AN ABSTRACT OF THE THESIS OF

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Title: Substratum-Aerated-Biofilm Reactor for Treatment of Carbonaceous and Nitrogenous Wastewaters

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Dr. Kenneth J. Williamson

This study involves the development of a biofilm reactor that supports growth of a deep biofilm on a gas permeable membrane. The reactor solution is not aerated, and oxygen is supplied through the membrane. The reactor is termed a substratum-aerated-biofilm reactor or SAB. With adequate concentrations of electron-donors and electron-acceptors, a deep biofilm grows on the membrane and is comprised of different layers of bacterial activity. The aerobic layers are near the membrane support, while the anaerobic layers are near the biofilm-liquid boundary.

In the SAB, the substrate diffuses from the bulk liquid into the biofilm to react. Oxygen diffuses through the membrane into the biofilm. All products likewise are transported by molecular diffusion through the biofilm and into the bulk liquid.

The reactors consisted of a reactor wall made of a plexiglass cylinder with the gas permeable membrane supported on a shallow rotating cup. The cup was designed so

that the cup and the membrane function as a flat plate. The flat plate was utilized for support of the biological growth, transfer of oxygen, and mixing of the bulk liquid and the gas phase.

The experiments were conducted in completely mixed, continuous-flow reactors maintained at 25°C with a hydraulic detention of 8 hours. Pure oxygen was delivered to a gas compartment under the membrane. All reactors were fed a synthetic waste buffered to pH 7.0. The background solution for the feed solution was made from distilled water combined with adequate inorganic nutrients and vitamins. The background solution was supplemented with acetate and ammonia to obtain the desired substrate compositions.

Combined nitrification and heterotrophic oxidation activity resulted when the SABs where fed 5 or 10 mg/l acetate, and 10 mg-N/l ammonia. Combined nitrification, heterotrophic oxidation, and denitrification resulted with acetate concentration of 20, 40, and 100 mg/l, and 10 mg-N/l ammonia. Combined heterotrophic oxidation and fermentation resulted with acetate concentration of 800 mg/l, and 10 mg-N/l ammonia. A series of mass balances were developed to determine the fate of the nitrogen compounds and acetate. These results showed that the flux values for carbon oxidation, nitrification, denitrification, and fermentation are higher than those reported for competing technologies such as rotating biological contactors.

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Substratum-Aerated-Biofilm Reactor for Treatment of Carbonaceous and Nitrogenous Wastewaters

by

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I. INTRODUCTION

Description of Biofilms

A biofilm is a collection of microorganisms including bacteria, algae, fungi, and/or protozoa and their extracellular products bound to a solid surface (substratum). Biofilms can develop on almost any surface exposed to an aqueous environment such as those found in streambeds, ground water aquifers, sediments, and water and wastewater treatment systems. The metabolic activity of biofilms has been exploited successfully in several wastewater treatment processes including trickling filters, rotating biological contractors (RBCs), biological filters and biological fluidized beds, such treatment systems result in the production, alteration, or elimination of many organic and inorganic compounds.

Bryers and Characklis (1987) has suggested that biofilm formation is a net result of several processes. One process is the sorption of organic molecules onto surface which occurs within minutes of exposure to the aqueous environment. Another process is the transport of suspended microbial cells to the surface which depends on the flow regime. In a quiescent system, cells are primarily transported to the surface by sedimentation;

however, motility and chemotaxis may also be important. In laminar flow systems, molecular diffusion is expected to be the dominant process for bacterial deposition. In a turbulent flow regime, eddy diffusion may be the dominating mechanisms for cell transport to near the surface with sedimentation and motility allowing for transport through the boundary layer. The attachment of cells to a surface is often associated with the production of extra cellular binding materials or polymers. Costerion et al.(1987) showed that bacterial adhesion to surfaces is mediated by glycocalyx. The glycocalyx that surrounds bacteria is formed from polysaccharide fibers or sugar molecule branches.

The growth of a biofilm is a net result of cellular growth and reproduction, substrate utilization, decay, and extracellular polymer production. The rate of biofilm growth depends on environmental conditions such as temperature, pH and toxicity, and the availability of required substrates and nutrients through diffusion into the biofilm.

The detachment of biofilm is the result of shearing or/and sloughing. Rittman (1982) reported that shear loss is caused by fluid shear stress at the fluid biofilm interface. Sloughing occurs when large amounts or entire sections of biofilms are dislodged from the supporting surface.

The Support-Aerated-Biofilm Reactor (SAB)

Water and wastewater treatment systems which utilize suspended bacterial growth have been used since the early 1900's. Recently, interest has shifted toward the development of a variety of new types of biofilm reactors because of a number of advantages as compared to earlier suspended-growth and attached-growth technologies. These advantages include:

1. better biomass-liquid separation;
2. higher bacterial cell concentration;
3. larger cell residence time;
4. greater protection against biomass loss due to shock hydraulic loadings; and
5. greater flexibility as to types of bacteria within the treatment process.

It has been a common practice in environmental engineering to categorize bacteria into "types" based upon the use of defined electron-donor and electron-acceptor pairs. This practice has been adopted in the research described herein. Specifically, the types are defined as follows:

Type	Electron-donor(s)	Electron-acceptor(s)
Nitrifiers	ammonia/nitrite	oxygen
Heterotrophs	organic carbon	oxygen
Denitrifiers	organic carbon	nitrite/nitrate
Fermenters	organic carbon/hydrogen	carbon dioxide

Spatial location of different bacterial types within a reactor can be an advantage of a biofilm reactor involving one or more bacterial types. The biological depletion of electron-donor/electron-acceptor pairs in an order reflecting their relative yields of free energy can result in gradations of masses of different bacterial types as a function of depth within a biofilm or as a function of distance within a plug-flow reactor. For example, nitrification and denitrification can proceed simultaneously within a biofilm, which suggests significant spatial gradients of oxygen with depth (Ito and Matsuo, 1980; Masuda et al., 1982; Strand et al., 1985). As an example of spatial distribution within a biofilm reactor, heterotrophic oxidation and nitrification in a segmented rotating biological contactors (RBCs) has been shown to occur sequentially with nitrification limited to only later stages of the reactor (Mueller et al., 1980; Torpey et al., 1972; Weng and Molof, 1974).

This study involves the development of a substratum-aerated-biofilm reactor (SAB) that supports the growth of a deep biofilm on a semi-permeable membrane. A biofilm is defined deep when either an electron-donor or an electron-acceptor decreases to a low concentration so that metabolism for that electron-donor/electron-acceptor pair ceases. The semi-permeable membrane (Gore-tex[a] marketed by Gore, Inc.) was a thin sheet of polytetrafluoroethylene

sandwiched between Teflon and nylon layers. In the SAB, oxygen is supplied through the semi-permeable membrane. The aqueous phase in the reactor is not aerated and remains in an anoxic to anaerobic state.

Conceptually, the deep biofilm which forms on the membrane has four different layers of bacterial activity: (a) a nitrification layer near the membrane, (b) a heterotrophic oxidation layer, (c) a denitrification layer, and (d) a anaerobic fermentation layer closest to the reactor solution. Conceptually, there is a continuous gradient of populations of the bacterial types between layers, with each layer dominated by a particular bacterial type. A schematic diagram for nitrification, heterotrophic oxidation, denitrification, and fermentation in the biofilm is shown in Figure (I-1).

In the SAB, the substrates diffuse from the liquid phase into the biofilm. Oxygen diffuses through the semi-permeable membrane into the biofilm and, consequently, in a direction countercurrent to the other substrates and nutrients. All metabolism products likewise are transported by molecular diffusion through the biofilm and into the liquid or gas phases.

Timberlake et al. (1988), conducted a preliminary experiments with the SAB involving a three-layer biofilm

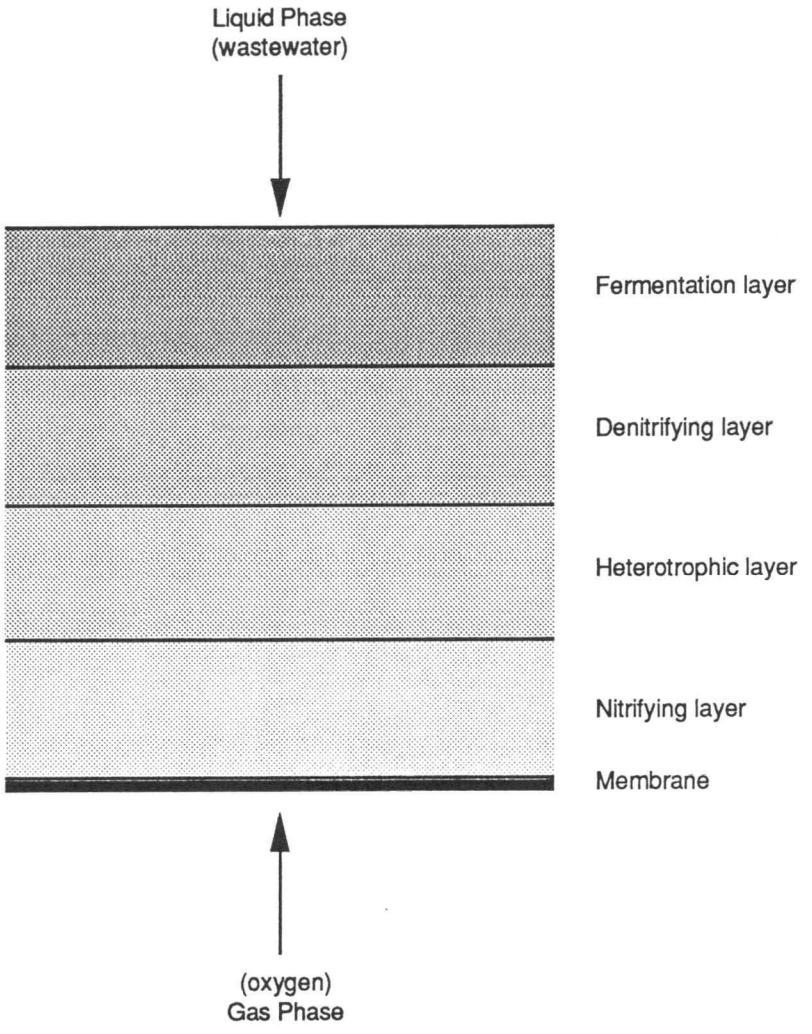


Figure I-1. Schematic diagram for nitrification, aerobic heterotrophic oxidation, denitrification, and fermentation processes in a biofilm.

of heterotrophic oxidation, nitrification, and denitrification. The reactor was fed filtered sewage supplemented with nutrient broth. The concentration of organic compounds (200 mg COD/l) were not large enough to support anaerobic fermentation. The bulk solution was anoxic and mixed at a low rate with nitrogen gas. All tests were performed on a non-steady state biofilm which continued to grow in thickness over the two-month experiment. A mass balance on nitrogen species showed that nitrification and denitrification were occurring simultaneously within the biofilm. This study by Timberlake, et al. (1988) served as the preliminary impetus for the research described herein.

