactor through an inlet opening at the bottom of the reactor. The effluent overflowed from the top of the inner cylinder into the outer arrangement, and entered the waste collection bottle through the outlet.

An inverted plastic funnel was placed inside the inner cylinder near the top, providing a water seal for trapping the product gases. This funnel was connected to a Teflon gas storage bag to collect the escaping gases from the reactor. A line sketch and photograph of the UASB reactor are shown in Figures 2 and 3 respectively.

3.2.1.2 Attached Film Expanded Bed Reactor. The AFEB reactor was made of a styrene acrylonitrile Imhoff cone with a plastic cylindrical tube attached to the top. The overall height of the reactor was 60 cm, of which the cylindrical portion at the top was 15 cm and the tapered cone was 45 cm. An outlet was made in the cylindrical tube by melting a hole in it and fixing a polyethylene tubing connector in place with epoxy cement. This outlet was about 10.0 cm from the top of the tube. The total volume of the Imhoff cone was 1350 ml and that of the cylinder 400 ml for a total reactor volume of 1750 ml.

An inert support medium consisting of diatomaceous earth was used for attachment of biomass. Diatomaceous

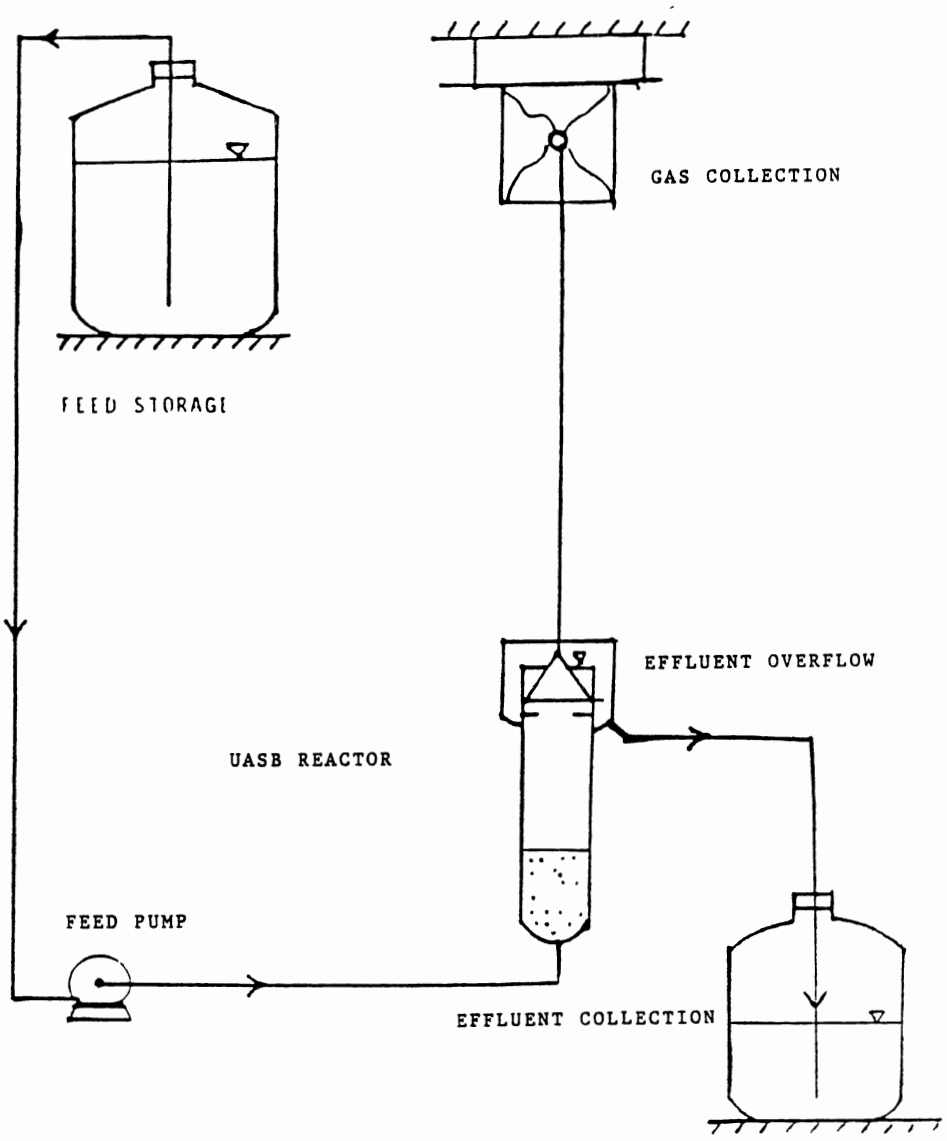


Figure 2. Schematic Diagram of the UASB Reactor System

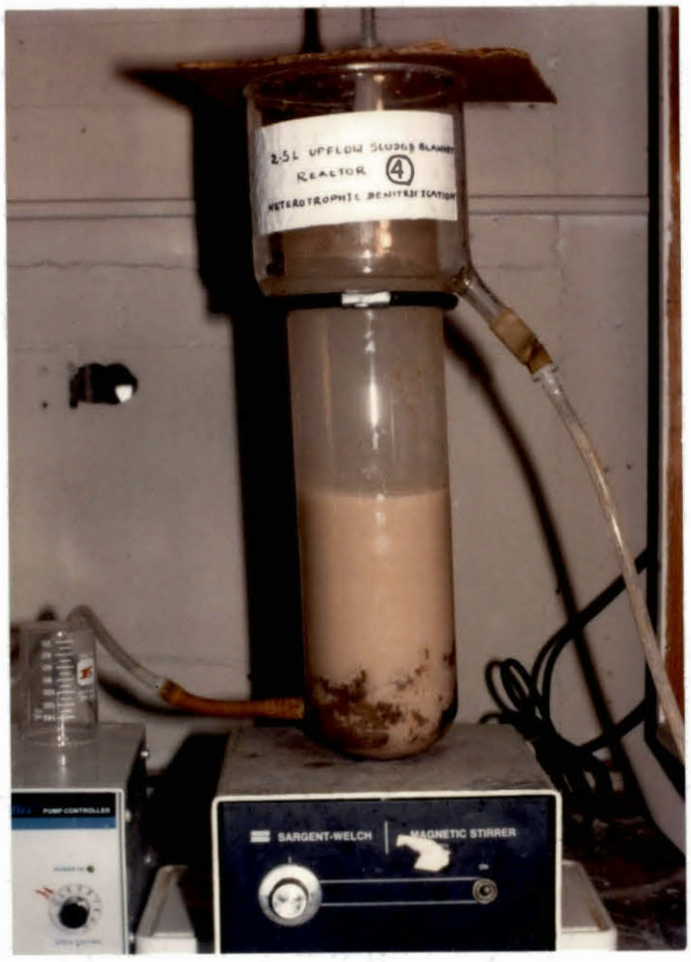


Figure 3. Photograph of the UASB Reactor

earth is composed of porous, siliceous particles which are the remains of diatoms. This is also known as 'infusorial earth', 'kieselguhr', or, 'triptolite' (Salle, 1973; Clarkson, 1986). This material was the choice for use in the reactor because of its low density, high porosity, high surface area to volume ratio, inertness, low cost, and resistance to ignition at 550° C (Clarkson, 1986).

Prior to placing this support medium into the reactor, the inert particles were sieved dry through a 28 mesh (589 μm) screen and washed well to eliminate very fine particles. This was done to select particles of size between 300 μm and 600 μm. The reactor was then filled with 350 ml of these particles and expanded to 20 % above its static volume by recycling the supernatant through the bottom of the reactor using a Masterflex pump (model No. 7553-60) fitted with a pump head (model No. 7015-20).

The feed solution from a 25 L glass bottle was pumped to the reactor with another Masterflex pump (model No. 7553-60) fitted with a Masterflex pumphead (model No. 7014-20). The feed entered at the bottom of the reactor axially upward. The recycling tube had its inlet end in the interior of the reactor, well below the outlet, but sufficiently above the expanded bed to avoid carryover of bioparticles along with the recirculated supernatant.

Similar to the UASB reactor configuration, an inverted plastic funnel was kept below the water surface and connected to the Teflon gas bag for collection of evolved

gases. A schematic diagram of the AFEB reactor configuration is shown in Figure 4, and a photograph of the actual bench scale reactor used in this study in Figure 5.

3.2.1.3 Apparatus for Gas Measurement. As mentioned in Sections 3.2.1.1 and 3.2.1.2, gases evolved as a result of denitrification in AFEB and UASB reactors were collected in Teflon gas bags. These bags were measured for gas volume with the use of a simple apparatus designed for this purpose.

This apparatus consisted of a graduated plastic tube floating in water inside a glass cylinder. There was an erect rigid glass pipe inside the glass cylinder along its axis, glued to its bottom, and placed inside the plastic tube. The gas from the Teflon bag passed through the glass pipe which would lift the floating tube by pressure. In order to facilitate the inward and upward movement of gas during measurement, and outward and downward expulsion of the measured gas, two plastic tubes with two plastic valves serving as inlet and outlet respectively, were connected to the pipe through the bottom of the cylinder. Two strings affixed to the tube passed through two smooth arms made of glass, and counterweights were attached to the end of these strings to make the tube buoyant and keep it stationary at any level in water. The configuration of this apparatus is shown as a line sketch in Figure 6 and photograph in Figure 7.