Research Objectives

The goal and scope of this research was to determine the feasibility of the SAB for the treatment of municipal and industrial wastewater. To achieve this goal, experimental work was performed to determine removal rates of various electron-donors and electron-acceptors. A synthetic waste was chosen to allow control of the feed concentrations of organic carbon and ammonia. The synthetic waste was made from distilled water combined with adequate inorganic nutrients and vitamins and was supplemented with acetate and ammonia to obtain the desired substrate composition for each run.

Sodium acetate and ammonium chloride have been selected as the two major constituents of the synthetic wastewater based on several considerations. Ammonium is common in most wastewater and can be oxidized to nitrate which can be subsequently reduced to nitrogen gas. Acetate is a good surrogate for various simple organic compounds since it can be both oxidized to carbon dioxide or fermented to methane. Data is available for rates of transformation of both acetate and ammonia in a variety of wastewater treatment reactors. Consequently, basic data were available for planning the experiments and for comparison with experimental results. The kinetic constants are available for future modeling.

Specific objectives were:

1. To model the composition of primary effluent, secondary effluent, and high-strength industrial waste, using acetate and ammonium as model compounds.
2. To determine removal rates of various electron-acceptors and electron-donors by the SAB under conditions where the biofilm activity was dominated by:

   A-nitrification,

   B-combined nitrification and heterotrophic oxidation,

   C-combined nitrification, heterotrophic

oxidation, and denitrification, and

D-combined nitrification, heterotrophic oxidation, denitrification, and anaerobic fermentation.

These conditions were controlled by varying the concentration of ammonia and acetate in the influent feed.

II. EXPERIMENTAL METHODOLOGY

General

The experimental apparatus was selected so that the biofilm would be supplied oxygen through the gas permeable support. Anoxic conditions were maintained in the liquid compartment and pure oxygen was provided by diffusion through permeable support surface. A complete mass balance of all dissolved species was conducted around the biofilm and the liquid compartment. Metabolism was assumed to be the major source for nitrite and nitrate, and the major sink for ammonia, nitrite, nitrate, and acetate.

The SAB reactor was chosen for biofilm growth experiment based on the work of Timberlake et al. (1988). The reactor was designed to achieve:

1. complete mixing for both the liquid phase and the gas phase,
2. uniform mass transfer over the entire biofilm surface,
3. uniform bacterial growth,
4. definable hydraulic properties, and
5. gas containment.

System Description

The SAB was designed to be completely-mixed, continuous flow reactor (Figure II-1). Four identical

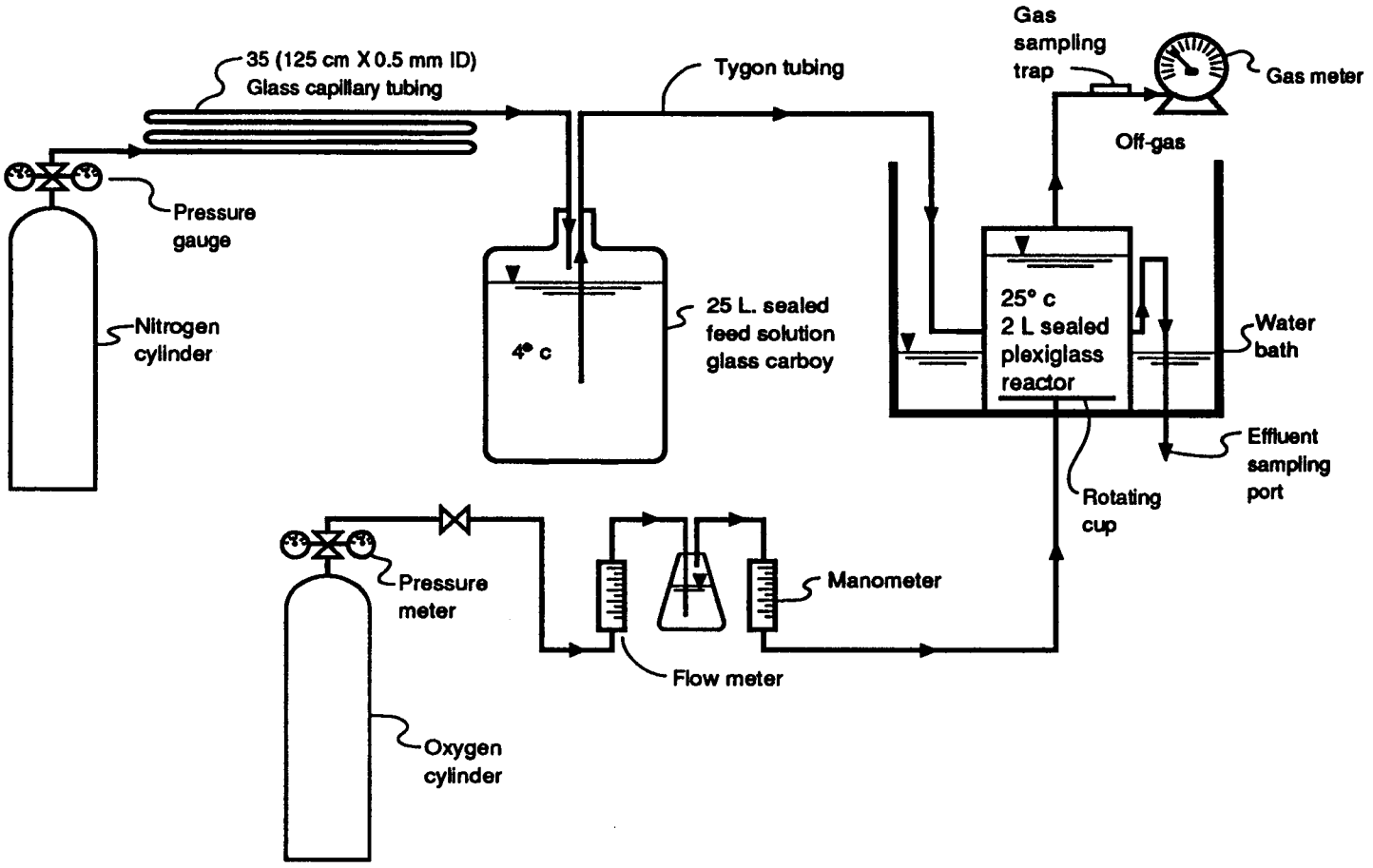


Figure II-1. Schematic diagram of the SAB system.

units were used; all were immersed in a 25°C water bath.

The SAB consisted of two parts. The body was made form a 5" ID (0.5" thick) plexiglass cylinder; the reactor volume was 2 liters. The influent and effluent ports were opposite each other at half the height of the cylinder. The top of the cylinder was fitted with an airtight plexiglass cover secured by eight stainless steel screws and a rubber gasket. A port was provided in the top to facilitate off-gas collection. The bottom of the cylinder was fitted with a watertight plexiglass cover using a waterproof sealant.

The rotary union was 4.9" OD and 4.15" ID. It consisted of a 0.125" thick stainless steel ring, a 0.5" thick plexiglass ring, a rubber gasket, a 1.0" thick plexiglass cup with a flat bottom, and a 0.5" OD X 0.26" ID X 7.5" long stainless steel shaft. The membrane fabric was sandwiched between the stainless steel ring and the plexiglass ring. The stainless steel ring, the membrane fabric, the plexiglass ring, the rubber gasket, and the plexiglass cup were connected together with twelve stainless steel screws. Every other screw was threaded into the rotary cup. The other six screws were threaded into the plexiglass ring only, forming a removable membrane holder. The cup was mounted on the stainless steel shaft. The shaft was mounted in two stainless steel roller bearing and was driven using a pulley and a timing belt, by a 1/8

horsepower Bodine motor. The rotation speed was 58 rpm throughout the experiment. The shaft was fitted through the bottom cover with a 1.25" OD and 0.5" ID oil seal. Physical properties of the SAB are summarized in Table (II-1) and illustrated in Figure (II-2).

The rotating union was utilized to support the biological growth, and to provide aeration, mixing, contact of microorganisms with the wastewater, shear to strip the excess biomass from the surface.

Mixing

Both the aqueous phase and the gas phase were mixed as the union was rotated at 58 rpm. The aqueous phase was mixed by the rotation of the rotary union as a flat plate. Agitation of oxygen resulted from shear forces between the rotary union and the stationary T-shaped oxygen feed tube. The 58 rpm rotation rate did not result in significant shearing of the biofilm at the growth surface.

Sampling

The influent aqueous samples were collected directly from the feed carboys, while the effluent aqueous samples were collected under gravity from the side of the SAB. The

TABLE II-1. Physical Properties of the SAB Reactor

Reactor radius (ID), cm	12.7
Rotary cup radius (OD), cm	12.4
Gortex radius, cm	10.5
Gap width, cm	0.127
Reactor height, cm	15.2
Reactor surface area, cm^2	126.7
Rotary surface area, cm^2	120.8
Gortex surface area, cm^2	86.6
Reactor Volume, cm^3	1925.8
Flow rate, l/day	5.78
Rotary union speed, rpm	58
Hydraulic retention time, hr	8
Reactor temperature, °C	25
Oxygen pressure in rotary cup (H_2O in)	0.2

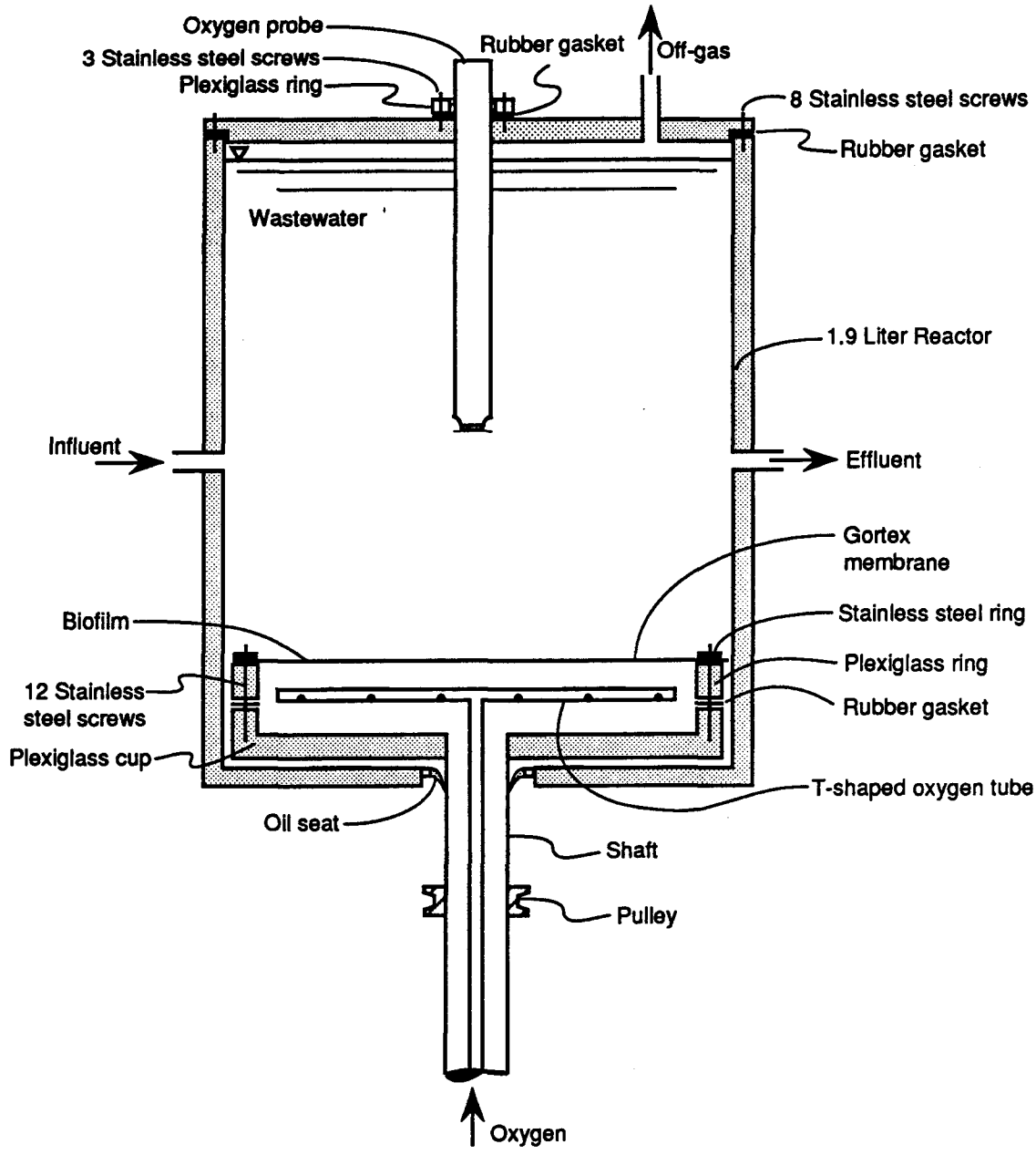


Figure II-2. Cross section of the SAB reactor.