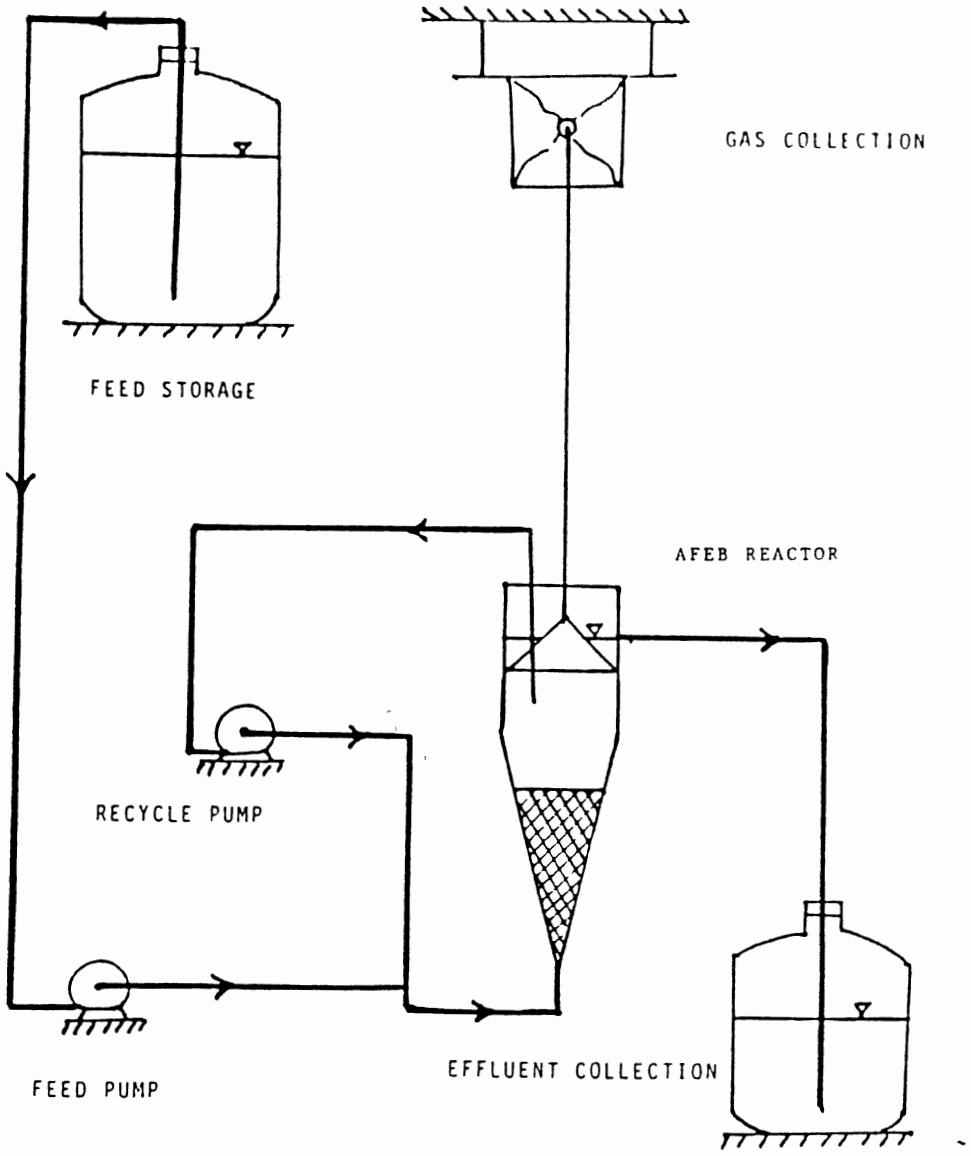


Figure 4. Schematic Diagram of the AFEB Reactor System

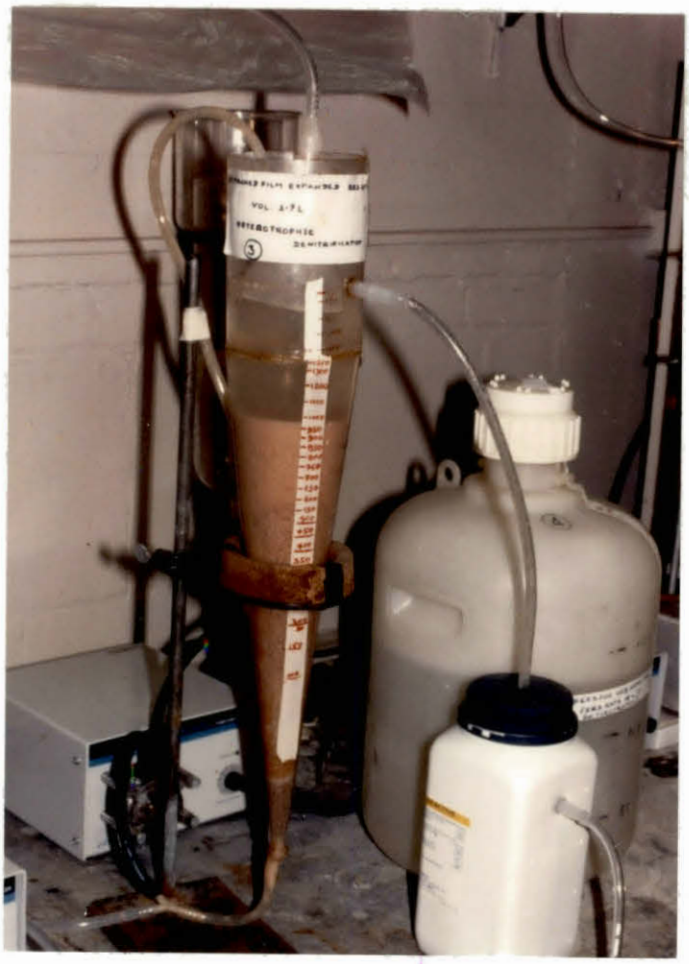


Figure 5. Photograph of the AFEB Reactor

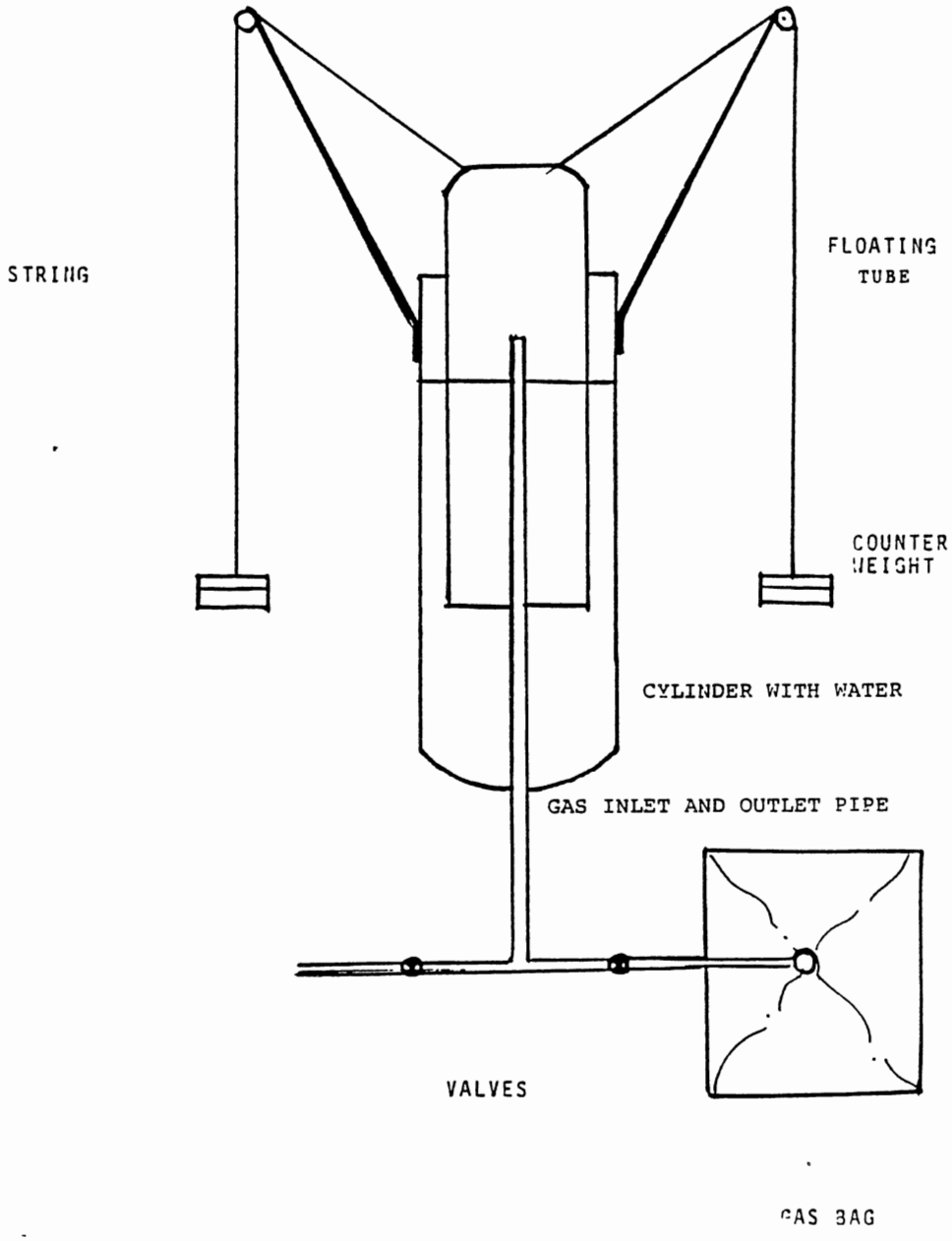


Figure 6. Schematic Diagram of the Gas Measuring Apparatus

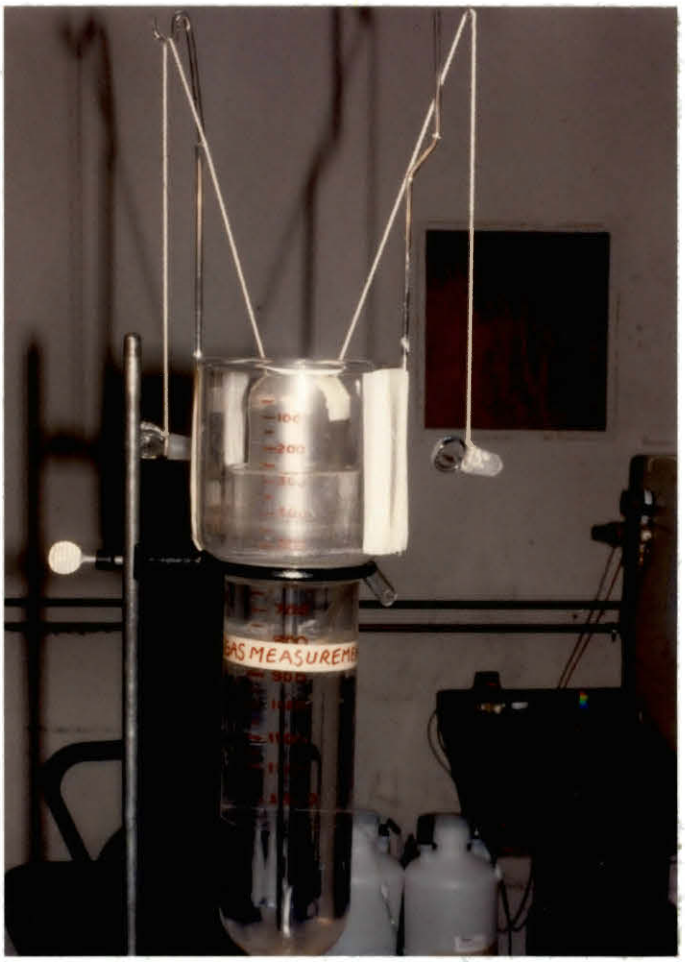


Figure 7. Photograph of the Gas Measuring Apparatus

3.3 Start-up Procedure

The seeding for heterotrophic denitrifiers was done in an inoculum developed for 2 weeks. This inoculum was obtained from the return activated sludge (liquid) from the secondary clarifier of the treatment plant at Ponca City, Oklahoma. The mixed liquor was collected in a 25 L glass bottle. The supernatant was decanted every day and refilled with the feed solution containing 4.0 g KNO_3/L and 1.66 g/L (2.1 ml/L) methanol. This batch feed was done for 2 weeks to select for heterotrophic denitrifiers.

At the end of 2 weeks, the acclimated biomass was fed into the AFEB and UASB reactors and filled up to half their volumes. Continuous operation of feed solution started at an initial feed concentration to both these reactors of 500 mg NO_3^-_N/L with a fluid retention time of 6 hours. To account for the loss of biomass from these reactors in the initial days, acclimated sludge from the seed culture was added every day into the reactors.

Development of a sludge blanket in the UASB reactor was delayed due to the need for appropriate mixing. The mixing had to be high enough to keep the bed in suspension, and slow enough to avoid washout of biomass. It took approximately 12 weeks for a clear blanket formation inside the UASB reactor. On the other hand, it took only 7 to 8 weeks for the AFEB reactor to display noticeable bed expansion due to biofilm attachment on the inert support media. The biomass was growing steadily by attaching

itself to the diatomaceous earth particles. The bed gradually expanded from 420 ml to 700 ml during this period.

3.4 Make-up of Feed Solution

Feed solution to both the reactors was made every day. The feed solution was made of potassium nitrate, methanol, magnesium sulfate ($MgSO_4.7H_2O$), ferrous sulfate ($FeSO_4.7H_2O$), and monobasic potassium phosphate (KH_2PO_4) dissolved in tap water. Each reactor had a feed tank capacity of 25 L. The necessary substrates and nutrients were dissolved separately in required amounts, and mixed well to make up 25 liters of solution.

The stoichiometric methanol requirement for denitrification in strict anoxic conditions was shown to be 2.47 mg per mg of NO_3^-_N [equation (11), Section 2.4]. However, when dissolved oxygen (DO) is present, a ratio of 3:1 has been proposed by several investigators, for denitrification and deoxygenation. Since tap water (DO ~8.0 mg/L) was used here to make up the feed solution, this ratio was adopted throughout this study. This ensured that methanol was not the limiting substrate. The composition of 500 mg NO_3^-_N/L feed solution is given in Table II.

3.5 Experimental Methods

3.5.1 Upflow Anoxic Sludge Blanket Reactor

The performance of the UASB reactor was not as

TABLE II

FEED RECIPE FOR 500 mg NO_3^-_N/L FEED SOLUTION

Ingredients	Concentration, g/L
Potassium nitrate	3.6
Methanol	1.5 (1.9 ml/L)
Magnesium sulfate	0.004
Ferrous sulfate	0.002
Monobasic potassium phosphate	0.035

expected in this study. Problems arose in maintaining a sludge blanket with the right speed of stirring. When stirring was slow, the blanket would become very dense and settle at the bottom of the reactor which halted the motion of the stirrer. If stirring speed was increased to provide continuous mixing, the blanket would be disturbed and excessive biomass was washed out. As a result, it took about 3 months before a clear thick blanket formed. Even at this stage, the denitrification efficiency was very low (~50 %), hence feed concentrations had to be decreased from 750 to 200 mg NO_3^-_N/L to achieve complete denitrification. Once steady operating conditions were established, the maximum concentration reached was 1500 mg NO_3^-_N/L at a constant HRT of 6 hours before failure occurred. In the second series of experiments, the feed concentration was kept constant at 500 mg NO_3^-_N/L and the HRTs varied from 6 hours to 1 hour.

3.5.2 Attached Film Expanded Bed Reactor

Contrary to the performance of the UASB reactor, the AFEB reactor maintained stable operating conditions from the beginning. Initial bed expansion due to 20 % fluidization was 420 ml and the bed volume gradually increased to 700 ml due to biofilm growth on the support media in a period of 8 weeks. Over the next six weeks, the growth was very rapid and steady. The unrestricted bed volume reached 1500 ml during this period before one third

volume of the bed was removed for conducting tests at steady-state conditions. The bioparticles were near spherical, light, densely coated, and mostly uniform in size. In order to maintain a constant bed volume within the reactor, the extra growth was removed periodically.

In the first batch of operations, the concentrations were increased from 500 to 1750 mg NO_3^-_N/L for a constant HRT of 3.43 hours before failure conditions were noticed. Although the system was stable, a drastic drop in efficiency prompted the termination of this first set of experiments.

The second set of experiments was performed by varying the HRTs from 3.43 hours to 0.51 hour at a fixed concentration of 900 mg NO_3^-_N/L. In this series of tests, operational difficulties such as bioparticle wash-out and clogging of the effluent port were encountered at the last two HRTs (1.03 and 0.51 hour). As a result, the effective bed volume decreased to 600 ml. The operations had to be stopped at this point due to such problems.

3.5.3 Analytical Techniques

3.5.3.1 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). In order to determine TSS, about 300 ml of effluent was collected from the reactors. This was well shaken, and a known volume, usually 100 ml, was

taken for solids analysis. Samples were filtered in Whatman glass microfibre filters (4.25 cm), and ignited at 103° C in an oven (Thelco-Precision Scientific) for 2 hours (minimum). Desiccators (Boekel) were used for cooling all samples.

3.5.3.2 Volatile Suspended Solids. The amount of volatile suspended solids (VSS) present in the effluent samples was determined according to the methods described in Standard Methods, Section 209 D.3 (APHA et al., 1985). The filtered residue from samples taken for TSS determination (Section 3.5.3.1) was used for VSS determination. Ignition at 550° C was done in a muffle furnace (Moldatherm - Lindberg, serial No. 878041).

3.5.3.3 Attached Biomass. The attached biomass analysis was done according to the procedures described by Clarkson (1986). Samples for this analysis were taken from the center of the expanded bed by a wide mouth 25 ml pipet and transferred to 10 ml wide bore graduated cylinders (Kimble). These cylinders were then gently tapped and spun several times to pack the samples. During the process of compaction, particles were added or subtracted and the tamping procedure continued until exactly 5.0 ml of the packed bed particles was obtained in each sample. The supernatant was poured off and the sample was then transferred to an ashed, preweighed, porcelain drying dish by sluicing it out with a jet of deionized water from a

wash bottle. The stream of water from the wash bottle was used to vigorously shake the particles to loosen the entrapped solids from the attached biomass. The supernatant containing loose solids was shifted to other drying dishes. Special attention was paid not to remove the support particles from their original dish. These processes were repeated until further washing produced no additional loose solids.

The dishes containing these samples were subjected to total solids and volatile solids determination procedures described in Section 3.5.3.2. Tests for blanks were also run simultaneously whenever attached biomass analysis was done. Blanks consisted of diatomaceous earth particles without biomass that had been sieved and prepared along with those which were used in the AFEB reactor. These blanks were kept in a buffer solution at room temperature. Tests on blank particles were essential to account for the hygroscopically bound water in the diatomaceous earth in performing the solids calculations. Duplicates of blanks and samples were analyzed each time. The ashed and desiccated samples were finally rehydrated with deionized water and transferred to the graduated cylinders. After necessary tamping for consolidation, the final volume of the rehydrated sample was recorded.

3.5.3.4 pH. pH readings of influent and effluent (filtered) samples were recorded using an Orion digital ion-analyzer (model No. 501). This pH meter had a single

electrode and digital display.

3.5.3.5 Alkalinity. The influent and effluent (filtered) samples were also tested for alkalinity by titration with 0.025 N sulfuric acid. The end point of titration was determined with phenolphthalein (for phenolphthalein alkalinity) and bromcresol green-methyl red (for total alkalinity). These indicators were available in pillows (Hach). The methods followed for titration were according to procedures described in Standard Methods, Section 403.4.a (APHA et al., 1985).

3.5.3.6 Nitrate and Nitrite. The influent and effluent (filtered) samples were analyzed for nitrate and nitrite using a Dionex ion chromatograph, series 2000 i/SP. These anions were measured according to the procedures outlined in Standard Methods, Section 429.4 (APHA et al., 1986).

3.5.3.7 Chemical Oxygen Demand. Methanol was the only organic carbon source used for denitrification in this study and this was measured in terms of COD. The influent COD (COD_{in}) and effluent soluble COD (CODs) correspond to the methanol and nitrite (if any) amounts present in them. Particulate COD (CODp) was calculated by subtracting CODs from effluent total COD, and this was related to the effluent cell mass expressed in terms of VSS.

COD analysis was made according to the procedures described in Standard Methods, Section 508 C (APHA et al.,

1986). The only deviation from the procedure described in this section was the transferring of digested, cooled samples from the culture tubes to open cuvets for measurement of absorbance in the spectrophotometer. This was done to facilitate the deposition of a white precipitate at the bottom of the cuvets which otherwise might interfere (if not tapped properly) with the absorbance readings taken directly with the culture tubes. In this method, the digested and cooled samples from the culture tubes were transferred to previously cleaned open cuvets and let stand for 1 or 2 hours before taking the readings on spectrophotometer. This method (transferring of contents) was adopted after making sure that the readings obtained in both the procedures (i.e. reading absorbance by directly placing the culture tubes in the spectrometer and by placing cuvets with the transferred contents) were the same.

Culture tubes 16 x 125 mm (Kimax) were used for COD samples and 13 x 100 mm cuvets were used in a Spectronic 20 spectrophotometer (Milton Roy Company) for absorbance measurement. Disposable teflon-lined screw caps (Kimax) were used for sealing the culture tubes. For digestion of samples for 2 hours at 150° C, a Thelco (Precision Scientific - Model 17) oven was used. In this study, the total final volume of each sample was 7.5 ml (diluted sample 2.5 ml, digestion solution 1.5 ml, and sulfuric acid reagent 3.5 ml). COD was measured on the total influent,

filtered effluent and unfiltered effluent samples. Because this colorimetric COD analysis is accurate in the range of 0 - 1000 mg/L, appropriate dilutions of the samples were prepared for each test.

The concentration of the initially prepared stock solution (potassium hydrogen phthalate) was 500 mg COD/L and standard solutions of concentrations 500, 250, 200, 150, 100, and 50 mg/L were prepared by dilution with deionized water. These standard solutions along with the blank were used for calibration of the standard curve each time COD analyses were performed. The diluted sample was measured in a 2.5 ml pipet (Fisher) using a pipet-pump (Bel-Art products), and separate pipets were used for different samples. The sulfuric acid reagent and the digestion solution were dispensed from Repipet-Dispenser (Lab Industries) containers of volume about 750 ml each. The repipet dispenser tubes had 20 ml and 10 ml capacities respectively. For soluble COD, samples were filtered with Whatman glass microfibre filters (4.25 cm).

3.5.3.8 Gas Volume Measurement. Gases evolved as a result of anaerobic respiration from the reactors were collected through the previously described inverted funnel arrangement connected to Teflon bags (Section 3.2.1.3). The amount of gas collected was measured at specified time intervals (usually for a 24 hour period). Prior to measuring the gas volume, the calibrated plastic tube inside the glass cylinder would be set at zero level in

water. After closing the outlet valve of this apparatus, the Teflon bag was connected to the inlet. Counterweights and small additional weights were then placed in the strings to withdraw gas from the Teflon bag thereby moving the floating tube upward. The gas would move upward through the glass pipe and push the tube upward in this process. Once the tube has reached the top, the inlet would be closed, additional weights from the strings removed, and the volume of escaped gas recorded. Now the outlet would be opened to let the entrapped gas (within the tube) into the atmosphere and bring the tube back to zero level. This was usually done by placing some small weights at the top of the tube. This process was repeated until all the gas in the bag had been measured.

CHAPTER IV

RESULTS

4.1 Study Objectives

The primary goal of this research was to study the feasibility of high-strength/high-rate heterotrophic denitrification using bench scale AFEB and UASB reactors. This investigation also addressed the maximum influent nitrate strength that could be treated, maximum possible rates of Ne and COD removal that could be achieved, and establishment of the relationship between operating parameters at steady state conditions. These objectives were accomplished by conducting the experiments in two phases.

The first set of operations was conducted at a constant HRT in order to determine the highest possible Ne feed concentration that could be treated before failure occurred. The second part of the study was conducted at a fixed lower concentration to achieve the highest loading/ removal rates in terms of Ne and COD before treatment efficiency dropped significantly.

Data were collected in each phase of this study and the relationship of the process parameters (as explained in Section 2.6 of Literature Review) was established. These

parameters were then compared with those obtained from other similar studies, and with the stoichiometric values. The performance of both the reactors was compared for their suitability and adaptability at high loading rates. These are discussed in detail in Chapter VI.

Steady state conditions were established prior to collecting data. As explained in the literature (Mulcahy, 1980), steady state conditions were considered to be attained when there was no significant change in the removal efficiency and other parameters for a particular loading rate, after at least 10 hydraulic retention periods. Since many readings (at least 3) were recorded for each steady state run, the average value of all data obtained for a particular loading rate is reported here.

4.2 Upflow Anoxic Sludge Blanket Reactor

The operation of the UASB reactor required careful supervision throughout this study. Especially, control of the stirring arrangement played a vital role in the biomass retention within the reactor. For example, when mixing speed was high, this caused an upheaval of the sludge blanket to the top of the reactor, and as a result, excess biomass was washed out with the effluent. On the other hand, if stirring was slow, the biomass settled at the bottom with the formation of a very dense, thick blanket, and consequently hindered the continuous mixing of the reactor contents. Therefore, setting the right speed for

the stirrer formed the baseline for starting the experiment in this reactor. However, once an adequate mixing speed was attained, operation and data collection were quite simple.

For the UASB reactor, at a constant HRT of 6 hours, the feed concentration varied from 200 to 1500 mg Ne/L before the efficiency dropped due to nitrite accumulation in the effluent. The loading rates increased from 0.8 to 5.94 kg Ne/cu.m-d while the removal rates ranged from 0.8 to 3.54 kg Ne/cu.m-d. For this period, COD loading rates varied between 5.21 and 27.54 kg COD/cu.m-d while the removal rates were between 4.39 and 11.57 kg COD/cu.m-d. The data gathered for this part of the study are summarized in Table III. Figures 8 and 9 depict the Ne removal rates and reduction efficiencies for this HRT of 6 hours. Figures 10 and 11 show the corresponding COD removal rates and efficiencies. As can be seen for both Ne and COD, the removal rates reached peak values before dropping, whereas the reduction efficiencies were continuously decreasing. The paths described by both Ne and COD for corresponding removal rates and efficiencies are quite similar.