off-gas samples were collected by connecting a sampling trap to the top of the reactor. The off-gas volume was measured with a gas test meter. Samples of the gas inside the rotary union were collected through a small tube that was extended through the T-shaped brass tube. Aqueous and gaseous samples were collected twice a week.

Reactor Operation

The SAB reactor was operated with no recycle. The influent and effluent were on opposite sides of the reactor, the off-gas exited from the top. The SAB was operated at an 8-hour hydraulic detention time.

The feed to the reactor was loaded in a step-wise fashion for Phases I, II, III and IV. In phase I, the SAB reactors were fed only 10 mg-N/l ammonia until the nitrifying layer developed (see Chapter IV). The reactor pH was buffered at pH 7.0. In phase II, the feed was supplemented with 5, and 10 mg/l acetate, for SABs #1 and #2, respectively, until they reached steady-state (see Chapter V). In phase III, the feed was supplemented with 20 and 40 mg/l acetate, for SABs #3 and #4, respectively, until they reached steady-state (see Chapter VI). Then the acetate concentration for SAB #4 was increased to 100 mg/l acetate until it reached steady-state. In phase IV the acetate concentrations for SABs #2, #3, and #4 were

increased to 800, 400, and 200 mg/l acetate, respectively, until the end of the experiment (see Chapter VII).

Bacteria were grown on the membrane and reached a steady-state depth of about 2 mm thickness. The biofilm was smooth and dense. No sloughing was observed during the run. Growth was determined by measurement of dissolved oxygen, three soluble nitrogen forms (ammonia, nitrite, and nitrate), acetate, and methane. The feed solutions were pumped by positive displacement with nitrogen to maintain anoxic conditions.

Pure oxygen was delivered to the SAB from a compressed gas cylinder through a stationary T-shaped brass tube, which extended out of the rotary shaft. The oxygen flow was controlled with a needle valve to keep 0.2" of water pressure in the rotary union.

Inoculation

Initially, each reactor was seeded with 1 liter of trickling filter effluent from Waste Water Reclamation Plant, Corvallis, Oregon. The trickling filter effluent was pumped into the SAB and the particles were allowed to settle onto the membrane for 4 hours without mixing.

Before starting the acetate feeds, the SABs were inoculated with denitrifying bacteria that were grown in a 2 liter batch reactor in the laboratory. One-half liter from the batch reactor was pumped into each SAB and again

the particles allowed to settle onto the membrane for 4 hours.

The SABs were inoculated for the third time before starting the fermentation process. The SABs were seeded with a one-half liter of anaerobic digester supernatant from the Waste Water Reclamation Plant, Corvallis, Oregon. The supernatant was pumped into the SAB and the particles were allowed to settle onto the membrane for 4 hours without mixing. After each inoculation the SABs were emptied and cleaned. The feeding and mixing then were started and maintained continuously.

Growth Surface

A gas-permeable membrane was used to support the microbial growth in the reactor. The membrane consisted of a thin sheet (216 micron thickness) of polytetrafluoroethylene (PTFE) with 0.2 micron pores sandwiched between the teflon and nylon layers. The membrane was positioned with the teflon side facing the liquid phase to support the microbial growth, and the nylon side facing the gas phase. This configuration was determined to maximize gas transfer.

Nutrient Media

All the reactors were fed a synthetic waste. The nutrient solution for the feed of each reactor was made

from distilled water combined with adequate inorganic nutrients and vitamins, and buffered at pH 7.0 with 12 mg/l potassium bicarbonate. The nutrient solution composition was adopted from Owen et al.(1979). This nutrient solution was supplemented with acetate and ammonia to obtain the desired substrate composition for each reactor. The feed solutions were prepared every week in 25-l glass carboys and kept in incubators at 4 °C to prevent bacterial growth. Samples of the feed solution were analyzed weekly for acetate and ammonia to verify that no degradation in the feed container. The preparation of the feed solution is discussed in Appendix A.

III. ANALYTICAL TECHNIQUES

General

Both aqueous and gaseous samples were analyzed. In aqueous samples, acetate, the three soluble nitrogen forms of ammonia, nitrite and nitrate, temperature, pH, and dissolved oxygen were measured. Oxygen, carbon dioxide, and methane were measured in gaseous samples. In addition, the mass and the depth of the biofilm and the suspended solids in the reactors were determined. In selecting a suitable analytical method several important points were considered, including specificity, sensitivity, sample size, reproducibility, detection limit, rapidity, and ease.

Aqueous Sample Analyses

Sample Preparation

The samples were treated to remove both column-fouling substances and interfering ions prior to ion chromatograph analysis. The extraction of column-fouling substances such as proteins, amino acids, folic acid, and vitamins, was accomplished with disposable, bond-phase, octadecyl reverse-phase solid extraction columns having a 200 mg sorbet mass and a 3 ml column volume, C18 (Bond Elut^{R}, Analytichem, Harbor City, CA part #607203). A vacuum

manifold capable of holding up to 10 disposable columns was used to facilitate elution. The removal of the interfering ion, chloride, was accomplished by the disposable OnGuard-Ag cartridge containing a silver form, high capacity, strong acid cation exchange resin (Dionex Corporation, Sunnyvale, CA, part #039598). The recovery of acetate, nitrite, and nitrate from both the clean-up procedures was greater that 98%. The collected samples from reactors were filtered through 0.45 um glass microfiber filters, and 3 ml of the filtrate were loaded into a disposable C18 column, that had been conditioned with one column-volume each of methanol and deionized water, respectively. The purified samples were collected in 5 ml test tubes. The purified samples were injected through an OnGuard-Ag cartridge that had been flushed with 5 ml deionized water, then frozen for a week before ion chromatograph analysis.

Ammonia Analysis

Ion chromatography was used for ammonia analysis. The ion chromatograph (Dionex 4000i) was equipped with a conductivity detector, a gradient pump, a cation guard column containing the same resin as the separator column (4 x 50 mm, Dionex P/N 037025, CG3), a cation separator column (4 x 250 mm, Dionex P/N 037024, CS3) packed with a pellicular low-capacity cation exchange resin constructed of polystyrene-divinylbenzene resin beads, cation

suppressor column (Dionex P/N 037076, CMMS, micro-membrane), and 100 ul sample loop. A Dionex automated sampler was used to deliver 0.5 ml sample to the 100 ul sample loop. Diaminopropionic acid monohydrochoride (0.25 mM) and hydrochloric acid (25 mM) were used as eluent solution at a flow rate of 2.0 ml/min. Fifty-five percent tetrabutylammonium hydroxide (100 mM) was used as a cation suppressor regenerant, at a flow rate of 15 ml/min. The detector response was recorded with a Dionex 4270 integrator. The detector was highly sensitive and linear over the working range. Typical chromatogram for ammonia is presented in Appendix F.

Acetate, Nitrite and Nitrate Analysis

Ion chromatography was used for analysis of acetate, nitrite, and nitrate. A Dionex 4000i ion chromatograph equipped with a conductivity detector was employed for samples in which acetate, nitrite and nitrate were present in mg/l concentration. The chromatograph was equipped with a gradient pump, an anion guard column containing the same resin as the separator column (4 X 50 mm, Dionex P/N 037042, AG4A), an anion separator column (4 X 250 mm, Dionex P/N 030985, AS4A), packed with a pellicular low capacity anion exchange resin constructed of polystyrene-divinylbenzene beads, an anion suppressor column (Dionex P/N 38019, AMMS, micro-membrane), and 100 ul sample loop.

A Dionex automated sampler was used to deliver 0.5 ml sample to the 100 ul sample loop. Sodium bicarbonate (1.7 mM) and sodium carbonate (1.8 mM) were used as eluent solution, at a flow rate of 2 ml/min. Sulfuric acid (25 mM) was used an anion suppressor regenerant, at a flow rate of 3 ml/min.

The detector response was recorded with a Dionex 4270 integrator. The detector was highly sensitive and linear over the working range. Typical chromatograms of acetate, nitrite, and nitrate is presented in Appendix F.

Temperature

Temperature was measured using a mercury thermometer that ranged from 0 to 70°C with an accuracy of ± 1.0°C.

Suspended Solids

Total and volatile suspended solids were determined by the procedures given in Standard Methods for the Examination of Water and Wastewater.

pH

An Orion 91-05 combination pH electrode was used to measure sample pH. The electrode was connected to an Orion 60/A digital ionizer, which had an accuracy of ± 0.01 pH.

Dissolved Oxygen

The dissolved oxygen (DO) concentration was determined with a YSI 5739 dissolved oxygen probe. The probe was connected to a YSI 57 dissolved oxygen meter with an accuracy of ± 0.1 mg/l. The meter was connected to an Omniscribe D5000 stripchart recorder.

Gaseous Sample Analysis

Carbon Dioxide, Oxygen, and Methane Analysis

Gas chromatography was used for analysis of carbon dioxide, methane, and oxygen. A Fisher 25V gas partitioner equipped with a thermal conductivity detector was employed for samples analysis. The gas partition was equipped with a 30" X 1/4" column packed with 30% HMPA on column (60 to 80 mesh) and a 6.5" X 3/16" column packed with 42-60 mesh molecular sieve 13X. The first column separated carbon dioxide from other gases and the second column separated oxygen, nitrogen, and methane, in that order. The instrument operated at ambient temperature. Purified helium was used as a carrier gas, at a flow rate of 80 ml/min. The detector response was recorded with a Hewlet Packard 3390A reporting integrator. A 250 ul pressure-lock, gas-tight syringe was used to collect 100 ul gas sample from each reactor, and immediately injected into the gas partitioner for analysis. Typical chromatograms of

carbon dioxide, oxygen, and methane is presented in Appendix F.