For the second batch of the study, feed concentration remained constant at 500 mg Ne/L while the HRTs varied from 6 hours to 1 hour. During this time, the loading rates increased from 2.05 to 11.95 kg Ne/cu.m-d, and the removal rates from 1.49 to 4.49 kg Ne/cu.m-d. In terms of COD, the loading rates changed from 9.72 to 55.10 kg COD/cu.m-d, and

TABLE III

SUMMARY OF RESULTS OF THE UASB REACTOR AT CONSTANT HYDRAULIC RETENTION TIME

Influent Concentration mg Ne/L	Ne Consumed mg/L	Ne Consumed %	HRT hr	Gas collected L/d	Loading Rate kg Ne/cu.m-d	Removal Rate kg Ne/cu.m-d	Total Alkalinity produced mg $CaCO_3$/L	Tot Alk/Ne
199	199	100	6		0.80	0.80	651	3.28
291	290	99.7	6		1.16	1.16	874	3.01
402	401	99.8	6		1.61	1.61	1420	3.54
592	409	69.1	6		2.37	1.64	1463	3.58
782	597	76.3	6		3.13	2.39		
1030	792	76.9	6	2.4	4.12	3.17		
1308	885	67.7	6	3.1	5.23	3.54	3161	3.57
1485	664	44.7	6	1.6	5.94	2.66		

TABLE III (Continued)

Influent COD mg/L	Influent CH_3OH mg/L	COD_{in}/CH_3OH	CODs Consumed mg/L	CODs Consumed %	COD/Ne	Loading Rate kg COD/cu.m-d	Removal Rate kg COD/cu.m-d	COD_P mg/L	VSS mg/L	COD_P/VSS
	600								28	
1302	900	1.45	1096	84.2	3.78	5.21	4.39		30	
1752	1200	1.46	1517	86.6	3.78	7.01	6.07	145	89	1.63
2472	1800	1.37	1453	58.8	3.55	9.89	5.82	158	103	1.53
3273	2325	1.41	2107	64.4	3.53	13.09	8.43	109	69	1.58
4275	3000	1.43	2828	66.2	3.57	17.10	11.32	169	112	1.51
5318	3900	1.36	2891	54.4	3.27	21.27	11.57	209	145	1.44
6885	4500	1.53	2281	33.1	3.44	27.54	9.12	151	109	1.39

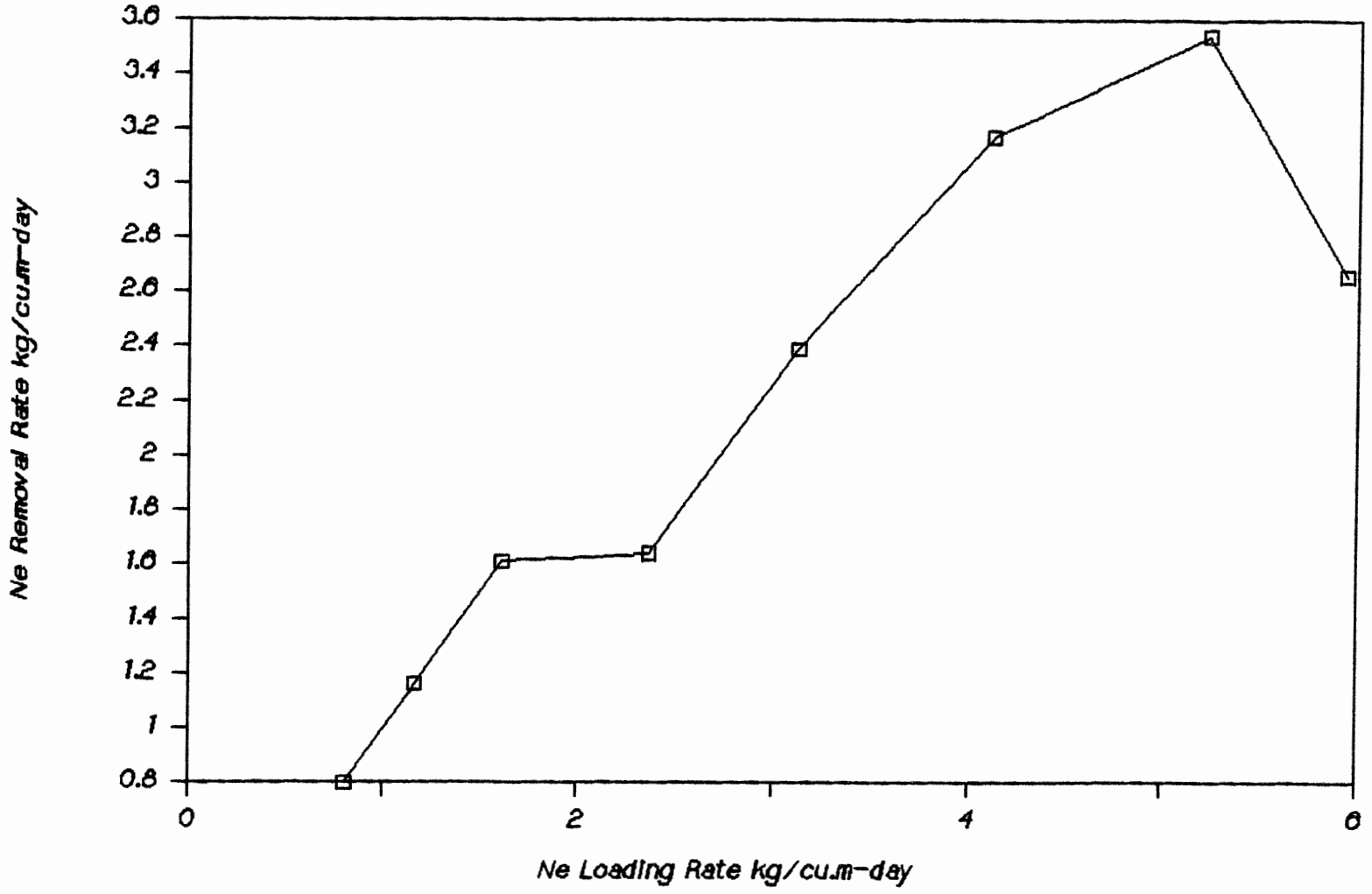


Figure 8. Denitrification Rates in the UASB Reactor for Constant 6 Hour HRT

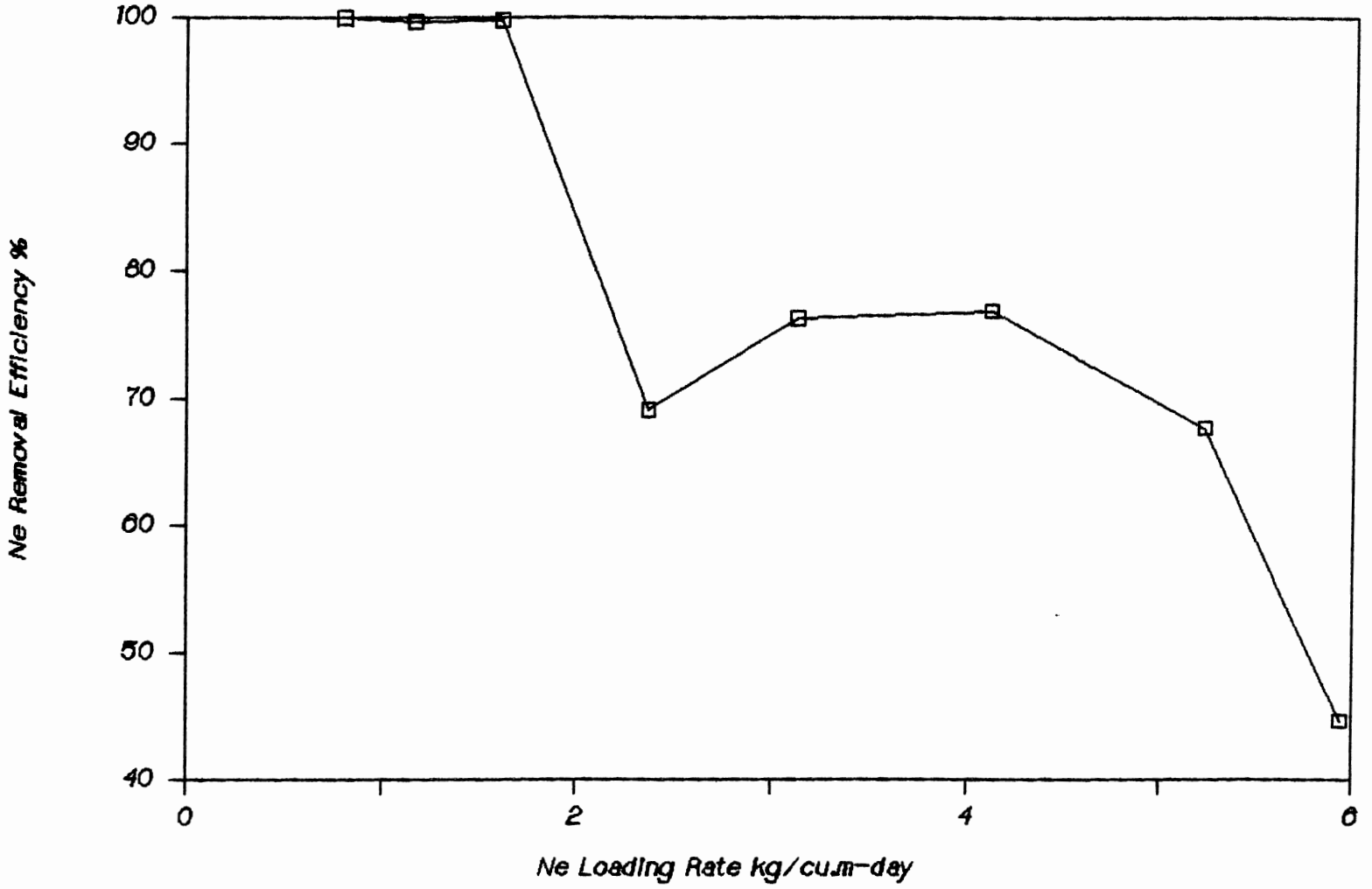


Figure 9. Denitrification Efficiencies in the UASB Reactor for Constant 6 Hour HRT

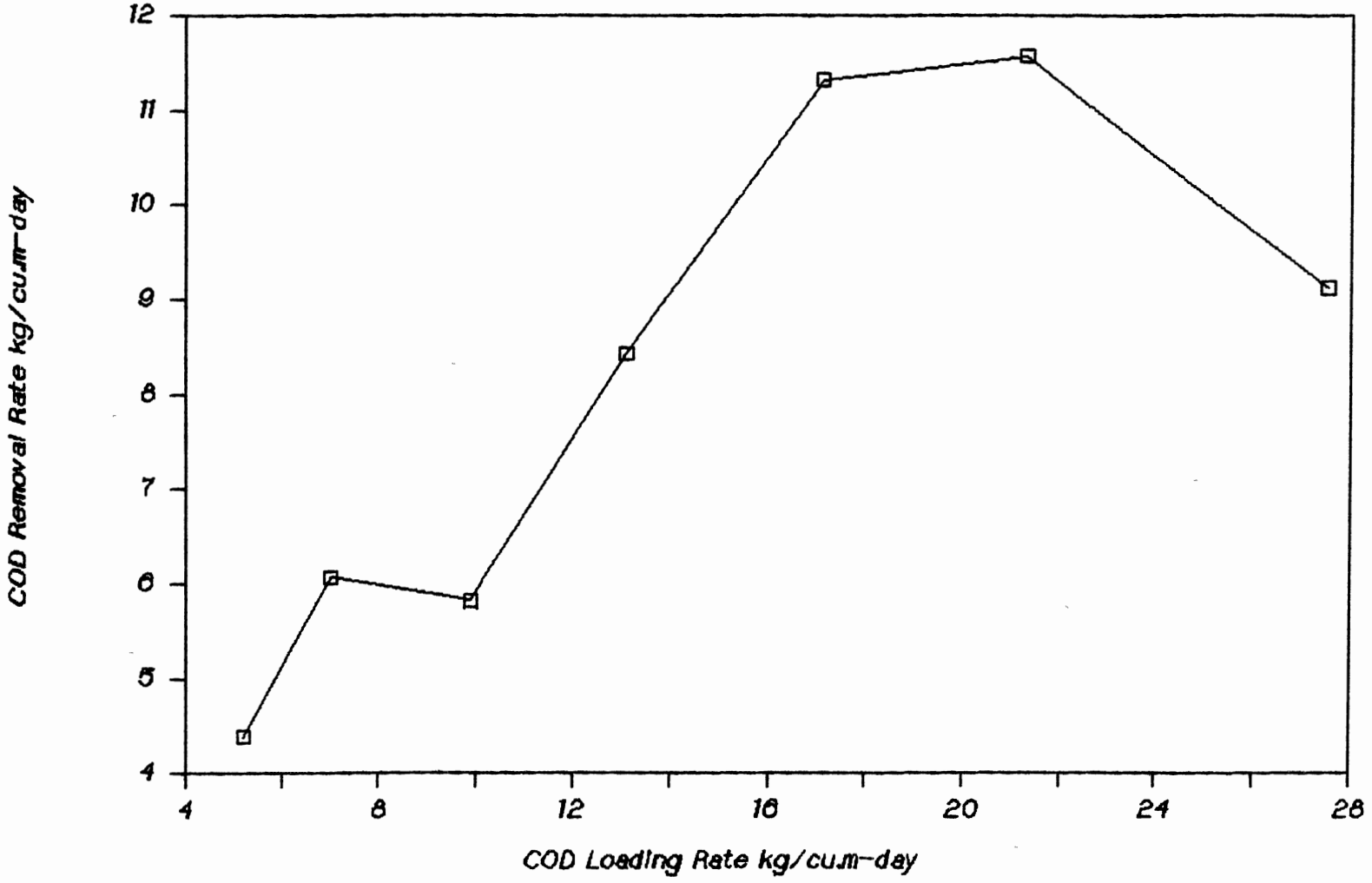


Figure 10. COD Removal Rates in the UASB Reactor for Constant 6 Hour HRT

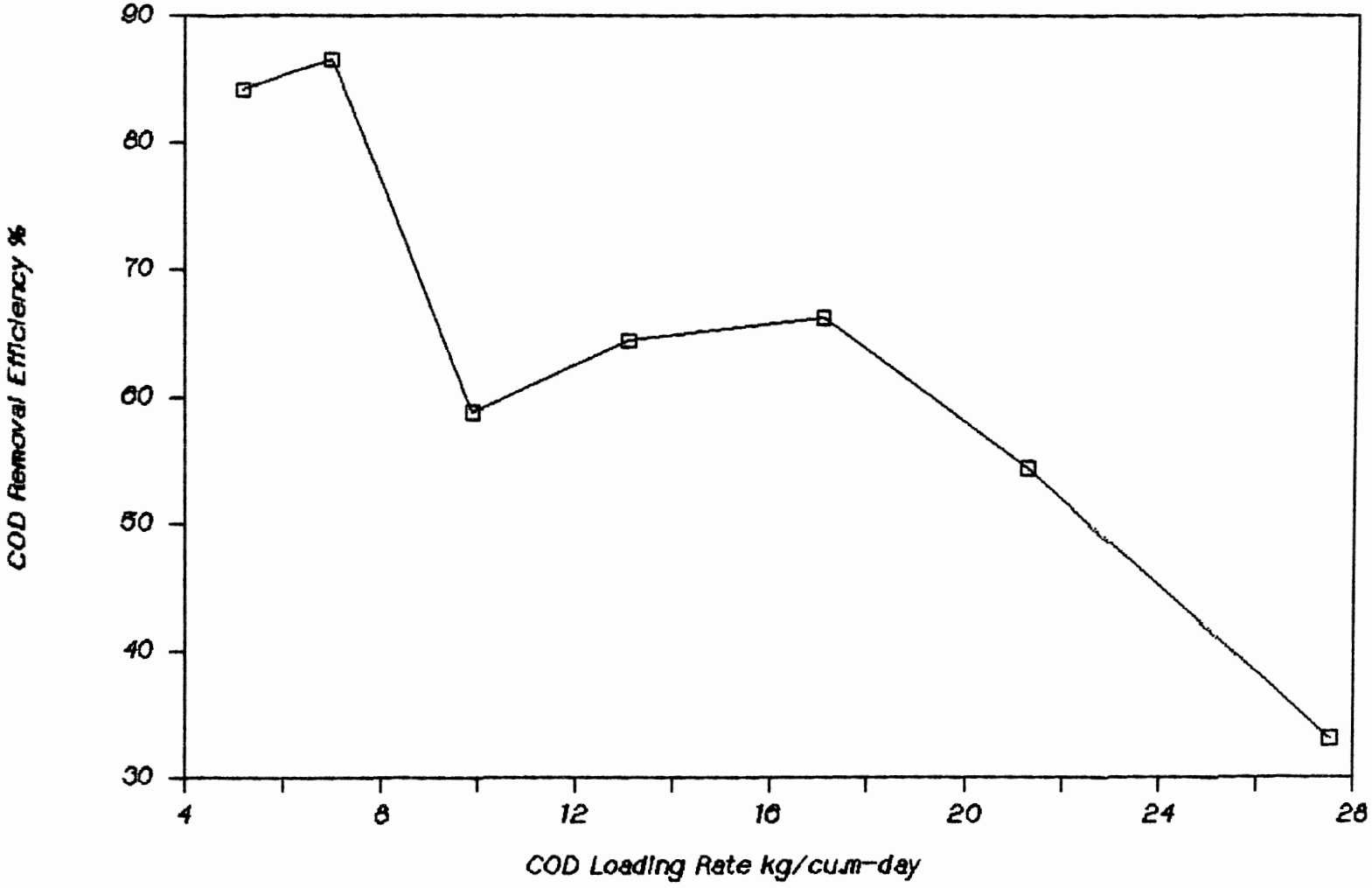


Figure 11. COD Removal Efficiencies in the UASB Reactor for Constant 6 Hour HRT

the removal rates between 5.30 and 15.74 kg COD/cu.m-d. These data are summarized in Table IV. Plots of loading vs. removal rates and loading vs. conversion efficiencies for Ne and COD are shown in Figures 12 - 15.

4.3 Attached Film Expanded Bed Reactor

Compared to UASB, the performance of the AFEB reactor was superior, and offered stable operating conditions for almost the entire study period. Experimental protocols for this reactor were similar to the two-phase UASB test procedure. When the first series of experiments was conducted, no operational difficulties were encountered, and the supernatant was almost clear.

Only during the penultimate run for the second set of tests (HRT 1.03 hours) did bioparticles start to escape in the effluent. This could possibly be due to the entrapment of a large amount of gas within the bed at this high loading rate (~22 kg Ne/cu.m-d), which attempted to escape and carried particles with it. Attached biomass was floating at the top of the reactor at this stage, and hence expanded bed volume decreased to 900 ml. When the HRT was reduced to 0.51 hour, a large mass of loose solids was seen to be occupying the top 300 ml of the bed besides some floating bioparticles near the effluent port. These loose solids were removed when tests were conducted and hence the effective bed volume was only 600 ml at this stage. Although the removal rates in terms of Ne and COD were very

TABLE IV

SUMMARY OF RESULTS OF THE UASB REACTOR AT CONSTANT FEED CONCENTRATION

Influent Concentration mg Ne/L	Ne Consumed mg/L	Ne Consumed %	HRT hour	Gas collected L/d	Loading Rate kg Ne/cu.m-d	Removal Rate kg Ne/cu.m-d	Total Alkalinity Produced mg $CaCO_3$/L	Tot Alk/Ne
513	373	72.7	6		2.05	1.49	1626	4.36
504	363	72.0	4	1.8	3.02	2.18	1498	4.13
520	286	55.0	3		4.16	2.29	1156	4.04
522	281	53.8	1.5	4.0	8.35	4.49	1258	4.48
498	133	26.7	1		11.95	3.19	603	4.53

TABLE IV (Continued)

Infl. COD mg/L	Infl. CH_3OH mg/L	COD_{in}/CH_3OH	CODs mg/L	Consumed %	COD/Ne	Loading Rate kg COD/cu.m-d	Removal Rate kg COD/cu.m-d	COD_p mg/L	VSS mg/L	COD_p/VSS
2429	1500	1.62	1326	54.6	3.55	9.72	5.30	82	57	1.44
2245	1500	1.50	1300	57.9	3.58	13.47	7.80	146.3	104	1.41
2227	1500	1.48	1009	45.3	3.53	17.82	8.07	151.1	134	1.13
2093	1500	1.40	983	47.0	3.50	33.49	15.74	138.8	104	1.33
2296	1500	1.53	468	20.4	3.52	55.10	11.24	204.3	142	1.44

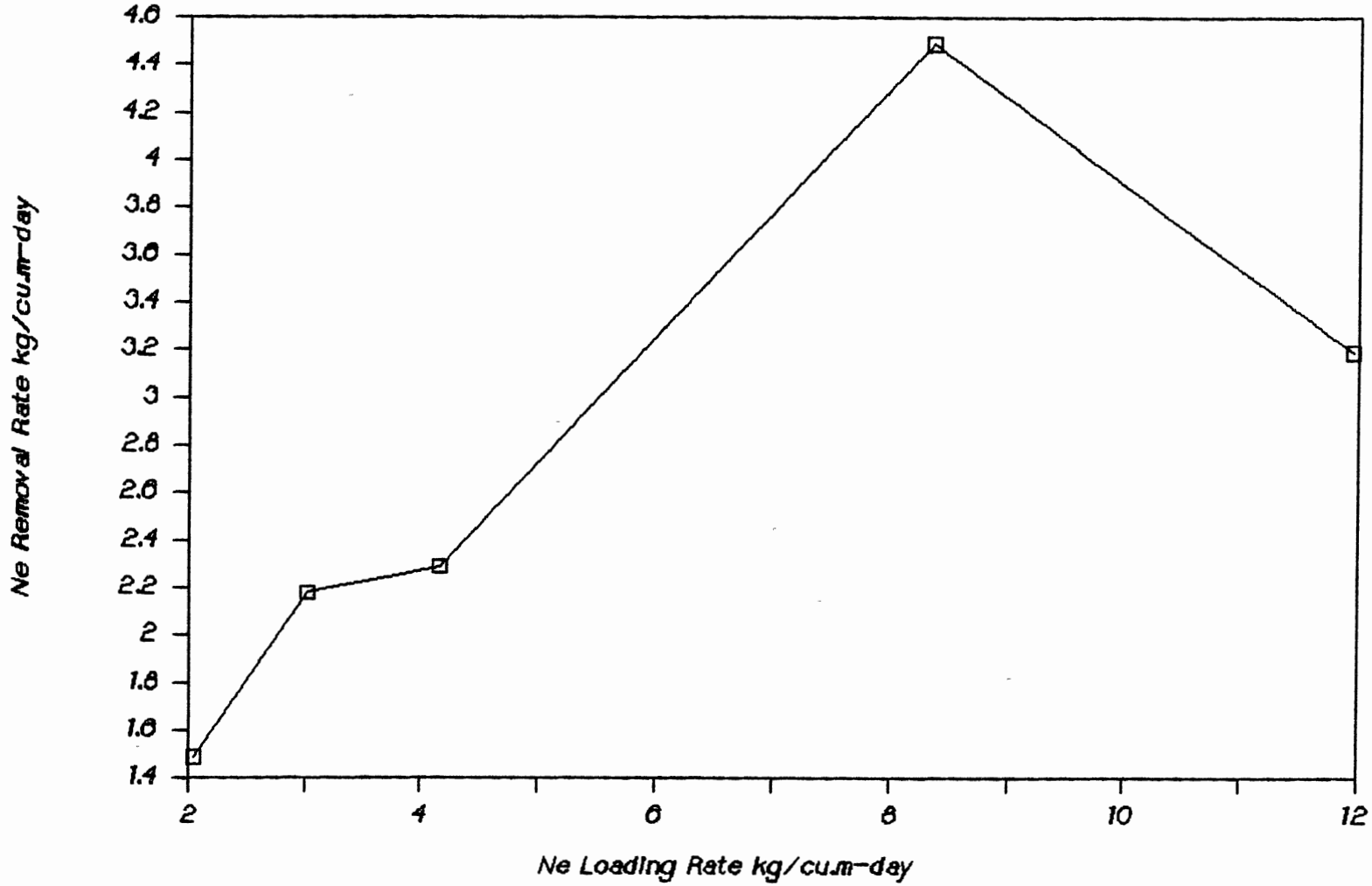


Figure 12. Denitrification Rates in the UASB Reactor for Constant 500 mg Ne/L Feed Concentration

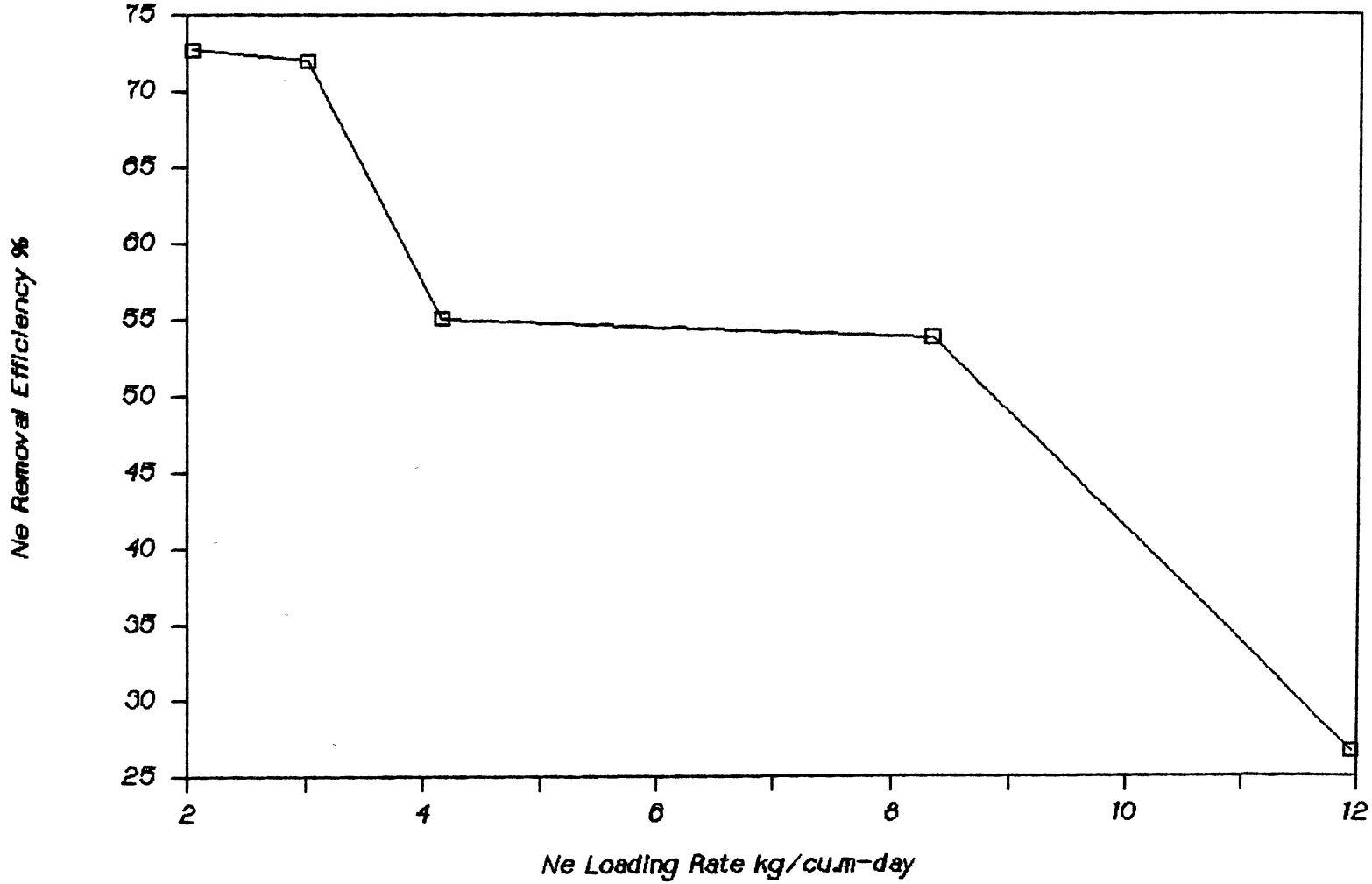


Figure 13. Denitrification Efficiencies in the UASB Reactor for Constant 500 mg Ne/L Feed Concentration

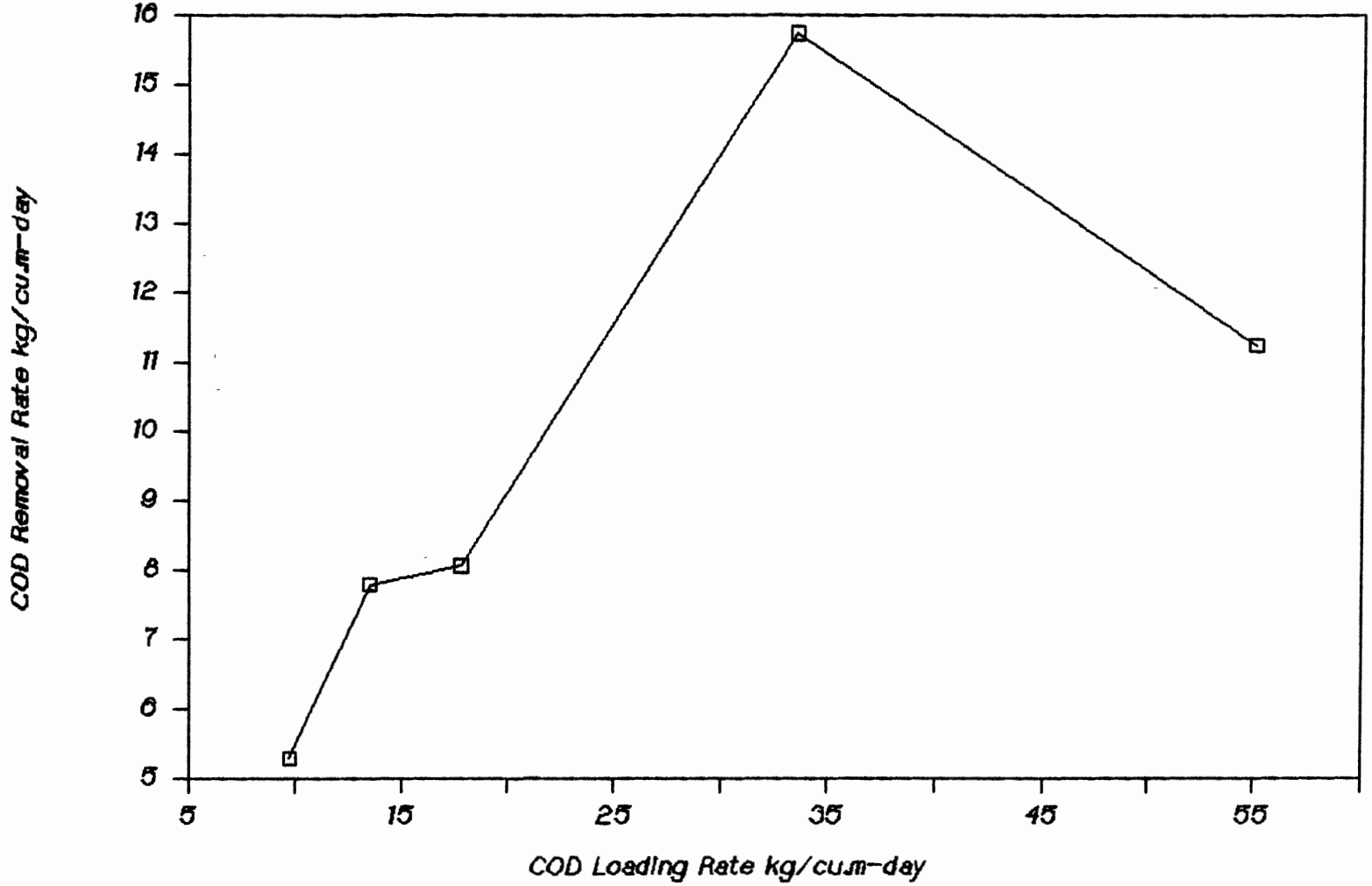


Figure 14. COD Removal Rates in the UASB Reactor for Constant 500 mg Ne/L Feed Concentration

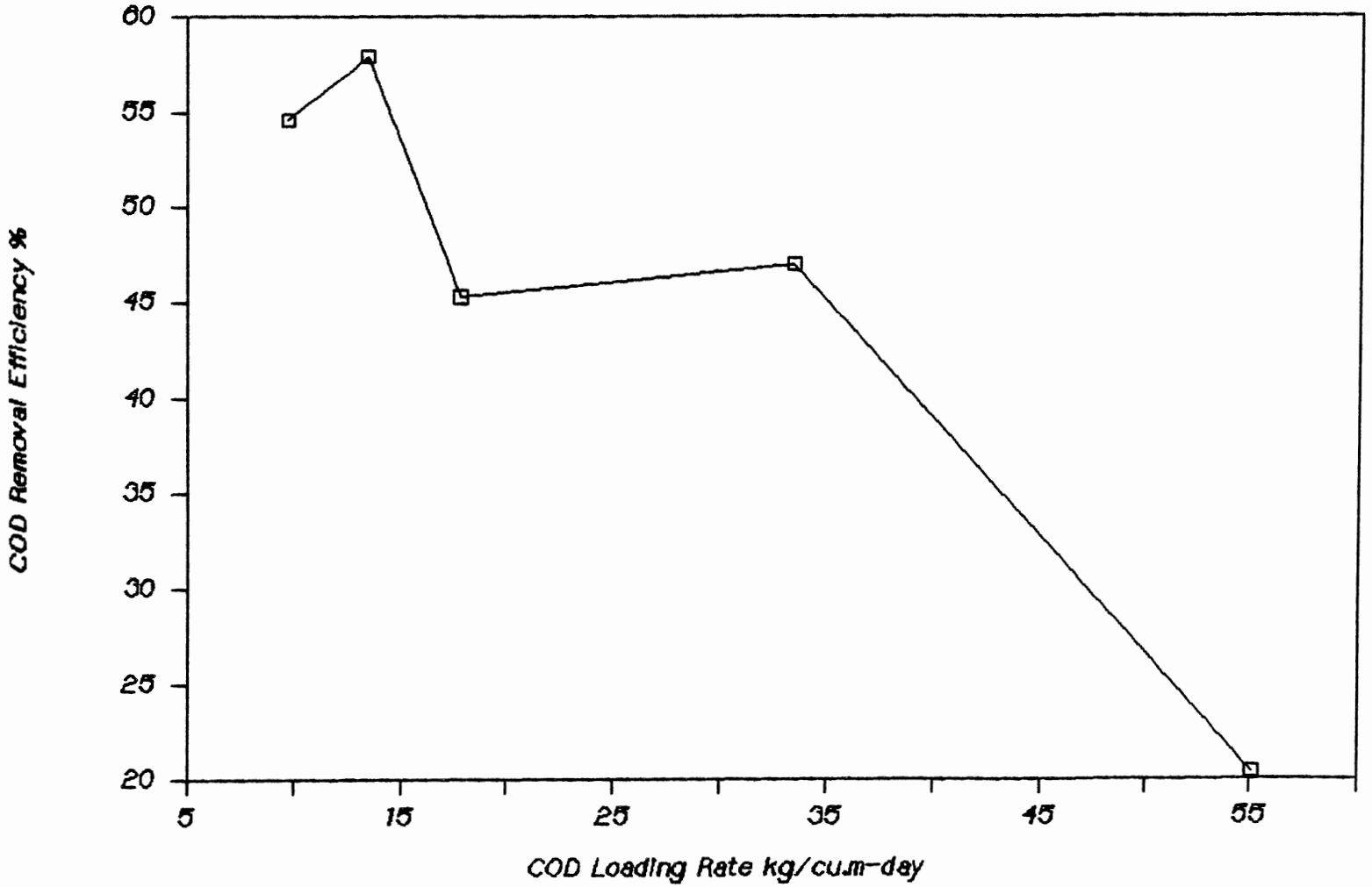


Figure 15. COD Removal Efficiencies in the UASB Reactor for Constant 500 mg Ne/L Feed Concentration

high, experiments at this stage had to be stopped owing to the biomass retention problems. The removal efficiencies, however, were very low at this point.

The total expanded bed volume was taken as the baseline for determining the loading and removal rates, and the hydraulic retention time. In doing so, no attempt was made for correcting the volume occupied by the inert particles and the biomass. This method is in accordance with that in previous AFEB studies (Clarkson, 1986). Such a method is actually more meaningful and comparable with other systems than counting the total volume of the reactor.

The data gathered during the first phase of this study are summarized in Table V. The HRT was fixed at 3.43 hours, and the feed strength was varied from 700 to 1750 mg Ne/L. Correspondingly, loading rates increased from 5.36 to 12.24 kg Ne/cu.m-d, and removal rates ranged between 5.35 and 10.61 kg Ne/cu.m-d. The Ne removal efficiencies were high for this duration and ranged between 90 and 100 % except the last feed concentration. Figures 16 and 17 were drawn to show the Ne removal rates and efficiencies, the values for which were obtained from Table V. In terms of COD, the loading rates averaged between 29.45 and 51.84 kg COD/cu.m-d while the removal rates ranged between 25.35 and 35.89 kg COD/cu.m-d. The corresponding COD removal rates and efficiencies are shown in Figures 18 and 19.