Biofilm Analyses

Biofilm Depth

Biofilm depth was measured by differential focussing using a Nikon, Model SBR-kt, microscope equipped with a fine focus knob calibrated in microns. The apparent difference between the top of the film and the Gortex membrane represents the biofilm depth. Biofilm depths determined in this fashion are approximate and non-destructive to the biofilm.

Biofilm Mass

At the end of each run, the biofilm mass was determined as the residue remaining on the glass filter after drying to 103°C for 12 hours.

IV. NITRIFICATION

Background

Biological nitrification has been used recently to control the discharge levels of ammonia in wastewater. Such controls reducing the impact of ammonia on the receiving surface waters as ammonia can exert considerable oxygen and chlorine.

Several types of attached and suspended growth reactors have been employed to achieve nitrification. The slow growth of nitrifiers makes attached-growth systems more feasible because of their longer solid retention times (SRTs) as compared with suspended-growth systems. Often the use of a suspended growth system for nitrification is limited by the required SRTs to maintain an active nitrifying population. Design SRTs for nitrification are typically considered to about 10 days (Jenkins and Garrison, 1968).

Biofilm reactors for nitrification have been successfully used with variety of configurations. Pretorius (1975) examined nitrification of an aerobically pretreated domestic waste water in a rotating disk system and observed nearly complete conversion of 40 mg NH_4^+-N/l to NO_3^--N with a hydraulic retention time of 10 hours. Haug and McCarty (1972) reported nitrification of a preoxygenated secondary effluent using submerged filters

and observed 90% removal of ammonia nitrogen (influent 20 mg NH_4^+-N/l) at temperature 25°C with a hydraulic detention time of 30 minutes. O'Shaughnessy, et al. (1980) studied the nitrification of an activated sludge effluent with rotating biological contactors and reported 95% removal of ammonia nitrogen (influent 10-20 mg NH_4^+-N/l) with a hydraulic detention time of 3 hours. Zenz, et al. (1980) employed four-stages rotating biological contactors for the nitrification of an activated sludge effluent and reported over 90% oxidation of ammonia nitrogen (influent 14 mg NH_4^+-N/l) with a hydraulic detention time of 5 hours and at 25°C.

The objective of this experiment was to provide evidence of the ability of the SAB to treat low strength soluble wastewater by nitrification.

Operating Conditions

Microbiology and Biochemistry

The family of bacteria mediating the nitrification reactions is named Nitrobacteraceae. It has been a common practice in environmental engineering to name all the ammonia oxidizer bacteria Nitrosomonas, and the nitrite oxidizers bacteria Nitrobacter. This practice has been adopted in this thesis.

Both Nitrosomonas and Nitrobacter are obligate autotrophs using carbon dioxide as their carbon source. The oxidation of ammonia is mediated by five genera of bacteria: Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosocystis, and Nitrosogloea. Painter (1970) indicated that the last three named genera are of doubtful validity. The oxidation of nitrite is carried out by two genera of bacteria, namely, Nitrobacter and Nitrocystis.

The biological oxidation of ammonia nitrogen is a two-step process in which Nitrosomonas bacteria oxidize ammonia to nitrite and then Nitrobacter bacteria convert nitrite to nitrate. The oxidation of ammonia and nitrite, according to Mcarty (1971), can be described as:

Nitrosomonas: $28.14\ NH_4^+ + 35.71\ O_2 + 5\ CO_2 = C_5H_7O_2N + 27.14\ NO_2^- + 55.28\ H^+ + 25.14\ H_2O$ (IV-1)

Nitrobacter: $95.28\ NO_2^- + 42.63\ O_2 + 5\ CO_2 + NH_4^+ + 2\ H_2O = C_5H_7O_2N + 95.26\ NO_3^- + H^+$ (IV-2)

The nitrification process produces acid and, as a result, the pH may decrease and adversely affect the rate of nitrification. Theoretically 7.14 mg/l alkalinity as $CaCo_3$ is required per mg of ammonia nitrogen oxidized to neutralize the acid produced.

The growth of nitrifiers is very slow as the energy required for cell growth is large. Oxidation of 10 mg NH_4^+-

N/l results in about 2.9 mg/l of Nitrosomonas and 0.85 mg/l of Nitrobacter. The stoichiometry of growth is shown in Appendix B.

Nutrients

The macronutrient requirement for the nitrifying bacteria are CO_2 and O_2 plus NH^+_4 in the case of Nitrosomonas and NO^-_2 in the case Nitrobacter. Equations (IV-1) and (IV-2) show that the stoichiometric oxygen requirement for complete oxidation of ammonia to nitrate are 2.9 mg oxygen/mg-N for Nitrosomonas and 1.02 mg oxygen/mg-N for Nitrobacter.

Apart from these micronutrients, other micronutrients are required for optimum growth conditions. For example, Lees (1952) and Lees et al. (1957), showed that phosphate, magnesium, and iron are required by Nitrosomonas and Nitrobacter, while calcium and copper are required by Nitrosomonas. Delwiche and Finstein (1965) showed that molybdenum stimulates the growth of Nitrobacter. Painter (1970) showed that Nitrosomonas is stimulated by the presence of sodium salts. Most of the micronutrients reported to be required for bacterial growth are listed in Appendix A with the range of concentration reported to be stimulatory.

Temperature

The effect of temperature on nitrification is summarized in U.S. EPA (1975). In general, nitrification increased as temperature increased. Weng and Molof (1974) found substantial increase in the nitrification process by increasing the RBC temperature from 20.7 to 29.3°C. Lue-Hing et al. (1976) showed a significant increase of nitrification rate of high strength ammonia waste, when temperature increased from 9 to 20°C.

pH

The optimum growth activity for Nitrosomonas and Nitrobacter occurs at pH 7-9 (U.S. EPA, 1975). Twelve mg/l of potassium bicarbonate was used to control the SAB pH at 7.0.

Theory of SAB Process

In the SAB process, ammonia and the required nutrients diffuse from the bulk liquid into the biofilm where the reaction occurs. Oxygen diffuses through the permeable membrane into the biofilm. All products likewise have to be removed by molecular diffusion out through the biofilm and into the bulk liquid. The diffusion of non-electrolytes such as O_2 and CO_2 are not influenced by the presence or absence of other dissolved compounds. However,

the diffusion of electrolytes such as ammonia, nitrite, nitrate and hydrogen must be balanced by either a concurrent flow of opposite charge or a counter-current flow of the same charge.

In the SAB, oxygen diffuses through the permeable membrane into the biofilm along a concentration gradient formed by the reduction of oxygen in the biofilm. Ammonia ions diffuse from the bulk liquid into the biofilm along a concentration gradient formed by the oxidation of ammonia in the biofilm. Nitrite is produced and quickly transformed to nitrate. Both nitrite and nitrate diffuse from the biofilm along concentration gradients. Since nitrite is rapidly converted to nitrate, the amount of nitrate diffusing out of the biofilm must be larger in comparison to nitrite diffusion. The hydrogen ions produced during the oxidation of ammonia diffuse out of the biofilm to the bulk liquid along their concentration gradient and are eventually neutralized by the bicarbonate ions in the water. In addition bicarbonate ions diffuse into the biofilm along a concentration gradient formed by their neutralization in the biofilm by hydrogen ions. A schematic diagram for nitrification in the SAB is shown in Figure (IV-1), while the concentration profile in the biofilm is illustrated in Figure (IV-2).

At the permeable membrane interface, oxygen concentration is maximum and in equilibrium with O_2 in the

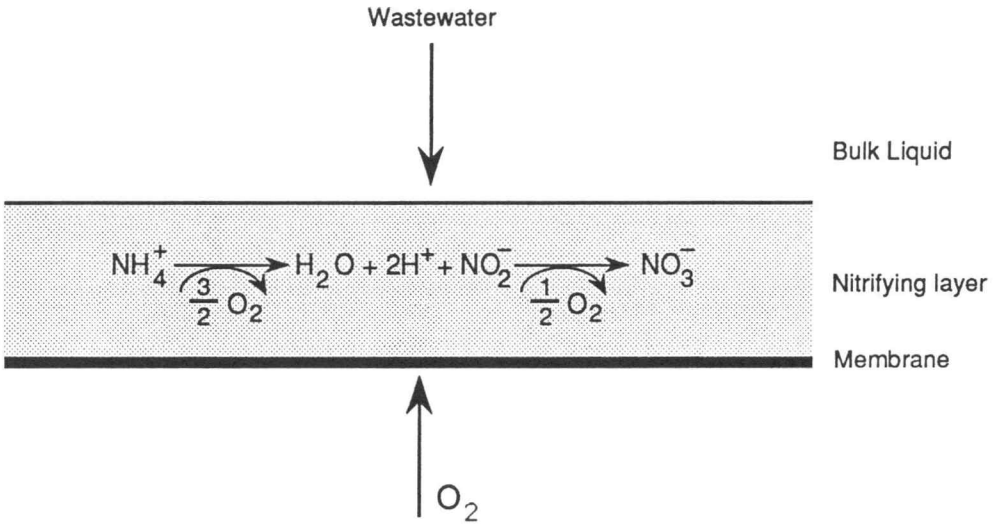


Figure IV-1. Schematic diagram for nitrification processes in a biofilm.

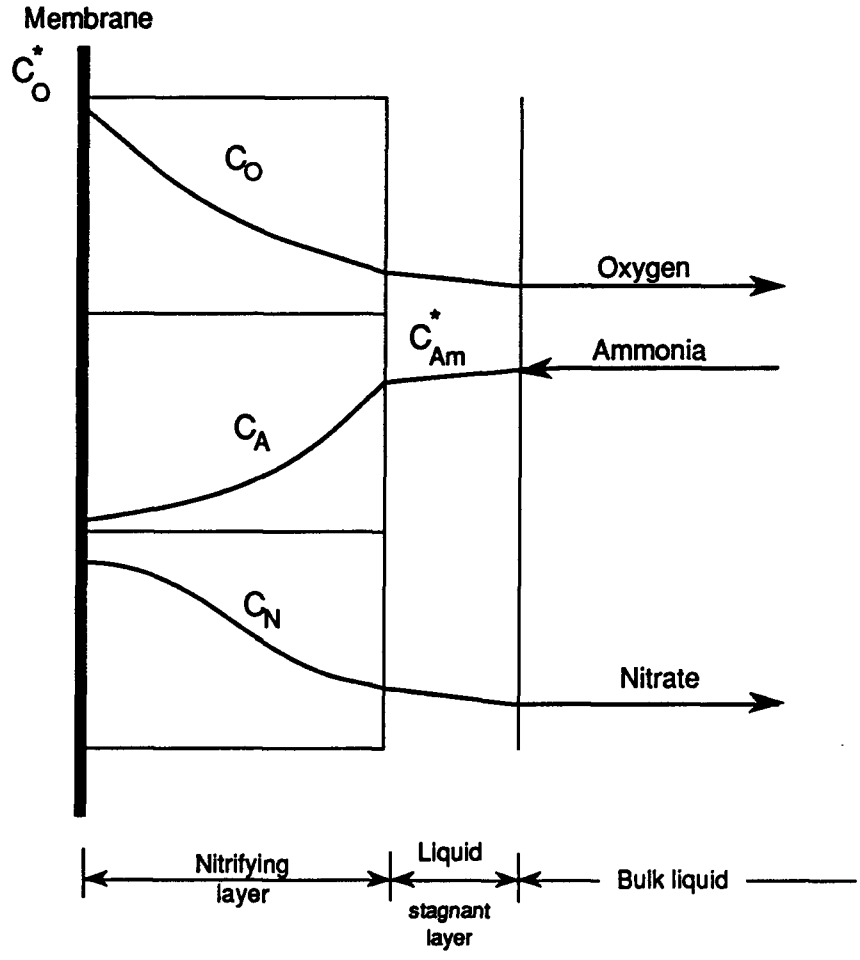


Figure IV-2. Conceptual concentration profile in a biofilm illustrating nitrification.

gas phase, and ammonia is at minimum. In contrast, ammonia is at maximum and oxygen concentration is at minimum at the biofilm liquid interface.