For the second phase of experiments, feed

TABLE V

SUMMARY OF RESULTS OF THE AFEB REACTOR AT CONSTANT HYDRAULIC RETENTION TIME

Infl. Conc. mg Ne / L	Ne Consumed mg / L	Ne Consumed %	Exp. Bed Vol. ml	HRT hour	Att. Biomass mg VS / ml	Gas Coll. L / d	Load. Rate kg Ne / cu.m-d	Rem. Rate kg Ne / cu.m-d	Substr. Removal Rate g Ne / g VS-d	Total Alk. Prod. mg $CaCO_3$ / L	Alk. / Ne
689	688	99.9	900	3.09	54.70	3.1	5.36	5.35	0.098		
986*	926	93.9	1000	3.43			6.90	6.48		3260	3.52
1004	999	99.5	1000	3.43	82.46	6.1	7.03	6.99	0.085		
1495	1412	94.4	1000	3.43			10.47	9.88		5071	3.60
1671	1516	90.7	1000	3.43	83.30	9.4	11.70	10.61	0.127		
1748	1211	69.3	1000	3.43	80.24		12.24	8.48	0.106	4413	3.64

TABLE V (Continued)

Infl. COD mg/L	Infl. CH_3OH mg/L	$\frac{COD_{in}}{CH_3OH}$	CODs Consumed mg/L	CODs Consumed %	COD/Ne	Load. Rate kg COD/cu.m-d	Rem. Rate kg COD/cu.m-d	Substr. Removal Rate g COD/g VS-d	COD_{mg}^{P} L	Effl. VSS mg/L	$\frac{COD_P}{VSS}$
	2100										
4168	3000	1.39	3078	73.8	3.32	29.18	21.53		109.4	73	1.50
4206	3000	1.40	3620	86.1	3.62	29.45	25.35	0.307	44.7	29	1.54
6446	4500	1.43	4610	71.5	3.26	45.12	32.26		46.8	31	1.51
7355	4950	1.49	5125	69.7	3.38	51.49	35.89	0.431		49	
7406	5250	1.41	4101	55.4	3.39	51.84	28.72	0.358	79.5	59	1.35

*This feed concentration was tested between 1671 and 1748 mg Ne/L influent concentration.

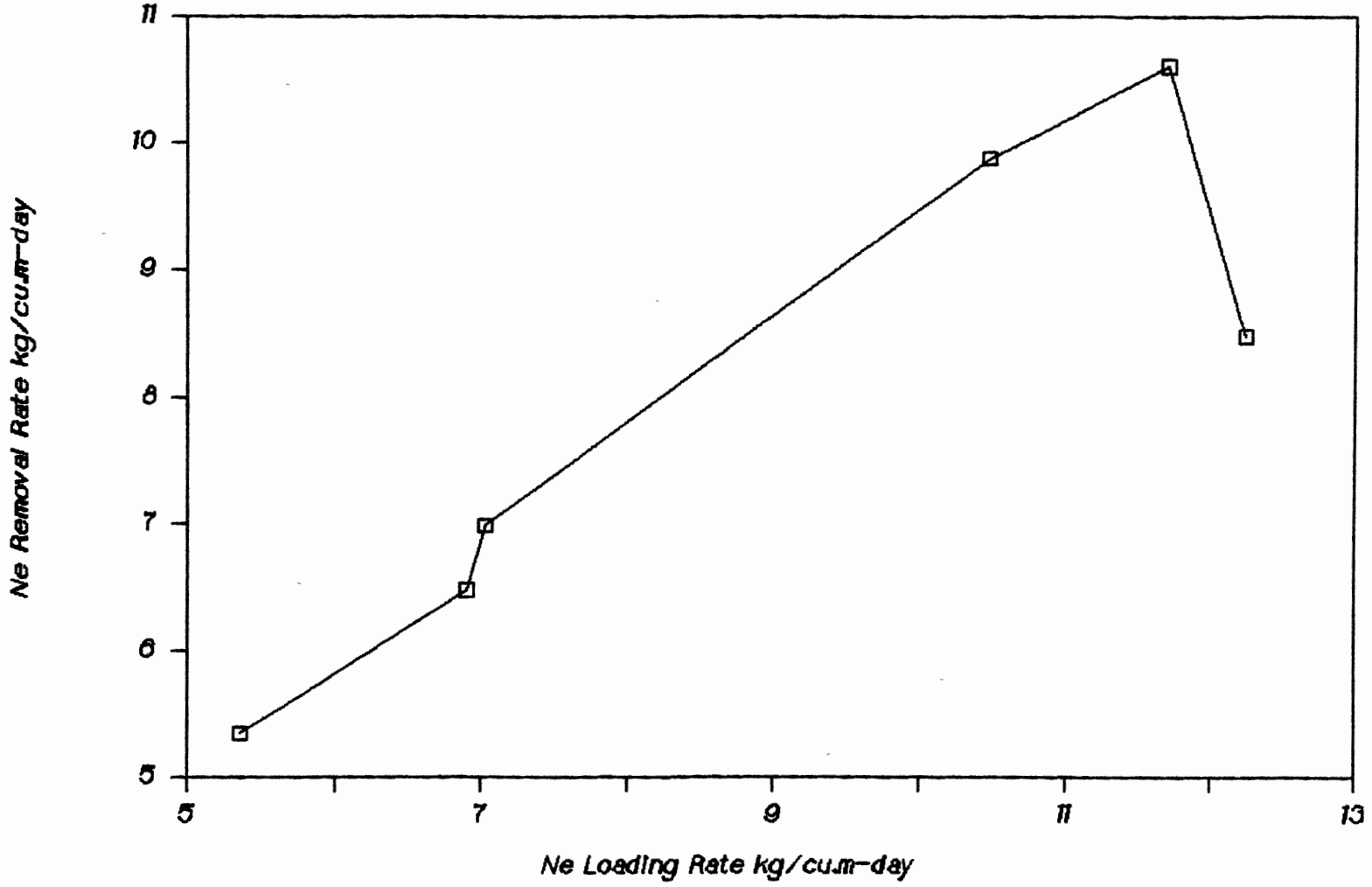


Figure 16. Denitrification Rates in the AFEB Reactor for Constant 3.43 Hour HRT

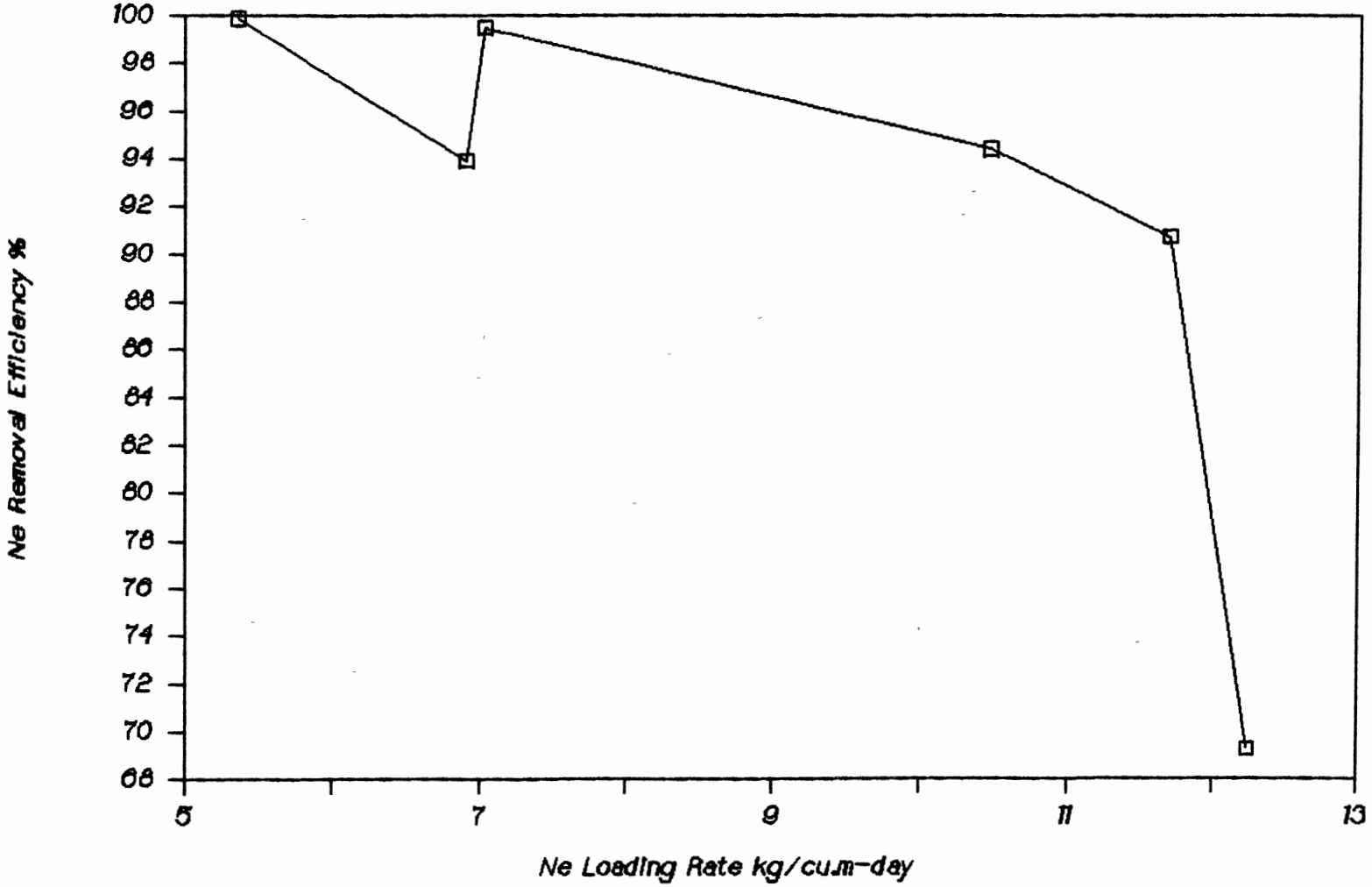


Figure 17. Denitrification Efficiencies in the AFEB Reactor for Constant 3.43 Hour HRT

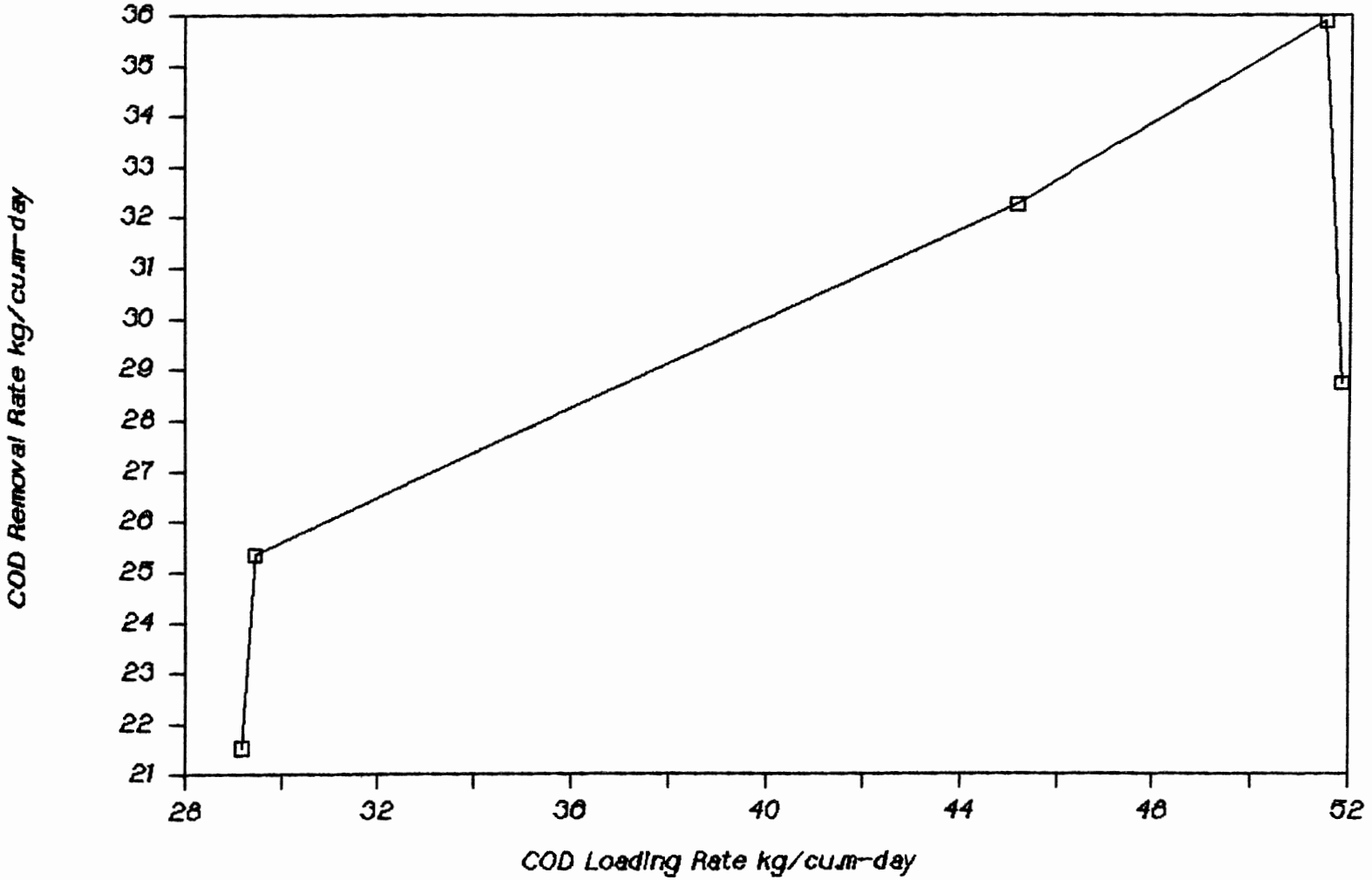


Figure 18. COD Removal Rates in the AFEB Reactor for Constant 3.43 Hour HRT

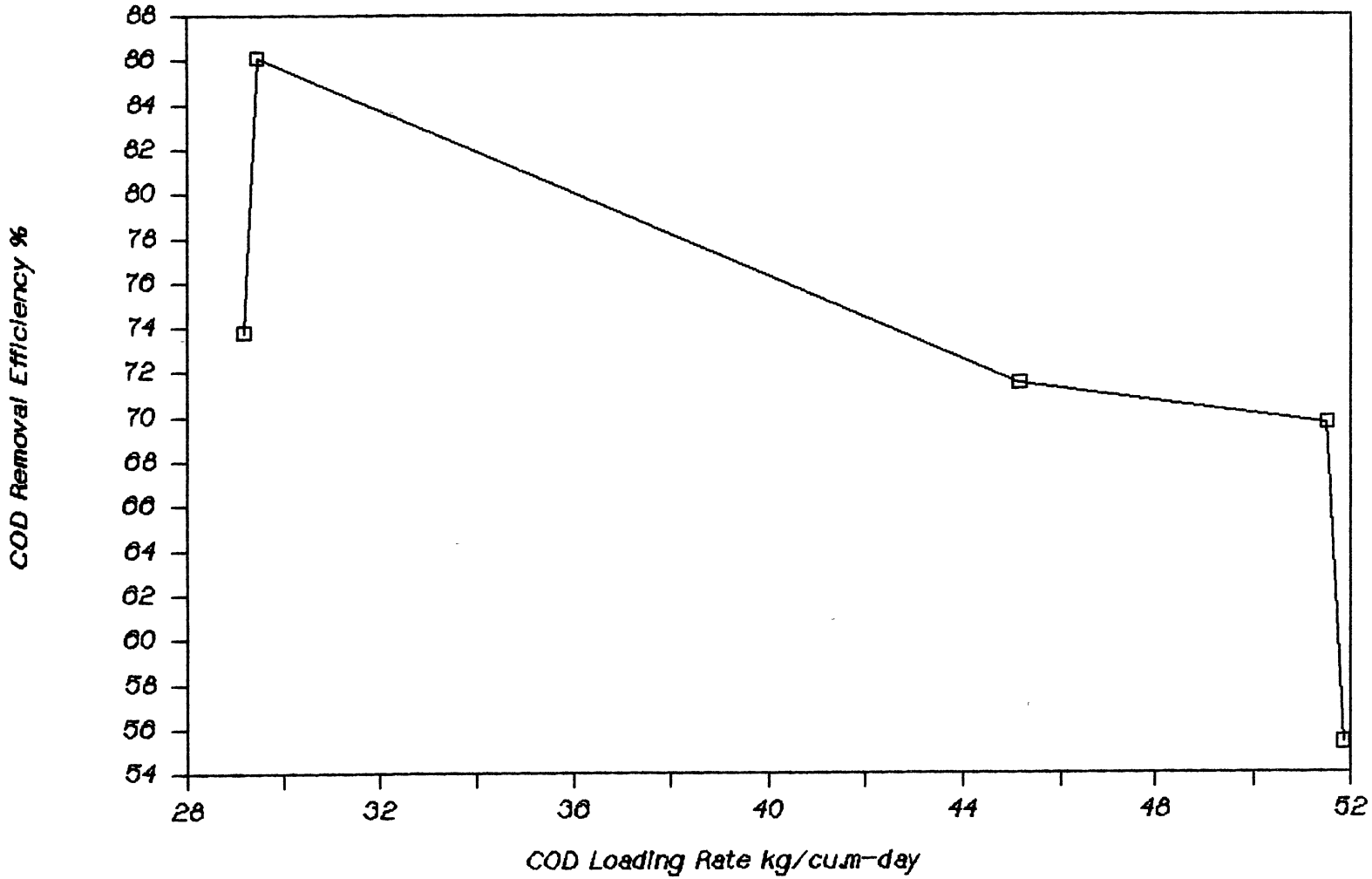


Figure 19. COD Removal Efficiencies in the AFEB Reactor for Constant 3.43 Hour HRT

concentration remained constant at 900 mg Ne/L while the HRT varied from 3.43 to 0.51 hour as shown in Table VI. For this period, loading rates increased from 6.54 to 42.19 kg Ne/cu.m-d and the corresponding removal rates from 6.16 to 22.02 kg Ne/cu.m-d. Figures 20 and 21 depict the Ne loading vs. removal rates, and Ne loading rates vs. reduction efficiencies respectively. Simultaneous COD loading rates were from 40.45 to 174.72 kg COD/cu.m-d, and the removal rates were from 27.38 to 68.67 kg COD/cu.m-d. Figures 22 and 23 show the COD removal rates and efficiencies. For this part of the research, although the removal rates in terms of Ne and COD were continually increasing, the corresponding removal efficiencies were steadily dropping as can be witnessed from these figures.

TABLE VI

SUMMARY OF RESULTS OF THE AFEB REACTOR AT CONSTANT FEED CONCENTRATION

Infl. Conc. mg Ne / L	Ne Consumed mg / L	Ne Consumed %	Exp. Bed Vol. ml	HRT hour	Att. Biomass mg VS / ml	Gas Coll. L / d	Load. Rate kg Ne / cu.m-d	Rem. Rate kg Ne / cu.m-d	Substr. Removal Rate g Ne / g VS-d	Total Alk. Prod. mg $CaCO_3$ / L	Alk. / Ne
934	880	94.2	1000	3.43			6.54	6.16			
943	796	84.4	1000	2.29	81.31	7.1	9.90	8.36	0.103	2876	3.61
912	787	86.3	1000	1.71	81.56		12.77	11.02	0.135		
941	675	71.7	900	1.03		14.0	21.96	15.74		2385	3.53
904	472	52.2	600	0.51			42.19	22.02		1816	3.85

TABLE VI (Continued)

Infl. COD mg/L	Infl. CH_3OH mg/L	$\frac{COD_{in}}{CH_3OH}$	CODs Consumed mg/L	CODs Consumed %	COD/Ne	Load. Rate kg COD/cu.m-d	Rem. Rate kg COD/cu.m-d	Substr. Removal Rate g COD/g VS-d	COD_P mg/L	Effl. VSS mg/L	$\frac{COD_P}{VSS}$
	2700										
3852	2700	1.43	2609	67.7	3.28	40.45	27.38	0.337	65.5	43	1.52
4007	2700	1.48	2493	62.2	3.17	56.10	34.89	0.428	58.8	35	1.63
3904	2700	1.45	2304	59.0	3.41	91.09	53.75				
3744	2700	1.39	1473	39.3	3.12	174.72	68.67				

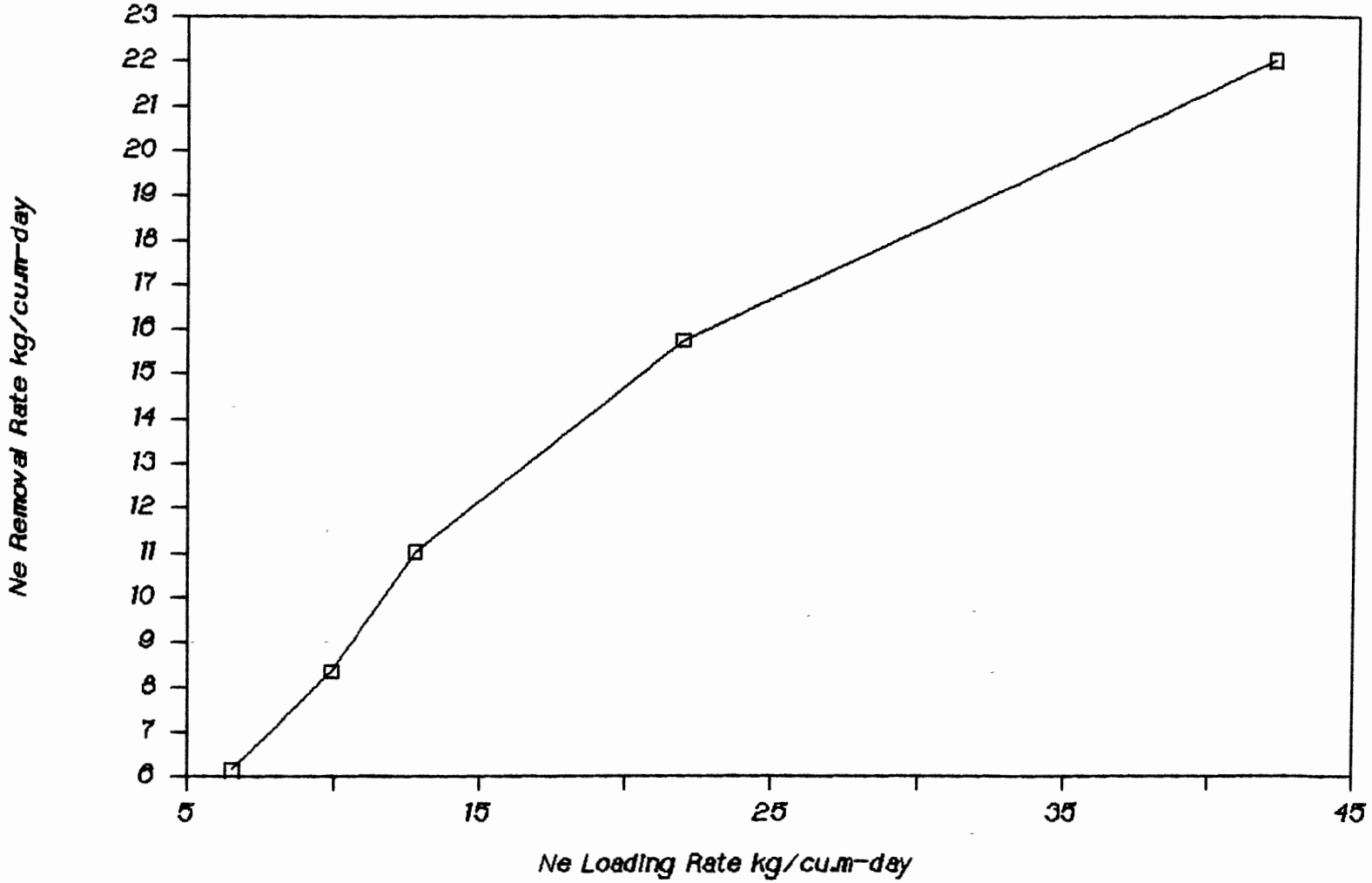


Figure 20. Denitrification Rates in the AFEB Reactor for Constant 900 mg Ne/L Feed Concentration

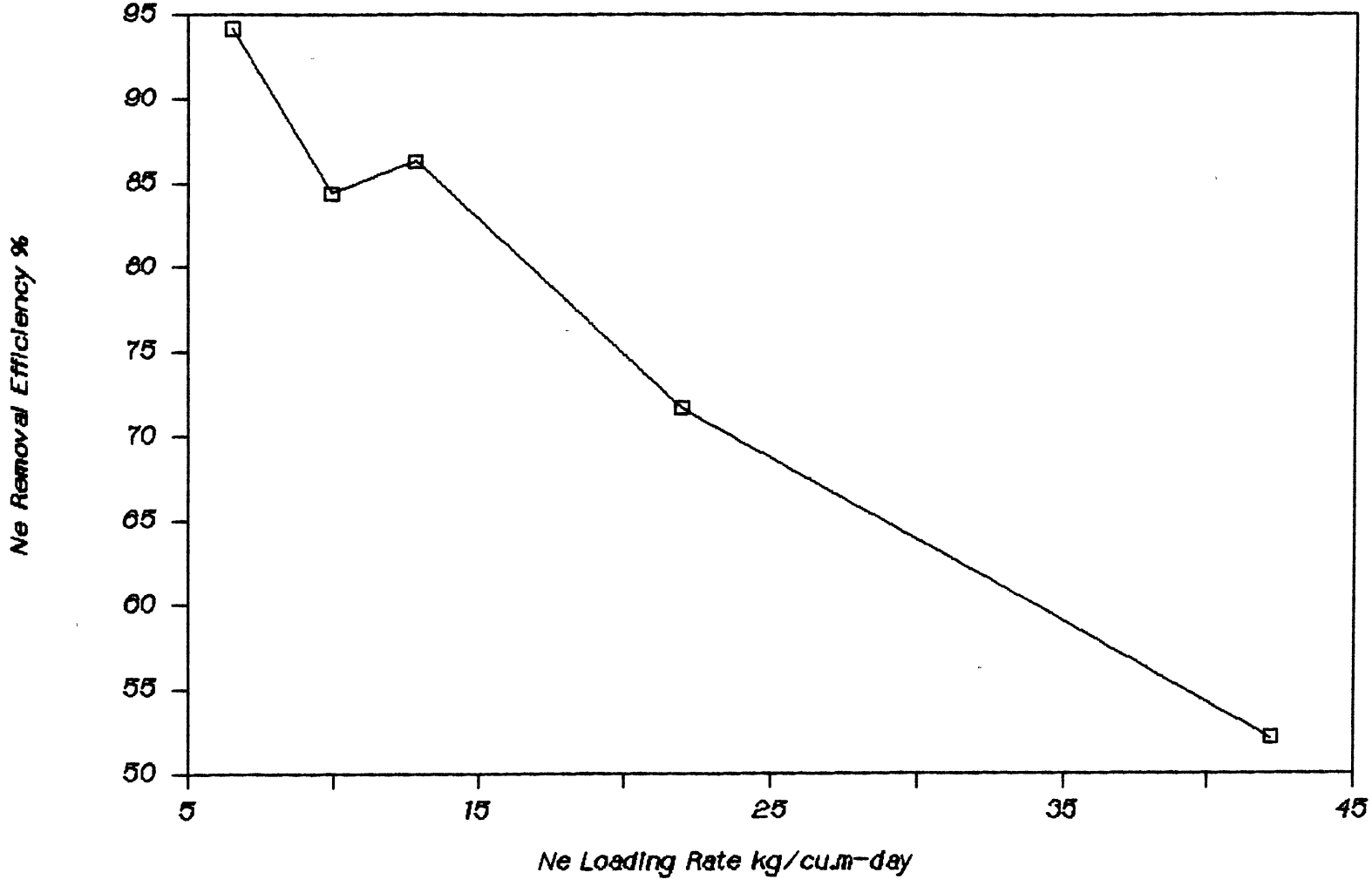


Figure 21. Denitrification Efficiencies in the AFEB Reactor for Constant 900 mg Ne/L Feed Concentration

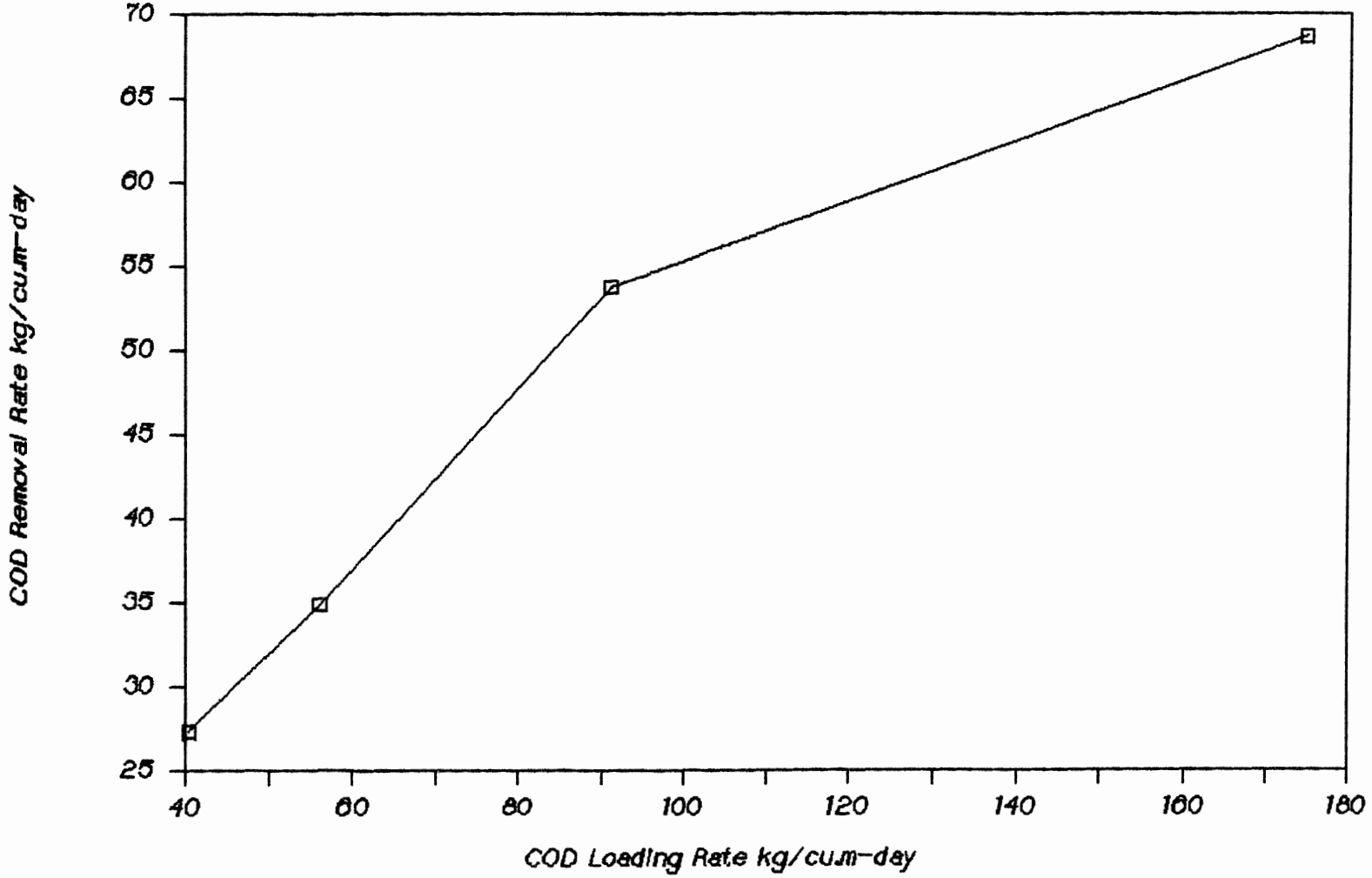


Figure 22. COD Removal Rates in the AFEB Reactor for Constant 900 mg Ne/L Feed Concentration

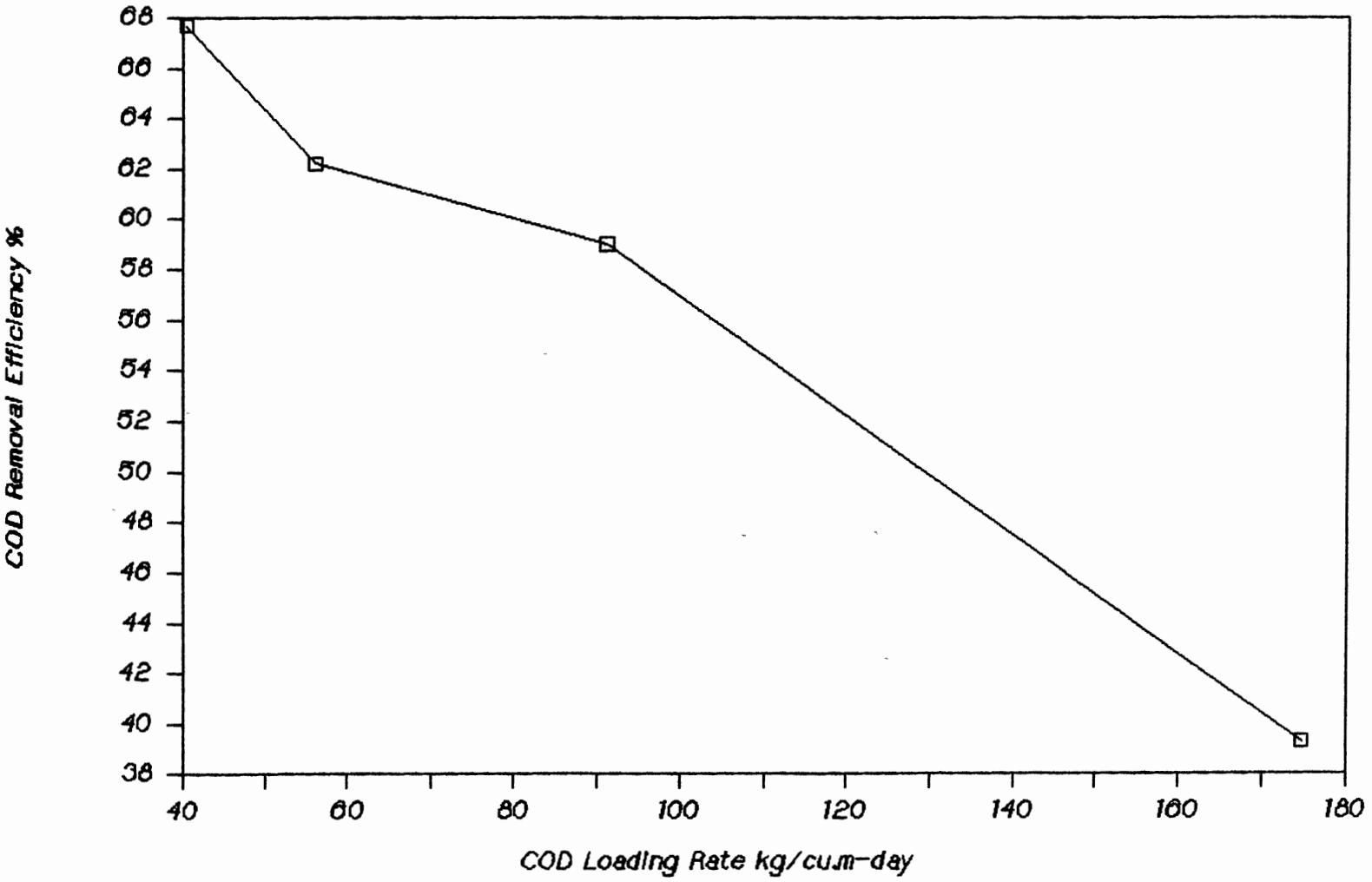


Figure 23. COD Removal Efficiencies in the AFEB Reactor for Constant 900 mg Ne/L Feed Concentration

CHAPTER V

DISCUSSION

This research was carried out to explore the possibility of high-strength/high-rate heterotrophic denitrification using bench scale AFEB and UASB reactors. The operating parameters were also established and their stoichiometric relationship determined in this process.

Experiments were conducted in two phases for achieving these goals. The first part of the investigation, which formed the basis for the high feed strength operation, was done by keeping the HRT fixed and increasing the feed NO_3^-_N concentration. The concentration was increased up to a point at which a significant drop in the rates of removal of both Ne and COD was noted.

The second series of experiments could be termed the high-rate denitrification studies. In these experiments a fixed, lower feed concentration was chosen for each reactor and the flow rates were increased by decreasing the HRTs gradually. The loading rates increased up to a point at which removal rates of Ne and COD deteriorated. Data in each step of both phases of the experiments were obtained when steady state conditions could be established.

The operating conditions for each reactor, sustenance

of the reactors at high loading/removal rates, and their susceptibility to failure conditions are discussed in the following sections. The performance of the reactors is evaluated in comparison to each other and to other relevant studies.

5.1 Performance of UASB

The UASB reactor was unstable in the initial three month start-up period of this study. It was somewhat difficult to maintain distinct sludge blanket and clear supernatant zones inside the reactor, primarily due to little or no control over the mixing arrangement. Although there was a speed adjustment knob with the stirring equipment, setting the right speed was not possible. The speed had to be slow enough to avoid washout of biomass, but high enough to maintain continuous mixing of the reactor contents. Therefore, acquiring the required speed of the stirrer was the major criterion for an effective biomass retention and blanket formation within the reactor. Such a difficulty was also experienced in a concurrent study on autotrophic denitrification with the UASB reactor, as part of the same project (Ross, 1989).