Results and Discussion

The nitrification experiment (Phase I) was started in November 1, 1988 and lasted 2.5 months. The ammonia concentration in the aqueous phase continued to decrease during the first 50 days of operation and then became constant. This was assumed to represent the quasi-steady-state performance of the SAB. The steady-state results of the SAB experiments are summarized in Table (IV-1). The performance of the SAB as a function of time is shown in Figures (IV-3) to (IV-5). The establishment and maintenance of the nitrifying biofilms followed a similar pattern for all SABs, in that about 30 days was required to achieve the establishment and acclimation of nitrifying biofilm.

Initially ammonia oxidation dominated the biofilm with a build-up of nitrite. At about 23 days following the peak of nitrite production (Figures IV-3 to IV-5), a Nitrobacter population appears to have become firmly established in the biofilm. After 42 days, nitrate predominated in the effluent. Nitrite was below 1.0 mg NH_4^+-N/l for the remainder of the study period. There are no significant sources or sinks of nitrogen in the SABs, and, as expected,

Table IV-1. SAB Steady-State Data for Phase I

SAB number	#2	#3	#4
NH_4^+-N, mg/l inf.	10.4±0.25	11.33±0.12	10.29±0.14
NH_4^+-N, mg/l eff.	0.046±0.026	0.83±0.7	1.31±0.36
conversion, %	99.6	92.7	87.3
NO_2^--N mg/l eff.	1.69±0.47	0.71±0.15	1.02±0.14
NO_3^--N, mg/l eff.	8.27±0.56	8.31±0.27	7.22±0.26
pH	7.09±0.17	7.02±0.09	7.03±0.09
DO, mg/l	4.0±0.4	1.8±0.1	2.03±0.6
Biofilm Depth μm	39	43	54

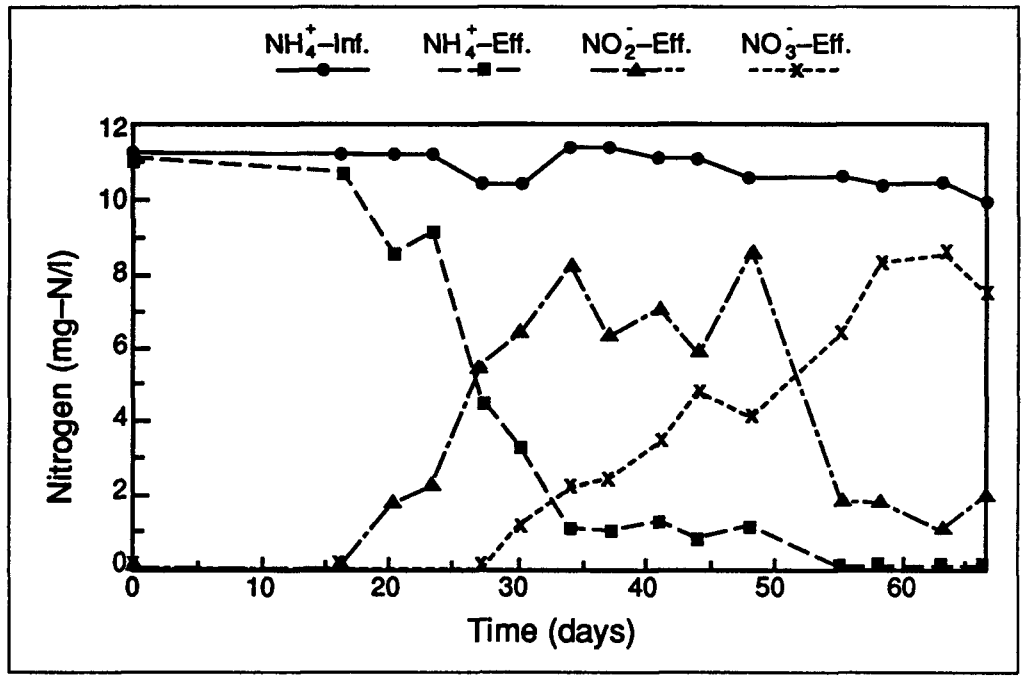


Figure IV-3. Nitrogen influent and effluent concentration, SAB #2 (influent concentration = 10 mg NH_4^+-N/l).

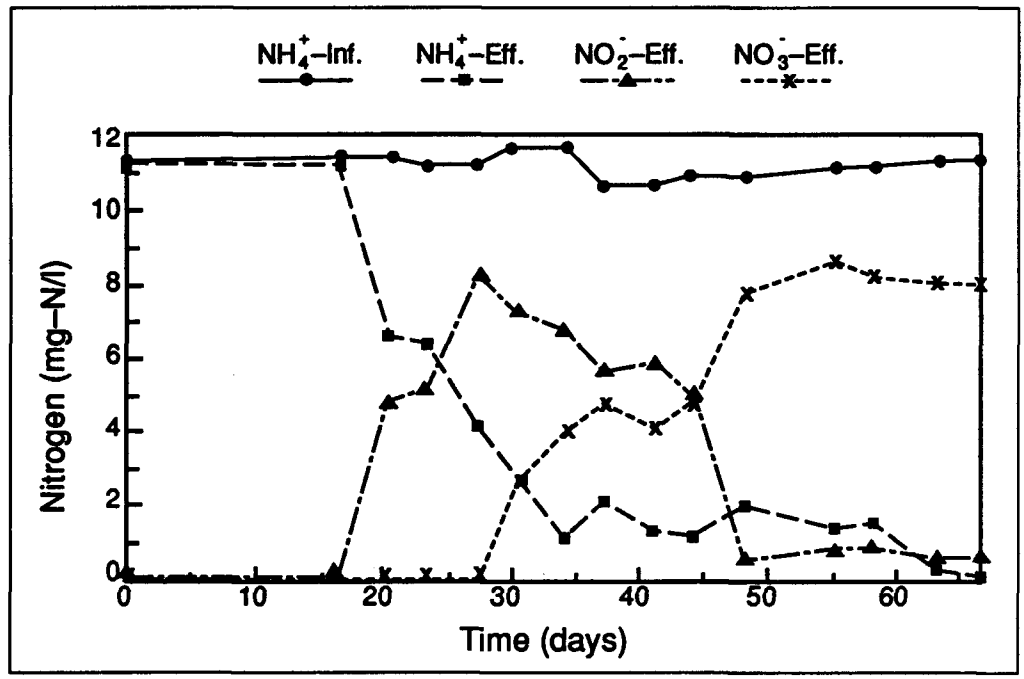


Figure IV-4. Nitrogen influent and effluent concentration, SAB #3 (influent concentration = 10 mg NH_4^+-N/l).

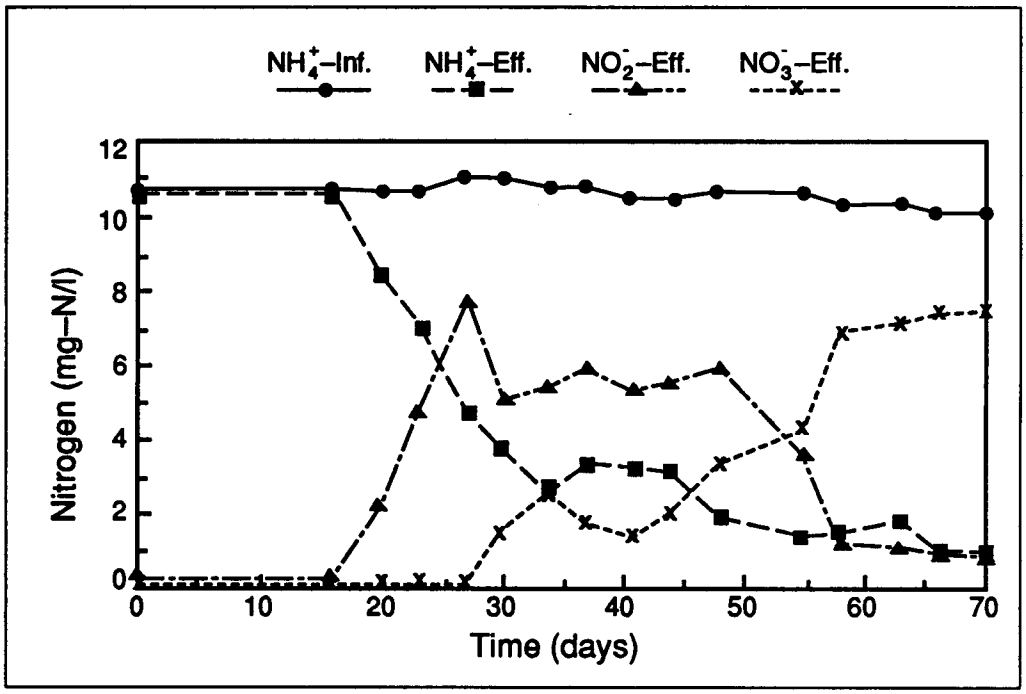


Figure IV-5. Nitrogen influent and effluent concentration, SAB #4 (influent concentration = 10 mg NH_4^+-N/l).

the total nitrogen concentration (sum of ammonia, nitrite, and nitrate) remained relatively constant even though the concentration of the individual nitrogen forms changed.

The mass loading rate applied to the SAB was 0.71 mg NH_4^+-N/cm^2.d with 10 mg NH_4^+-N/l influent concentration. The efficiency of ammonia removal was about 96% at a was 0.66 mg NH_4^+-N/cm^2.d which was higher than the values of 0.29 mg NH_4^+-N/cm^2.d observed by Zenz et al. (1980) and 0.37 mg NH_4^+-N/cm^2.d reported by O'Shaughnessy et al. (1980) for rotating biological contactors.

Twelve mg/l potassium bicarbonate was used as a buffer and also to insure that sufficient inorganic carbon would be available for nitrification. The average pH value for the reactors was 7.1. The temperature of the SAB was kept constant at 25°C throughout the run. The average effluent total suspended solids was below 3 mg/l during the data collection period. Since no significant sloughing of the biofilm was observed, cell growth in the reactor probably continued to accumulate during the experimental runs.

The reactors and biofilms were completely aerobic during Phase I. The average dissolved oxygen and ammonia concentrations at steady-state in the liquid phase were 2.6 mg/l and 0.73 mg-N/l, respectively. Williamson and McCarty (1976a, b) proposed that the rate of substrate utilization within biofilms follows the Monod equation.

The rate limiting substrate at any point within the biofilm may be determined as the minimum of:

$$S_a/(K_{sa}+S_a) \quad \text{or} \quad S_d/(K_{sd}+S_d) \qquad \text{(IV-3)}$$

In which K_{sa} and K_{sd} are the half-velocity coefficients for the electron-acceptor and the electron-donor, respectively. S_a and S_d represent the concentrations of the electron-acceptor and the electron donor at a specified depth in the biofilm, respectively. Williamson (1973) found K_s for oxygen and ammonia to be 0.3 and 0.5 mg/l, respectively. Based on equation (IV-3), ammonia would be the limiting nutrient throughout the biofilm. If the biofilm was sufficiently deep for oxygen to be depleted before all the ammonia had been oxidized, ammonia would be the limiting nutrient near the permeable membrane-biofilm interface, while oxygen would be limiting near the biofilm-bulk liquid interface. However, the 10 mg-N/l of ammonia could not support enough organisms to produce anoxic conditions.

V. NITRIFICATION AND HETEROTROPHIC OXIDATION

Background

The biological oxidation of organic compounds and ammonia-nitrogen has been used in recent years to control carbonaceous and nitrogenous oxygen demands. The heterotrophic bacteria are responsible for carbon oxidation and use carbon as an electron-donor and oxygen as an electron-acceptor. The nitrifying bacteria use ammonia as an electron-donor and oxygen as an electron-acceptor. In biofilms where a competition exists between heterotrophs and nitrifiers for dissolved oxygen, the nitrifiers are often outgrown within the biofilm because of their smaller growth rate.

Activated sludge systems often combine carbon removal and nitrification by maintaining long solids retention time (SRTs) above 5 to 7 days. However, these long SRTs result in increased oxygen requirements due to high levels of endogenous respiration, and may require high recycle rates from the secondary clarifier to maintain low solid fluxes in the secondary clarifier. Long SRTs may also result in poorer settling characteristics of the activated sludge.

In biofilm reactors, nitrification often cannot be achieved because of oxygen limitations. The nitrifying organisms must have sufficient solids retention time to multiply in the aerobic zone of the biofilm. Nitrification

in combined carbon oxidation/nitrification biofilm reactors is often limited by the difference in growth rates between the heterotrophic and nitrifying bacteria. The nitrifiers in a growing biofilm can quickly be submerged beyond the limit of oxygen penetration.

This problem can be overcome by either increasing the oxygen concentrations so that oxygen can penetrate deeper or by reducing the biochemical oxygen demand (BOD) of the wastewater to below 20 mg/l. For example, heterotrophic oxidation/nitrification in trickling filters and rotating biological contactors (RBC) most commonly occurs sequentially with carbon oxidation near the influent and nitrification near the effluent where the BOD level is below 20 mg/l. This phenomena has been reported by several authors. Weng and Molof (1974) used a six-stage biological fixed-film rotating disk to treat synthetic wastewater and found that the soluble BOD is removed in the first few stages. Nitrification occurred in later stages where BOD levels were less than 14 mg/l and DO was greater than 2 mg/l. Similarly, Torpey et al. (1972) employed a ten-stage rotating biological contractors and observed nitrifying activity only in the later stages after the bulk of carbonaceous matter had been removed. Also, he observed nitrification at earlier stages of the RBC when biofilm was exposed to an atmosphere enriched with oxygen. Tomlinson and Snaddon (1966) showed that heterotrophs extinguished

nitrification in a well established nitrifying biofilm when organic carbon was increased in the feed. He observed that the nitrification activity could be restored by increasing the oxygen concentration of the atmosphere. Mueller et al.(1980) and Surampalli and Baumann (1989) reported on the performance of a full scale rotating biological contactors (RBC) with carbon oxidation and nitrification. The reported that carbon oxidation and nitrification varied significantly from the entrance to the exit of the reactor, with nitrification limited to the later stages of the reactor where the carbon levels are low. Mueller et al. (1980) concluded that carbon level must fall to 10-15 mg/l before nitrification becomes significant. Strand (1986) indicated that carbon and ammonium oxidation are sequential in rotating biological contactors.

The SAB differs from typical biofilm reactor in that oxygen is provided by diffusion through a permeable support surface from one direction, and substrates are provided from the opposite direction. This configuration isolates the slow growing nitrifying bacteria in a zone high in ammonia and dissolved oxygen, maximizing conversion of ammonia to nitrate.

The objective of this portion of the study is to provide evidence of the ability of the SAB to treat soluble wastewater by combined nitrification and heterotrophic oxidation.

Operating Conditions

Biochemistry

Acetate is aerobically oxidized by several groups of heterotrophic bacteria. These bacteria use acetate as a carbon source for cell synthesis as well as for energy. According to McCarty (1971), the energy reaction is as follows:

$$\frac{1}{8}\ CH_3COO^- + \frac{1}{4}\ O_2 = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + \frac{1}{8}\ H_2O \qquad \text{(V-1)}$$

and the synthesis reaction is as follows:

$$\frac{1}{8}\ CH_3COO^- + \frac{3}{40}\ CO_2 + \frac{1}{20}\ NH_4^+ = \frac{1}{20}\ C_5H_7ON + \frac{3}{40}\ H_2O + \frac{3}{40}\ HCO_3^- \qquad \text{(V-2)}$$

The overall metabolic reaction for acetate under optimal conditions for the bacteria, becomes:

$$4.27\ CH_3COO^- + 3.54\ O_2 + NH_4^+ = C_5H_7O_2N + 0.27\ CO_2 + 3.27\ H_2O + 3.27\ HCO_3^- \qquad \text{(V-3)}$$

Equation (V-3) gives a maximum yield of 0.45 g cell/g CH_3COO^-, oxygen consumption of 0.45 g O_2/g CH_3COO^-, and ammonia utilization of 0.056 g NH_4^+-N/g CH_3COO^-

The biochemistry of nitrification is discussed in Chapter IV. The stoichiometry of growth is in Appendix B.

Theory of SAB Process

The biofilm in the SAB reactor can be described schematically as two layers of deep biofilms. The biofilm nearest to the membrane will be dominated by the nitrification involving oxygen as the electron-acceptor and ammonia as the electron-donor. Oxygen concentrations will be in stoichiometric excess for oxidation of ammonia. The biofilm nearest the bulk liquid will be dominated by heterotrophic oxidation with oxygen as the electron-acceptor and acetate as the electron-donor. Figure (V-1) shows a schematic diagram for nitrification and heterotrophic oxidation in the SAB reactor.

Acetate, ammonia and the micronutrients diffuse from the bulk liquid into the biofilm. Oxygen diffuses through the membrane into the biofilm. All products (nitrite, nitrate, carbon dioxide and hydrogen ions) likewise have to be removed by molecular diffusion out through the biofilm and into the bulk liquid.

Oxygen diffuses through the membrane into the biofilm along a concentration gradient formed by the reduction of oxygen, due to nitrification in the first layer and carbon oxidation in the second layer, in the biofilm. Ammonia ions diffuse from the bulk liquid into the biofilm along a concentration gradient formed by the oxidation of ammonia in the nitrifying layer and the uptake in biomass synthesis

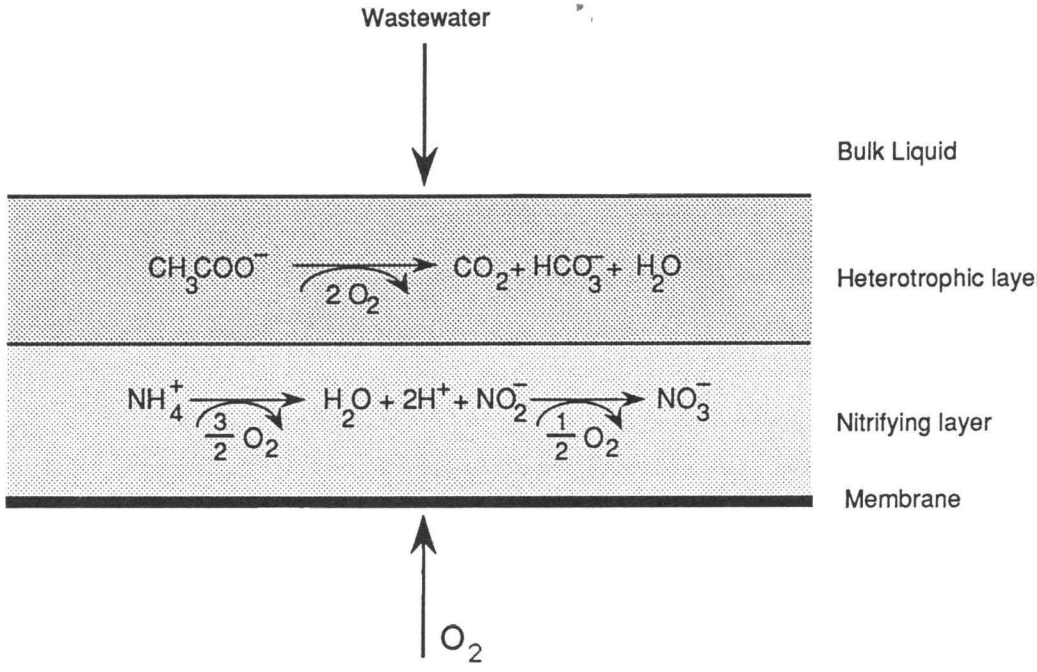


Figure V-1. Schematic diagram for nitrification and aerobic heterotrophic oxidation processes in a biofilm.

in the heterotrophic layer. Nitrite is produced and, often with an adequate Nitrobacter population quickly transformed to nitrate in the nitrifying layer. Both nitrite and nitrate diffuse from the biofilm along concentration gradients. Since nitrite is rapidly converted to nitrate, the amount of nitrate diffusing out of the biofilm must be larger in comparison to nitrite diffusion. The hydrogen ions produced during the oxidation of ammonia diffuse out of the biofilm to the bulk liquid along their concentration gradient and are eventually neutralized by the bicarbonate ions in the bulk liquid. The bicarbonate ions diffuse into the biofilm along a concentration gradient formed by their neutralization in the biofilm by hydrogen ions. Figure (V-2) shows a conceptual description of the concentration profile in the biofilm.