To establish steady state conditions in the reactor and achieve a stable sludge blanket, feed concentration and flow rate were adjusted besides manipulating the stirrer speed. The feed concentration for the continuously mixed reactor started with 500 mg Ne/L immediately after the

batch acclimation. The feed strength was increased up to 750 mg Ne/L without any improvement in the removal efficiency. Because of the regular washout of biomass during this period, Ne reduction efficiency averaged 50 % during this period with equal concentrations of NO_3^- and NO_2^- in the effluent. The feed concentration was then brought back to 500 mg Ne/L, the HRT was increased from 6 to 9 hours, and this resulted in a slight improvement in the process performance. Although removal efficiencies were only ~60 % for this duration, the effluent NO_3^- was less than NO_2^-. Steady state conditions could not be achieved owing to fluctuations in removal efficiencies.

A caged stirrer was obtained at this stage for use within the UASB reactor which replaced the ordinary stir bar that was used previously. This cage, however, had to be delicately placed inside the reactor, and any slight disturbance of its orientation hindered the operation of the stirrer. At the same time, the feed concentration was dropped to 200 mg Ne/L, and the HRT to 6 hours. The reactor responded dramatically at this point with complete denitrification (~100 % efficiency), and there was a distinct interface between the sludge bed and the supernatant. The blanket occupied approximately one fourth of the reactor volume at the bottom. Data were collected from this point onwards until the reactor finally succumbed to excess nitrite accumulation in the effluent at a feed concentration of 1500 mg Ne/L.

In the first set of tests, feed strength increased from 200 to 1500 mg Ne/L at a constant HRT of 6 hours. For the second battery of experiments, influent concentration remained fixed at 500 mg Ne/L while the HRTs were varied between 6 hours and 1 hour.

The highest Ne removal rate achieved in the UASB reactor was 4.49 kg Ne/cu.m-d at a feed concentration of 522 mg Ne/L, and a HRT of 1.5 hours. In terms of COD, the highest removal rate was 15.74 kg COD/cu.m-d at the same operating conditions mentioned above.

Failure in both the phases of this study with the UASB reactor occurred due only to an excessive accumulation of nitrite in the effluent. The causes of such a failure are dealt with in detail in Section 5.3.1.

5.2 Performance of AFEB

Contrary to the difficulties faced with the UASB reactor, the AFEB reactor functioned under steady and stable operating conditions throughout this study. Such a superior performance was evident by the gradual attachment and growth of biomass to the inert diatomaceous earth particles from the day the acclimated sludge was transferred to the reactor.

As with the UASB, concentration of feed solution was increased at a constant HRT. In this case, waste concentration ranged from 700 to 1750 mg Ne/L at 3.43 hours HRT. The Ne removal efficiency averaged more than 90 % for this

period, and only when the concentration reached 1750 mg Ne/L did the efficiency drop to 70 %.

When the feed strength was ~1650 mg Ne/L, nitrite began to accumulate in the effluent (~150 mg NO_2^-_N/L), and the COD/Ne ratio dropped to 3.3 from 3.7, possibly due to nitrite accumulation. At this stage, it was decided to decrease the feed concentration to ~1000 mg Ne/L for two reasons, firstly to test if COD/Ne ratio remained at 3.3, and secondly to see if this would eliminate NO_2^- formation in the effluent. The former condition was confirmed (i.e. COD/Ne = ~3.3), but the latter could not be achieved, and some nitrite was always present in the effluent. The feed strength was then gradually increased up to 1750 mg Ne/L before failure occurred, and care was taken to duplicate the operating conditions established previously for each feed concentration. The highest removal rate obtained for this part of the study was 10.61 kg Ne/cu.m-d at a feed strength of 1671 mg Ne/L and removal efficiency of 90.7 %.

In the second set of operations, the feed concentration was kept at 900 mg Ne/L to eliminate NO_2^- presence in the effluent and achieve high removal rates in terms of both Ne and COD. The range of HRTs was 3.43 to 0.51 hours for this study.

As already explained in Section 4.3, when HRT was 1.03 hours, corresponding to a loading rate of ~22 kg Ne/cu.m-d, gases produced had been entrapped within the bed. The gas bubbles attempted to carry away the attached biomass with

them to the top of the reactor. The bed volume was reduced to 900 ml because of some biomass washout. For the next HRT (0.51 hour), more bioparticles were washed out with the effluent, and the top 300 ml of bed volume was occupied by loose solids. These loose solids might have been the result of rapid synthesis of new cells at such a high loading rate (~42 kg Ne/cu.m-d) that were not attached to the media plus the previously entrapped biomass that was sheared from the media due to evolving gas bubbles. The bioparticles that were being washed out with the effluent at this stage were collected in a glass beaker. Figure 24 shows a photograph of these captured particles. Effluent total COD values were not recorded for the last two HRTs since the effluent contained washed-out bioparticles.

The loose biomass was removed prior to conducting the tests at this HRT, and hence the effective bed volume decreased to 600 ml. The removal rate of 22.02 kg Ne/cu.m-d obtained under these conditions was the highest achieved in this entire study on heterotrophic denitrification. In terms of COD removal rate, this corresponded to 68.67 kg COD/cu.m-d which was also the highest obtained in this study. The reduction efficiencies of both Ne and COD were, however, relatively low (52.2 and 39.3 % respectively). Operations were discontinued at this stage due to the difficulties stated above. Biomass washout at the highest loading rates was also noted by Ross (1989) in a simultaneous study of autotrophic

Figure 24. Photograph of the Captured Bioparticles that Escaped in the AFEB Effluent

denitrification.

5.3 Failure Conditions

A drop in the removal rates of both Ne and COD compared to the corresponding immediately preceding values was considered to represent the occurrence of failure conditions for this study (the only exception was the AFEB study with constant Ne concentration and variable HRTs). However, this reduction in removal rates was always accompanied by increasing nitrite concentration in the effluent except for the last run in the second set of experiments with the UASB reactor. Equal concentrations of NO_3^- and NO_2^- appeared in the effluent when failure occurred in this case. The HRT for this condition was 1 hour, and the effluent was turbid with ~150 mg VSS/L.

5.3.1 Nitrite Accumulation

It was quite interesting to observe that in this study, most conditions of failure were accompanied by effluent nitrite build-up, the effluent nitrate being much less. For example, in the AFEB reactor, at 3.43 hour HRT when the feed concentration reached 1750 mg Ne/L, the effluent had ~550 mg NO_2^-_N/L, and less than 6 mg NO_3^-_N/L. In the UASB, nitrite in the effluent was evident at a feed concentration of 600 mg Ne/L and increased with an increase in feed strength.

Because of consistent build-up of nitrite in the

effluent as the research progressed, it was decided to conduct a simple test to identify the microorganisms present in the reactor. This was done with the coordination of the OSU Microbiology Department. For this test, two samples from each reactor were obtained from the center of the expanded bed and the sludge blanket with sterile pipets. These samples were streaked on agar plates containing nutrients, and bacterial colonies were grown on these strips. Tests on these colonies were then conducted for identification of the genus according to the procedures described in the Manual of Methods for General Bacteriology (ASM, 1981). The results of these tests have thrown some light on the possible cause of nitrite accumulation in both the reactors.

Results from these experiments revealed the presence of *Achromobacter* denitrifying species in both the reactor samples. However, all the denitrifying species under the genus *Achromobacter* are now classified under the closely related *Alcaligenes* according to Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Taxonomic comments in the manual point out that both the genera have frequently been 'a dumping ground' for a variety of bacteria,

> due to the lack of an adequate description of both genera and to the inactivity of these bacteria in the commonly used biochemical tests.

It may be recalled from Section 2.7.1 of Literature Review (Chapter II) that the possibility of nitrite

reduction rate falling behind that of nitrate reduction could result in nitrite accumulation. Such a kinetic explanation was in fact confirmed by experiments on *Alcaligenes* and *Pseudomonas* by Betlach and Tiedje (1981), and later supported with a mathematical model developed by Wilderer et al. (1987).

Therefore, at this stage with these limited clues, it can be said that nitrite could have accumulated due to the relative lag in the reduction rate of nitrite. Such a hypothesis is strengthened from the very fact that nitrate in the effluent was negligibly small compared to nitrite, and the presence of *Achromobacter* (*Alcaligenes*) denitrifying species is further circumstantial evidence. Also, the repressing effects on denitrification due to the presence of dissolved oxygen (refer Section 2.7.2) should also be taken into account here, because tap water (DO ~8 mg/L) was used for simulation of feed wastewater.

While the organisms recognized in these tests may not be the only species present in the reactors, their dominance is irrefutable. Such an argument can be based on the fact that identical colonies of bacteria grew vigorously and were present in greater numbers than others at the time the tests were conducted. However, in the heterogeneous mixture of population present in the reactors, the survival of microorganisms that reduce nitrate assimilatively to nitrite cannot be altogether ruled out at this stage, and more detailed investigation on

the microbiology of the reactor population would be required before any further conclusion can be reached.

5.3.2 Other Causes

In the second set of experiments with the AFEB reactor, when the feed strength was constant at 900 mg Ne/L, and HRTs were 1.03 and 0.51 hour, the attached biota was lifted due to vigorous gas production. This resulted in the reduction of bed volume by as much as 400 ml. Although the highest removal rates (22.02 kg Ne/cu.m-d and 68.67 kg COD/cu.m-d) were achieved during this time, operations were discontinued owing to excessive washout of bioparticles. Such functional difficulties were also experienced with an autotrophic AFEB reactor (Ross, 1989) and with static filter column and continuously mixed reactors (Jewell and Cummings, 1975).

5.4 AFEB Attached Biomass

The attached biomass in the AFEB reactor averaged 81.77 mg VS/ml. This value is quite high compared to that in the AFEB for autotrophic denitrification which averaged 22.6 mg VS/ml (Ross, 1989). The tests for attached biomass determination were done at steady state conditions. The attached biomass reached 54.70 mg VS/ml after 10 weeks of continuous feed operation. No comparable values are available in the literature for heterotrophic denitrification. Sample attached biomass calculations are

shown in Appendix A.

The substrate uptake rates for both Ne and COD (g Ne/g attached VS-d and g COD/g attached VS-d) were calculated under both testing conditions and are presented in Tables V and VI. These values indicate that the conversion rates on the basis of attached biomass are quite low compared to volumetric conversion rates which are high. Table VII shows a comparison of substrate removal rates for denitrification obtained from this study with values calculated from other similar studies. Specific substrate uptake rates are seen to be generally low. Such low substrate removal rates might possibly be due to the attachment of non-viable microbial biomass alongwith true denitrifiers to the inert media. Such a postulation could be confirmed if ATP analysis or enzyme bioassays had been conducted.

5.5 COD Calculations

Methanol in the influent and the effluent was measured in terms of COD. The oxidation of 1 mole of methanol would require 1.5 mole of O_2 as shown by the following equation.

$$CH_3OH + 3/2\ O_2 \text{ ----> } CO_2 + 2\ H_2O \qquad (15)$$

Thus 1 mg/L of CH_3OH would exert an oxygen demand of 1.5 mg O_2/L. Methanol was added in the feed solution as three times that of influent nitrate-nitrogen. Therefore, it was simple to correlate the influent COD to this methanol content in the feed solution. As against a

TABLE VII

COMPARISON OF SUBSTRATE REMOVAL RATES FOR DENITRIFICATION

Reactor Type	Substrate Removal Rates: g Ne/g MLVSS-d	g COD/g att.VS-d	Org. Subst.	Reference
CSTR	0.30 - 0.45	--	Methanol	Aitken (1983)
CSTR	0.21 - 0.52	--	Methanol	Beccari et al. (1983)
CSTR	0.32[a]	--	Methanol	Moore and Shoreder (1971)
CSTR	0.06	--	Methanol	Paskins et al. (1978)*
CSTR	0.28 - 0.31	--	THF alc.	Ramadori et al. (1983)
CSTR	--	10.3[d]	Methanol	Stensel et al. (1973)
CSTR	0.15	--	Methanol	Sutton et al. (1975)
PBR	0.015 - 0.08	--	Methanol	Huang et al. (1984)
UASB	0.40[b]	0.45 - 1.62[e]	Alcoholic Waste	Klapwijk et al. (1981)
Batch feed	0.09 - 0.20	--	Brewery Wastes	Monteith et al. (1980)
AFEB	0.21 - 0.67[c]	--	Auto-trophic	Ross (1989)
AFEB	0.09 - 0.14[c]	0.31 - 0.43	Methanol	This study

[a]units in g Ne/g SS-day
[b]units in g Ne/g TS-day
[c]units in g Ne/g attached VS-day
[d]units in g COD/g SS-day
[e]units in g COD/g TS-day
*adapted from Beccari et al. (1983)

stoichiometric ratio of 1.5, this value (mean) was found to be 1.45 and this could have been due to dilution by the feed water.

Culture tube (closed reflux) COD tests were conducted for this research (see Section 3.5.3.7). Although sample preparation and absorbance measurement were time consuming, this procedure was easier, less dangerous, and required smaller amounts of expensive reagents than the open reflux method. Besides, many samples could be tested at the same time using the closed reflux method. Data were collected each time the COD analysis was made, and are summarized in Table IX of Appendix B to demonstrate the accuracy and reliability of this method.

5.6 Stoichiometric Parameters

Table VIII presents the values of stoichiometric parameters obtained in this study compared to those found in the literature.

5.6.1 CODs Consumption/Ne Removal

The amount of methanol utilized for denitrification was measured as soluble COD consumed. Filtered effluent samples were analyzed for CODs and correlated with Ne reduced to yield CODs/Ne values. Appropriate deductions were made for the amount of NO_2^-_N present in the influent and/or effluent. Sample calculations explaining the correlation of CODs to Ne are shown in Appendix C.

TABLE VIII

COMPARISON OF OBSERVED AND CALCULATED STOICHIOMETRIC PARAMETERS

Parameter	Value	Reference
COD_s/N_e	2.8 - 3.2	Jewell and Cummings (1975)
	3.00	Miyaji and Kato (1975)
	4.95 (Molasses)	Bosman et al. (1978)
	3.66 (Fusel Oil)	Klapwijk et al. (1981)
	4.20 (2.6 - 7.0)	Aitken (1983)
	4.10	Beccari et al. (1983)
	3.6 - 14.0	Bode et al. (1987)
	3.00 (THF)	Ramadori et al. (1987)
	3.71	Stoichiometric value (See Section 2.4)
	3.45	Obtained value in this study
COD_{in}/CH_3OH	1.50	Stoichiometric value (See Section 5.5)
	1.45	Obtained value in this study
CH_3OH/N_e	2.40	Jeris et al. (1974)
	4.20	Jeris and Owens (1975)
	2.64	Claus and Kutzner (1985b)
	2.47	Stoichiometric value (See Section 2.4)
	2.38	Calculated value in this study
Alkalinity/Ne	2.95	Jeris and Owens (1975)
	3.57	Stoichiometric value (See Section 2.4)
	3.77	Observed value in this study

Against a stoichiometric CODs/Ne ratio of 3.71, the mean value observed in this study was 3.45. However, when Ne removal efficiencies were ~100 %, this value agreed well with the stoichiometric ratio (refer Tables III & IV). A shift in this ratio could have occurred due to the accumulation of nitrite in the effluent which rendered incomplete removal of nitrate. Another factor could be the incorporation of some nitrogen in cell mass which was not considered for calculating this ratio.

This ratio in terms of actual methanol consumption to Ne reduced thus would be 2.38 against the stoichiometric value of 2.47. This value of 2.38 was obtained by taking the mean COD_{in}/CH_3OH value of 1.45 obtained in this study as this would seem logical rather than the stoichiometric value of 1.5.

5.6.2 Total Alkalinity Production/Ne Reduction

An average value of 3.77 for Alkalinity/Ne was observed in this study, slightly higher than the stoichiometric value of 3.57. This could be due to two factors, first because influent tap water contained some alkalinity, and second due to the titration procedure using indicators. Although filtered effluent samples were used for titration, end points were sometimes difficult to see clearly and may have been passed resulting in slightly higher alkalinity values for effluent samples.

5.6.3 Effluent Particulate COD/Effluent VSS

The ratio of COD_p to VSS in the effluent was found to average 1.46 compared to a stoichiometric value of 1.42 if $C_5H_7O_2N$ was assumed for cell mass. However, this chemical composition might have been different, and no attempt was made in this study to define the chemical formula for the cells in the effluent.

All the particulate COD was considered to represent the cell mass expressed in terms of VSS as there was no other particulate matter in the influent. Generally in this research, VSS was 80 - 90 % of total suspended solids. Sample calculations on COD_p/VSS are shown in Appendix C.

CHAPTER VI

CONCLUSIONS

1. This research has shown that high-strength denitrification is very successful with high Ne removal rates using the AFEB reactor.
2. The highest Ne removal rate achieved in this study was 22.02 kg Ne/cu.m-d (corresponding COD removal: 68.67 kg COD/cu.m-d) with AFEB at HRT 0.51 hour and feed strength of 904 mg Ne/L. However, reactor performance was unstable at this HRT due to bioparticle washout.
3. The highest influent concentration treated in this study was 1748 mg Ne/L with the AFEB reactor at HRT 6 hours with removal efficiency 69.3 %. Ne removal efficiency at this HRT was > 90 % up to a feed strength of 1670 mg Ne/L.
4. The mean attached biomass concentration for AFEB in this study was 81.77 mg VS/ml.
5. For UASB, the highest Ne removal rate obtained was 4.49 kg Ne/cu.m-d, for a feed strength of 522 mg Ne/L at 1.5 hour HRT.
6. Removal efficiencies for UASB at HRT 6 hours were ~100 % up to feed strength of 400 mg Ne/L, and dropped to 44.7 % at 1485 mg Ne/L.
7. Reduction efficiencies for both the reactors decreased

due to effluent nitrite accumulation as the feed Ne concentration increased.

8. Nitrate in the effluent was always low (< 25 mg NO_3^-_N/L).
9. Volumetric Ne removal rates and mean attached biomass were both higher for the AFEB than those obtained in concurrent autotrophic denitrification by Ross (1989).
10. Of the two reactors used for this study, AFEB was more stable and performed better.

CHAPTER VII

SIGNIFICANCE OF THE STUDY

This is the first ever study on heterotrophic high-strength denitrification using AFEB. This investigation also included high-rate denitrification studies using UASB. AFEB yielded better results in terms of high-strength and high-rate Ne removal.

Denitrification would be necessary for nitrate-rich effluents of industries to prevent eutrophication of receiving water courses. Biological denitrification is simpler and more economical than alternative physico-chemical processes. Although many organic substrates are available for heterotrophic denitrification, methanol is a common choice due to its ease in availability and biodegradation, higher solubility, and lower vapor pressure and cell yield. Complete denitrification can be achieved with a CH_3OH:NO_3-_N ratio of 2.5:1, and this would ensure low COD in the treated effluent. Compared to autotrophic denitrification, heterotrophic activity generates more alkalinity stoichiometrically. Hence, pH of the effluent tends to rise if not adjusted. However, autotrophic oxidation of sulfur produces sulfate as an end product, and may warrant an additional sulfate removal unit. For autotrophic denitrification, more chemicals would be

required. Besides, autotrophic denitrification may cause odor problems due to dissolved sulfide in the final effluent.