Results and Discussion

The Phase II experiment started two weeks after the nitrification experiment reached steady-state. The reactor was inoculated with heterotrophic bacteria from the Wastewater Reclamation Plant, Corvallis, Oregon. One-half liter from the activated sludge liquor was pumped into each SAB and allowed to settle to the bottom. After 4 hours, the SAB were emptied and cleaned, then feed and mixing were started and maintained continuously. SAB #1 and #2 were

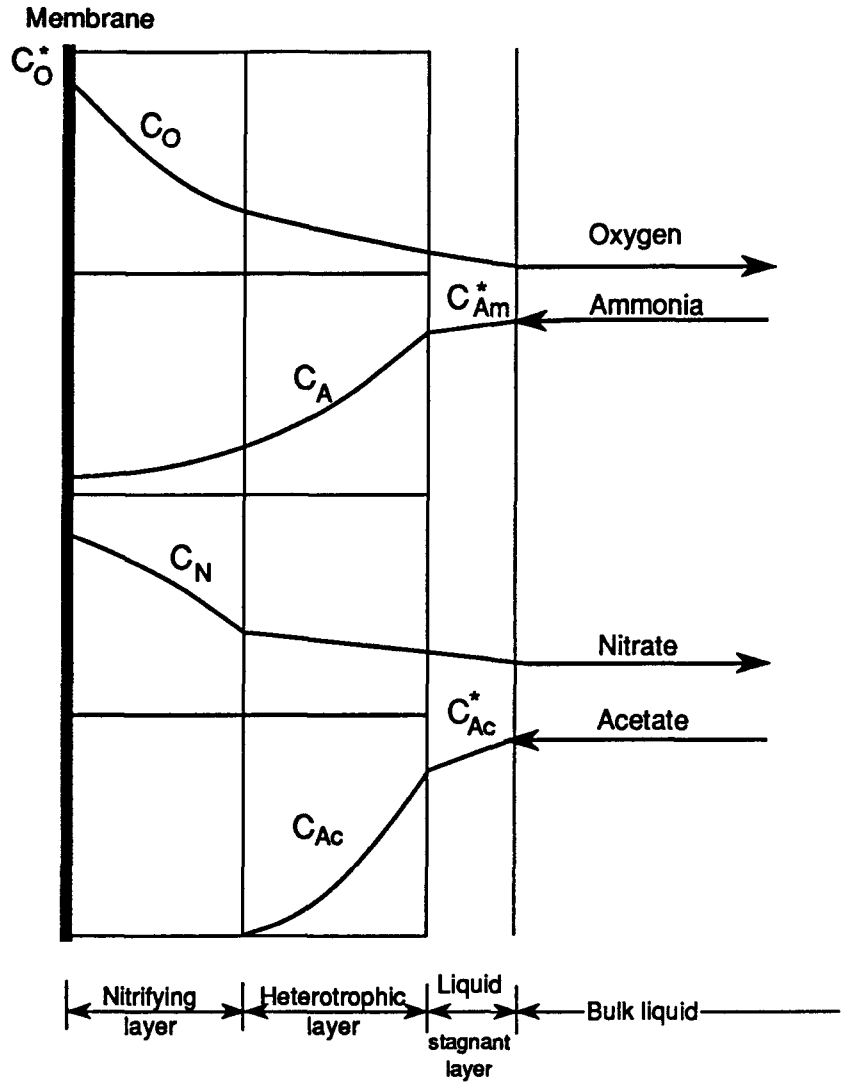


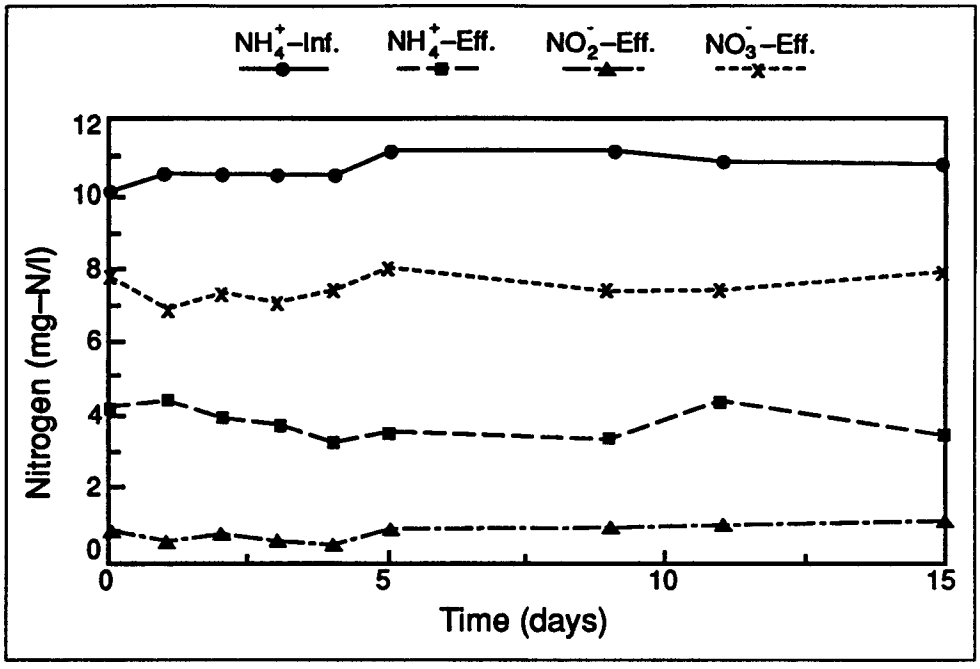
Figure V-2. Conceptual concentration profile in a biofilm illustrating nitrification and aerobic heterotrophic oxidation.

fed 5 and 10 mg/l acetate until steady-state was achieved, respectively.

In SAB #1 and #2 acetate concentration dropped to less than 1 mg/l two days after acetate feed begun (Figure VI-3 and VI-4). During this time nitrification rate was reduced by about 20%, compared to Phase I. This was assumed to represent the steady-state performance of the SABs. The steady-state results of the SAB experiments are summarized in Table (V-1).

A series of mass balances based on growth thermodynamics and energetics model (McCarty, 1971) were developed to follow the fate of the nitrogen compounds and acetate in the SABs. A sample calculation is in Appendix C. The acetate and nitrogen flux values are presented in Figures (V-5) and (V-6), respectively. The acetate loading rates applied to the SABs #1 and #2 were 0.384 and 0.758 mg acetate/cm^2.d with 5 and 10 mg/l acetate influent concentrations, respectively. The efficiency of acetate removal was 99% at a hydraulic detention time of a 8 hours. The acetate oxidation fluxes were 0.368 to 0.748 mg acetate/cm^2.d, respectively.

These fluxes values are within the lower range of values observed for rotating biological contactors (0.01 - 5 mg COD/cm^2.d) by Famularo (1978), Friedman et al. (1976), Mueller et al. (1980), and Weng and Molof (1974). This is



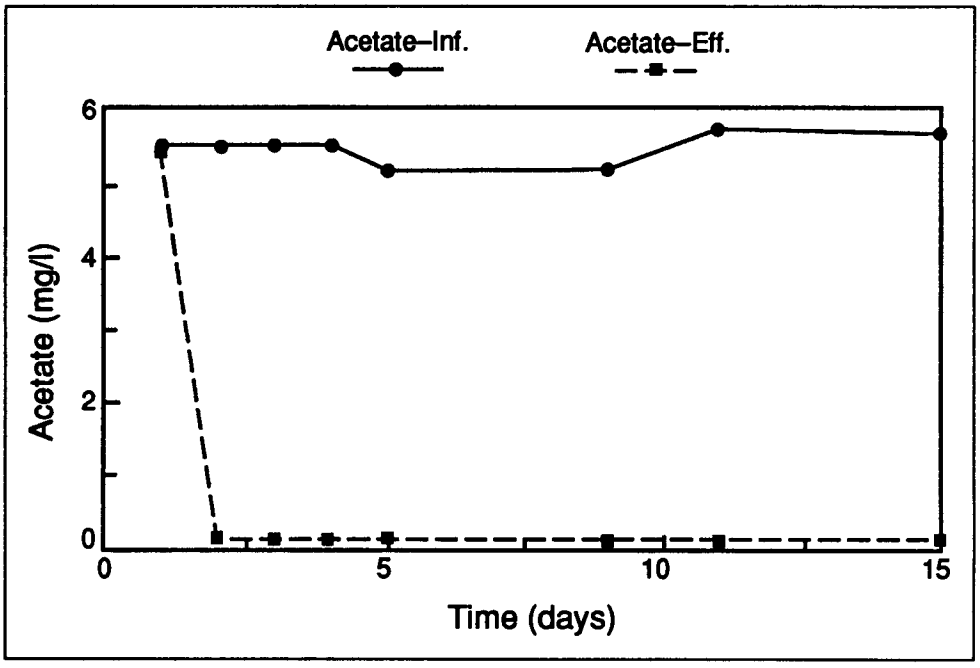


Figure V-3. Nitrogen and acetate influent and effluent concentration, SAB #1 (influent concentration = 10 mg/l NH_4^+-N/l and 5 mg acetate/l).

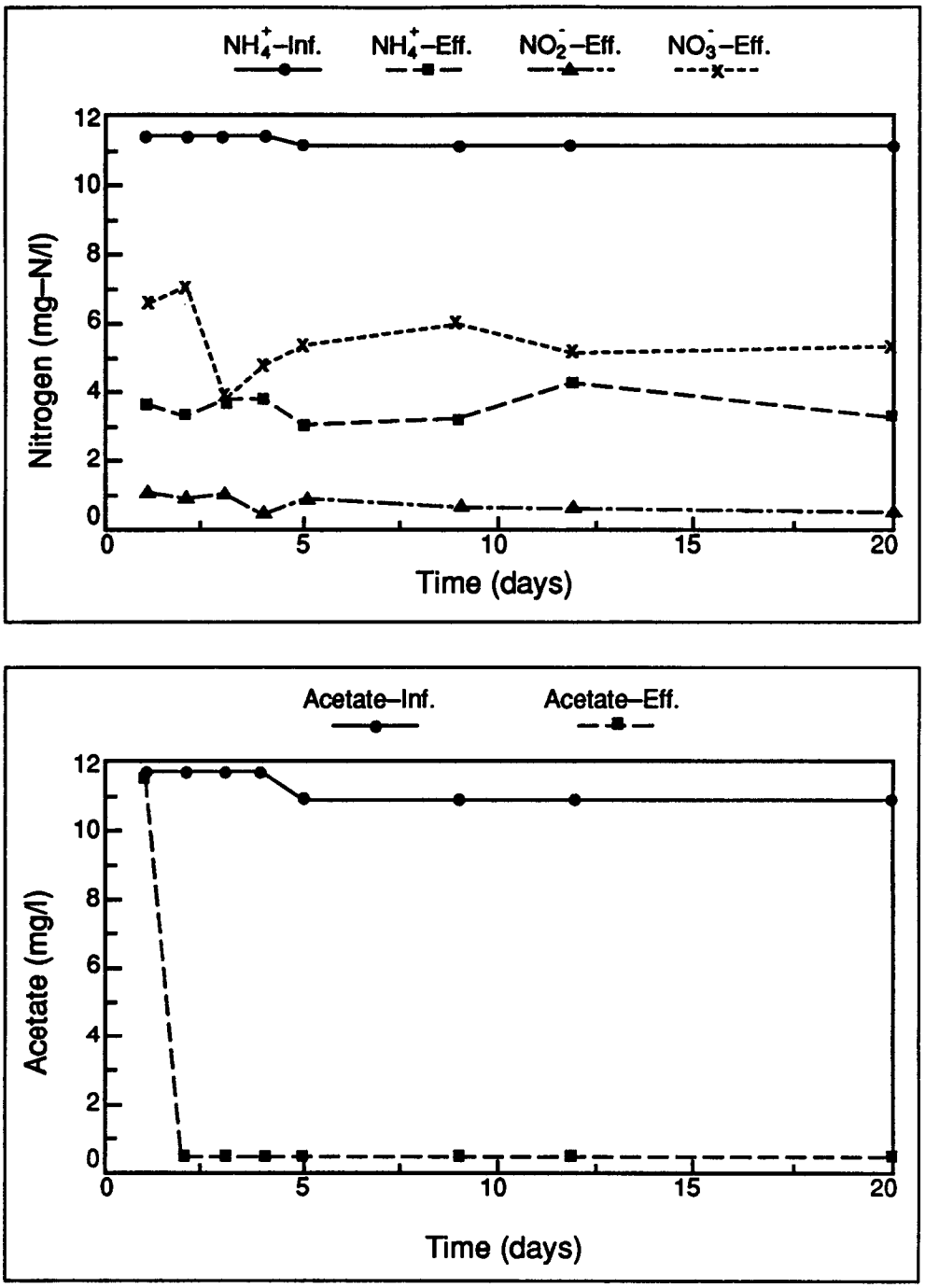


Figure V-4. Nitrogen and acetate influent and effluent concentration, SAB #2 (influent concentration = 10 mg/l NH_4^+-N/l and 10 mg acetate/l).