High Ne loading rates can be obtained by either decreasing the HRTs for a given Ne concentration or increasing the feed Ne strength at a fixed HRT. Therefore, initial and operating costs of a reactor would depend on feed concentrations and HRTs for constant reactor volume.

It was learned from this study that problems due to effluent nitrite accumulation, biomass escape at high Ne loading rates, and mixing arrangement for sludge blanket need to be addressed for high-strength/high-rate denitrification. Effluent nitrite build-up can be avoided and removal efficiency enhanced by either diluting the high feed strength or increasing the HRT. Both of these would, however, reduce the volumetric loading rates.

CHAPTER VIII

APPLICATIONS AND FUTURE RESEARCH NEEDS

This study indicated the potential feasibility of high-strength/high-rate denitrification using AFEB and UASB reactors. Although the bench scale studies conducted for this research were not part of any full scale operation, the results of this investigation can nevertheless be relied upon for any future full scale operation in this field. However, the following points must be considered for a successful implementation of heterotrophic denitrification:

1. Provision of the stoichiometric methanol amount to lessen effluent COD.
2. Comparison of suitability and economics of various organic substrates with methanol.
3. Feasibility of exploiting the organic substrates already present in the nitrogenous wastewater for denitrification.
4. Evaluation of the effect of HRTs over a wide range of feed Ne concentrations.
5. Analysis of constituents of gases collected from the reactors.
6. Comparison of costs for AFEB and UASB on requirement of pumps, volume, and dilution to select the optimum

reactor.

7. Design of an appropriate mixing arrangement for UASB and determination of the required speed to maintain the sludge blanket.
8. Adjustment of pH and subsequent control of alkalinity if influent wastewater contains calcium.
9. Technical solution to problem of bioparticle washout due to gas evolution at high loading rates.
10. Identification of the microbiology of the reactor contents to help understand denitrification mechanisms and causes of nitrite accumulation.
11. Need for ATP analysis and enzyme bioassays to correlate the specific substrate uptake rates with viability of biomass present in the reactor.
12. Investigation of the effects of residual NH_4^+_N and NO_2^-_N (or organic N) in the feed, on denitrification rates and efficiencies, in a two-phase biological nitrification/denitrification treatment system for the removal of ammoniacal nitrogen.

BIBLIOGRAPHY

American Society for Microbiology. 1981. Manual of Methods for General Bacteriology. Section V. Chapter 20. pp.409-443

APHA, AWWA, and WPCF. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Ed. Washington, DC: APHA.

Aitken, M. D. 1983. Discussion of: Kinetics of Dissimilatory Nitrate and Nitrite Reduction in Suspended Growth Culture, M. Beccari et al. J. Water Pollut. Control Fed. 55:1404.

Bailey, D. A. and Thomas, E. V. 1975. Nitrogen Removal by Biological Denitrification. Prog. Water Tech. 7:329-338.

Beccari, M., Passino, R., Ramadori, R., and Tandoi, V. 1983. Kinetics of Dissimilatory Nitrate and Nitrite Reduction in Suspended Growth Culture. J. Water Pollut. Control Fed. 55:58-64.

Betlach, M. R. and Tiedje, J. M. 1981. Kinetic Explanation for Accumulation of Nitrite, Nitric Oxide, and Nitrous Oxide during Bacterial Denitrification. Appl. Envir. Microbiol. 42:1074-1084.

Blaszczyk, M., Mycielski, R., Jaworowska-Deptuch, H., and Brzostek, K. 1980. Effect of Various Sources of Organic Carbon and High Nitrite and Nitrate Concentrations on the Selection of Denitrifying Bacteria. I. Stationary Cultures. Acta Microbiol. Polon. 29:397-406.

Blaszczyk, M., Galka, E., Sakowicz, E., and Mycielski, R. 1985. Denitrification of High Concentrations of Nitrites and Nitrates in Synthetic Medium with Different Sources of Organic Carbon. III. Methanol. Acta Microbiol. Polon. 34:195-206.

Bode, H., Seyfried, C. F., and Kraft, A. 1987. High-rate Denitrification of Concentrated Nitrate Wastewater. Wat. Sci. Tech. 19:163-174.

Bosman, J., Eberhard, A. A., and Baskir, C. I. 1978. Denitrification of a Concentrated Nitrogenous

Industrial Effluent Using Packed Column and Fluidized Bed Reactors. Prog. Wat. Tech. 10:297-308.

Bridle, T. R., Bedford, W. K., and Jank, B. E. 1980. Biological Nitrogen Control of Coke Plant Wastewaters. Prog. Wat. Tech. 12:667-680.

Clarkson, W. W. 1986. Fermentation of Particulate Organic Matter to Methane in a Thermophilic Anaerobic Attached Film Expanded Bed Reactor. Ph.D. Thesis, Cornell University, Ithaca, NY.

Claus, G. and Kutzner, H. J. 1985a. Physiology and Kinetics of Autotrophic Denitrification by *Thiobacillus denitrificans*. Appl. Microbiol. Biotechnol. 22:283-288.

Claus, G. and Kutzner, H. J. 1985b. Denitrification of nitrate and nitric acid with methanol as carbon source. Appl. Microbiol. Biotechnol. 22:378-381.

Grady, C. P. L. and Lim, H. C. 1980. Biological Wastewater Treatment. New York: Mercel Dekker, Inc.

Harada, H., Ando, H., and Momonoi, K. 1987. Process Analysis of Fluidized bed Biofilm Reactor for Denitrification. Wat. Sci. Tech. 19:151-162.

Huang, J. Y. C., Anderson, S. J., and Gabbita, K. V. 1984. Nitrogen Removal at Milwaukee. Effl. and Water Trmt. Journal, pp.143-153.

Jaworowska-Deptuch, H., Wieczorek J., and Mycielski, R. 1985. Quantitative Selection of Denitrifying Bacteria in Continuous Cultures and Requirement for Organic Carbon. II. Maltose. Acta Microbiol. Polon. 34:293-300.

Jeris, J. S., Beer, C., and Mueller, J. A. 1974. High rate Biological Denitrification Using a Granular Fluidized Bed. J. Water Pollut. Control Fed. 46:2118-2128.

Jeris, J. S. and Owens, R. W. 1975. Pilot-scale, High-rate Biological Denitrification. J. Water Pollut. Control Fed. 47:2043-2057.

Jeter, R. M. and Ingraham, J. L. 1981. The Denitrifying Prokaryotes. In: The Prokaryotes. Vol. 1. P. M. Starr et al., (Eds.) New York: Springer-Verlag, pp.913-924.

Jewell, W. J. and Cummings, R. J. 1975. Denitrification of Concentrated Nitrate Wastewaters. J. Water Pollut.

Control Fed. 47:2281-2291.

Jewell, W. J., Switzenbaum, M. S., and Morris, J. W. 1981. Municipal Wastewater Treatment with the Anaerobic Attached Microbial Film Expanded Bed Process. J. Water Pollut. Control Fed. 53:482-490.

Jewell, W. J. 1985. The Development of Anaerobic Wastewater Treatment. A paper presented at National Science Foundation Sponsored Seminar on Anaerobic Treatment of Sewage at the University of Massachusetts, Amherst, MA.

Kelly, R. C. and Switzenbaum, M. S. 1984. Temperature and Nutrient Effects on the Anaerobic Expanded Bed Treating a High Strength Waste. Proc. 38th Ind. Waste Conf., Purdue Univ., 10-12 May, 1983, pp. 591-601.

Klapwijk, A., Jol, C., and Donker, H. J. G. W. 1979. The Application of an Upflow Reactor in the Denitrification Step of Biological Sewage Purification. Water Res. 13:1009-1015.

Klapwijk, A., van der Hoeven, J. C. M., Lettinga, G. 1981. Biological Denitrification in an Upflow Sludge Blanket Reactor. Water Res. 15:1-6.

Knowles, R. 1982. Denitrification. Microbiol. Rev. 46:43-70.

Krieg, N. R. and Holt, J. G. 1984. Bergey's Manual of Systematic Bacteriology. Vol. 1. Baltimore: Williams & Wilkins. pp.361-390.

Lam, Y. and Nicholas, D. J. D. 1969. Aerobic and Anaerobic Respiration in *Micrococcus denitrificans*. Biochem. Biophy. Acta. 172:450-461.

Lettinga, G., van Velsen, A. F. M., Hobma, S. W., de Zeeuw, W., and Klapwijk, A. 1980. Use of the Upflow Sludge Blanket (USB) Reactor Concept for Biological Wastewater Treatment, Especially for Anaerobic Treatment. Biotech. Bioeng. 22:699-734.

McCarty, P. L., Beck, L., and Amant, P. S. 1969. Biological Denitrification of Wastewaters by Addition of Organic Materials. Proc. 24th Ind. Waste Conf., Purdue Univ., 6-8 May, 1969. Ext. Ser. 135:1271-1285.

Miyaji, Y. and Kato, K. 1975. Biological Treatment of Industrial Wastes Water by Using Nitrate as an Oxygen Source. Water Res. 9:95-101.

Monteith, H. D., Bridle, T. R., and Sutton, P. M. 1980. Industrial Waste Carbon Sources for Biological Denitrification. Prog. Wat. Tech. 12:127-141.

Moore, S. F. and Shroeder, E.D. 1971. The Effect of Nitrate Feed Rate on Denitrification. Water Res. 5:445-452.

Morris, J. W. and Jewell, W. J. 1982. Organic Particulate Removal with the Anaerobic Attached-Film Expanded-Bed Process. Proc. 36th Ind. Waste Conf., Purdue Univ., 12-14 May, 1981, pp.621-630.

Mulcahy, L. T., Shieh, W. K., and LaMotta, E. J. 1980. Kinetic Model of Biological Denitrification in a Fluidized Bed Biofilm Reactor (FBBR). Prog. Wat. Tech. 12:143-157.

Payne, W. J. 1981. Denitrification. New York: John Wiley & Sons.

Przytocka-Jusiak, M., Duszota, M., Matusiak, K., and Mycielski, R. 1984a. Intensive Culture of *Chlorella Vulgaris/AA* as the Second Stage of Biological Purification of Nitrogen Industry Wastewaters. Water Res. 18:1-7.

Przytocka-Jusiak, M., Blaszczyk, M., Kosinska, E., and Bisz-Konarzewska, A. 1984b. Removal of Nitrogen from Industrial Wastewaters with the Use of Algal Rotating Disks and Denitrification Packed Bed Reactor. Water Res. 18:1077-1082.

Ramadori, R., Fenoglio, F., and Pozzi, L. 1987. Biological Treatment for Low-Activity Nuclear Wastewaters. Wat. Sci. Tech. 19:345-353.

Ross, B. J. B. 1989. High-rate Autotrophic Denitrification of Simulated Industrial Wastewater. M.S. Thesis, Oklahoma State University, Stillwater, OK.

Sadana, J. C. and McElroy, W. D. 1957. Nitrate Reductase from *Achromobacter fischeri*. Purification and Properties: Function of Flavines and Cytochrome. Arch. Microbiol. 67:16-34.

Salle, A. J. 1973. Fundamental Principles of Bacteriology. 7th Ed. McGraw-Hill Book Co. pp.257.

Sperl, G. T. and Hoare, D. S. 1971. Denitrification with Methanol: a Selective Enrichment for *Hyphomicrobium* Species. J. Bacteriol. 108:733-736.

Stensel, H. D., Loehr, R. C., and Lawrence, A. W. 1973. Biological Kinetics of Suspended-growth Denitrification. J. Water Pollut. Control Fed. 45:249-261.

Strand, S. E., McDonnel, A. J., and Unz, R. F. 1985. Concurrent Denitrification and Oxygen Uptake in Microbial Films. Water Res. 19:335-344.

Sutton, P. M., Murphy, K. L., and Dawson, R. N. 1975. Low-Temperature Biological Denitrification of Wastewater. J. Water Poll. Control Fed. 47:122-134.

Switzenbaum M. S. and Jewell, W. J. 1980. Anaerobic Attached-Film Expanded-Bed Reactor Treatment. J. Water Pollut. Control Fed. 52:1953-1965.

Waki, T., Murayama, K., Kawato, Y., and Ichikawa, K. 1980. Transient Characteristics of *Paracoccus denitrificans* with Changes between Aerobic and Anaerobic Conditions. J. Ferment. Technol. 58:243-249.

Walker, J. F., Helfrich, M. V., and Donaldson, T. L. 1989. Biodenitrification of Uranium Refinery Wastewaters. Env. Prog. 8:97-101.

APPENDIXES

APPENDIX A

SAMPLE ATTACHED BIOMASS SOLIDS CALCULATIONS

The process of separation of attached biomass from the loose solids on AFEB samples was done by washing as explained in Section 3.5.3.3. These samples were then subjected to total and volatile solids analyses as described in Standard Methods (refer Sections 3.5.3.1 & 3.5.3.2). A control blank comprised of clean diatomaceous earth particles was tested for every analysis of attached VS to correct for the loss of weight due to evaporation of water of hydration in the mineral particles. Sample volumes were measured before drying and after ashing to account for the loss of biomass due to ignition at 550° C. Attached biomass is computed as follows:

Weight of sample, Ws = (Dried wt. + Tare) - Ashed wt.

Initial sample volume, V_{si} = 5.00 ml

Final sample volume, V_{sf} as measured.

Control blank concentration of hydrated water,

C=[(Blank Dried+Tare wt.)-Blank Ashed wt.](mg)/$V_{Blank\ f}$ (ml)

Then for each sample, Attached Biomass = (Ws-C.V_{sf})/V_{si}

If Dry & Tare wt. = 55.2804 g

Ashed wt. = 54.8664 g

Ws = 0.414 g = 414 mg

C = 3.3 mg/ml and V_{sf} = 1.00 ml

Attached Biomass = [414 - (3.3)1.00]/5.00 = 82.14 mg/ml

APPENDIX B

TABLE IX

LINEAR REGRESSION DATA FOR CULTURE TUBE COD ANALYSES

Number of Standards n	Slope Y = a X +	Intercept b	Regression Coefficient r	Number of Unknown Samples
7	2981.395	3.946818	0.999383	9
7	2969.947	4.193092	0.999467	9
7	3099.308	15.636350	0.998853	9
7	3140.714	0.896734	0.999783	9
7	3245.794	9.790126	0.999358	6
7	3152.386	2.938482	0.999927	6
7	3152.386	2.938482	0.999927	6
7	3150.629	3.486454	0.999922	6
7	3244.908	9.372651	0.999433	9
7	2898.991	-8.206420	0.999067	18
7	2901.085	-7.512460	0.999049	9
7	2822.781	-2.893110	0.999939	9
7	2880.838	-6.625310	0.999700	18
7	2902.276	-10.491100	0.999254	18
7	2923.856	-10.643800	0.999280	18
7	2899.856	-3.290990	0.999153	18
7	2965.199	-6.965360	0.999378	18
7	2911.545	-9.015300	0.999013	18
7	2868.618	-8.093690	0.999045	15
7	2890.099	-9.285050	0.999170	15
7	2874.149	-3.731790	0.999602	9
7	2892.342	-1.993370	0.999683	9
7	2980.849	-0.279560	0.999194	15
7	2979.577	3.840500	0.999629	15
7	2944.504	-5.670420	0.999701	9
7	2909.222	-6.371980	0.999247	9
7	3012.090	0.427761	0.999810	9
7	3026.030	2.629357	0.999821	9
7	2929.763	-8.514890	0.999142	9
7	2908.792	-5.098020	0.999490	9
7	2918.725	-7.393060	0.999292	9
	Mean Values for 31 COD Analyses:			
7	2898.021 (± 489.881)	-1.999318 (±6.538064)	0.999442 (±0.000313)	11

APPENDIX C

COD ANALYSES

Sample COD values obtained for AFEB reactor at HRT 1.71 hours at a constant feed strength of 900 mg Ne/L:

Influent NO_3^-_N = 959.9 mg/L

Influent NO_2^-_N = 18.2 mg/L

Effluent NO_3^-_N = 11.1 mg/L

Effluent NO_2^-_N = 120.5 mg/L

Hence Ne reduced = (959.9+18.2)-(11.1+120.5) = 846.5 mg/L

Influent methanol = 2700 mg/L

Influent COD = 4051.3 mg/L

According to Standard Methods, NO_2^-_N exerts an oxygen demand of 1.1 mg COD/L. Therefore deductions for NO_2^-_N must be made in COD calculations.

Deduction due to inf. NO_2^-_N = (18.2) (1.1) = 20 mg/L

Adjusted inf. COD = (4051.3 - 20) = 4031.3 mg/L

Adj. inf. COD/Inf. methanol = (4031.3)/(2700) = 1.49

Effluent CODs = 1507.1 mg/L

Deduction due to effl. NO_2^-_N = (120.5) (1.1) = 132.6 mg/L

Adjusted effl. CODs = (1507.1 - 132.6) = 1374.5 mg/L

Therefore CODs consumed = (4031.3 - 1374.5) = 2656.8 mg/L

Then CODs consumed/Ne removed = (2656.8)/(846.5) = 3.14

Effluent total COD = 1566.4 mg/L

Then Effl. particulate COD i.e. COD_p = (1566.4 - 1507.1)
= 59.3 mg/L

Effluent VSS = 36 mg/L

Therefore COD_p/VSS = (59.3)/(36) = 1.65

VITA

Srikanth Krishnamachari

Candidate for the Degree of

Master of Science

Thesis: HETEROTROPHIC DENITRIFICATION OF SIMULATED HIGH-STRENGTH INDUSTRIAL WASTEWATER

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Trichy, Tamil Nadu, India, May 23, 1964, the son of R. Krishnamachari and Thangamani Chari.

Education: Graduated from St. Thomas Higher Secondary School, Madras, Tamil Nadu, India, in 1982; received Bachelor of Science (Engineering) in Civil Engineering from Regional Institute of Technology (Ranchi University), Jamshedpur, India, in 1987; completed requirements for the Master of Science degree at Oklahoma State University in May, 1990.

Professional Experience: Instructor in the Civil Engineering Department at M. K. Polytechnic, Madras, India, from August, 1987, to July, 1988; Graduate Research Assistant for Dr. William W. Clarkson, Department of Civil Engineering, Oklahoma State University, from September, 1988 to December, 1989.

Membership in Professional Societies: American Society of Civil Engineers; Institution of Engineers (INDIA); Indian Society for Technical Education.