Table V-1. SAB Steady-State Data for Phase II

SAB number	#1	#2
NH_4^+-N, mg/l inf.	10.76±0.34	11.33±0.11
NH_4^+-N, mg/l eff.	3.79±0.39	3.49±0.47
conversion, %	64	69
NO_2^--N mg/l eff.	0.79±0.24	0.66±0.14
NO_3^--N, mg/l eff.	7.48±0.35	5.26±0.32
Acetate mg/l inf.	5.76±0.19	11.36±0.42
Acetate mg/l eff.	<1.0	<1.0
Conversion, %	>99	>99
pH	7.8	7.2
DO, mg/l	0.6	0.8
Biofilm Depth μm	58	76

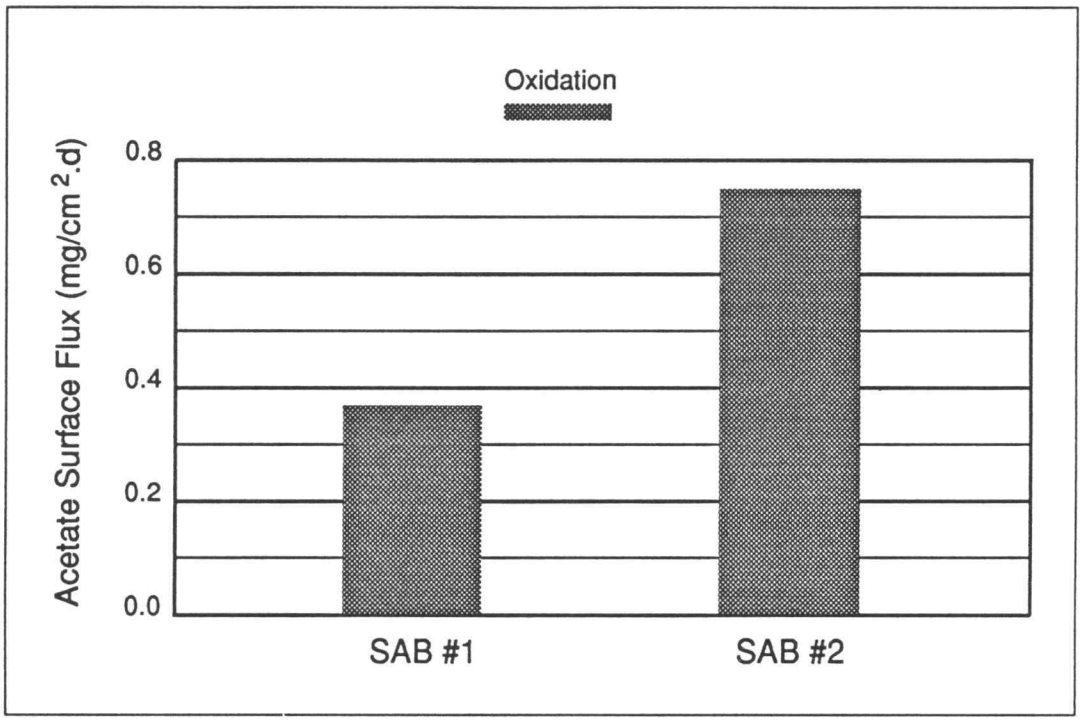


Figure V-5. Acetate flux for SAB #1 and SAB #2, calculated from mass balances.

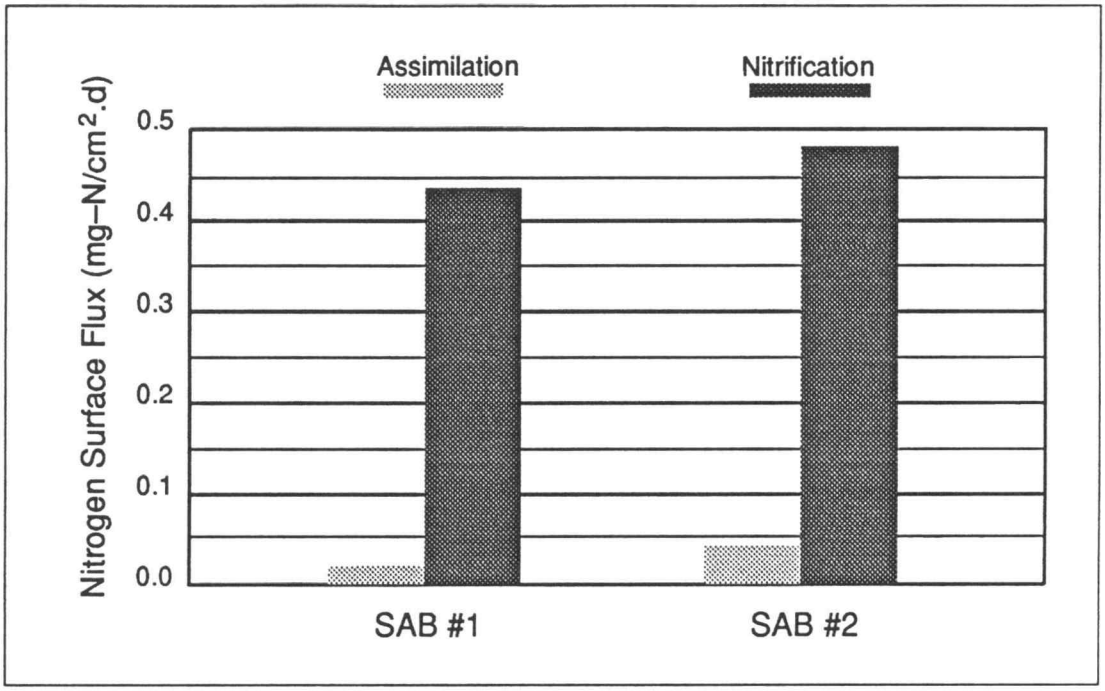


Figure V-6. Nitrogen flux for SAB #1 and SAB #2, calculated from mass balances.

probably due to lower acetate concentration levels than the organic levels in the RBCs.

The ammonia mass loading rate applied to the SABs was 0.71 mg NH_4^+-N/cm^2.d with 10 mg NH_4^+-N/l influent concentrations. The efficiency of ammonia removal were 64 to 69%. Nitrification fluxes were 0.481 to 0.436 mg NH_4^+-N/cm^2.d, while assimilation fluxes ranged from 0.021 to 0.042 mg NH_4^+-N/cm^2.d, respectively.

The nitrification flux values compare well with those values observed for rotating biological contactors of 0.01 to 0.42 mg ^{NH4}-N/cm^2.d by (Antonie, 1974; Mueller et al., 1980; Weng and Molof, 1974). However, Wantanabe et al. (1980) reported values up to 0.9 mg NH_4^+-N/cm^2.d. Higher acetate concentration up to 100 mg/l did not inhibit nitrification but resulted in denitrification activity.

The average pH value for the SABs was 7.6. Potassium bicarbonate was used as a buffer and also to insure that sufficient inorganic carbon would be available for nitrification. The temperature of the SABs was kept constant at 25°C throughout the run. The effluent total suspended solids was consistently, below 2 mg/l during the data collection period. No significant sloughing of the biofilm occurred and cell growth in the reactor continued to accumulate during the experimental runs. The reactors were in an aerobic condition during the study. The average dissolved oxygen concentrations in the liquid phase at steady-state were less than 1 mg/l.

VI. NITRIFICATION, HETEROTROPHIC OXIDATION, AND DENITRIFICATION

Background

Combined nitrification and denitrification has been developed as a means of removing nitrogen from wastewaters to control surface water eutrophication. The denitrification process is carried out by a number of heterotrophic bacteria include Microccus, Pseudomonas, Denitrobacillus, Spirillum, Bacillus, and Achromobacter. It has been a common practice in environmental engineering to name this family of bacteria denitrifiers. This practice has been adopted in this thesis.

Denitrifiers are obligate anaerobes, use organic carbon as their carbon source and derive their energy from the oxidation of organic carbon. Nitrate is reduced as the electron acceptor. An additional, but relatively small, quantity of nitrate is reduced to ammonia in order to supply nitrogen for cell synthesis. Nitrate, an organic carbon source, and an anaerobic environment are the requirements for denitrification (Painter, 1970; Christensen and Harremoes, 1977).

Denitrification Systems

Several designs of attached and suspended growth reactors are used in engineering practice to achieve

nitrification and denitrification. Christensen and Harremoes (1977), and Panzer (1984) described several flow schemes that combined nitrification/denitrification in suspended growth systems. These processes involved anoxic tanks for denitrification, preceded by aerated tanks for nitrification, recycle of various mixed liquors and sludge, and the use of external or internal carbon source. These processes are complex and difficult to operate. Strand et al. (1985) used a tubular reactor to provide evidence of denitrification in deep biofilm with aerobic surface. Ito and Matsuo (1980) demonstrated the simultaneous proceeding of nitrification and denitrification on the same rotating biological contractor, when methanol was used as the carbon source.

Combined nitrification/denitrificaton can occur in a biofilm reactor if the biofilm is deep enough for oxygen to be depleted, sufficient organic carbon exists to allow significant denitrification, yet adequate oxygen transfers to allow conversion of ammonia to nitrate. Under high organic loading both heterotrophic and nitrifying bacteria compete for dissolved oxygen. Nitrifying bacteria can be outgrown by the faster growing heterotrophic organisms often physically displaced from the aerobic zone of the biofilm. As a result the production of nitrate will cease and denitrification will stop.

Tomlinson and Snaddon (1966) showed that heterotrophs extinguished nitrification in a well established nitrifying biofilm when organic carbon was increased in the feed. He observed that nitrification activity could be restored by increasing the oxygen concentration of the gaseous phase. Timberlake et al. (1988) demonstrated the coexistence of aerobic heterotrophic, nitrifying, and denitrifying bacteria in a biofilm that was developed on a gas permeable membrane. The oxygen was supplied to the interior of the biofilm through the membrane and the bulk solution was anoxic.

In the SAB reactor oxygen is provided by diffusion throughh a gas permeable support surface from one direction, and substrates are provided from the opposite direction. This configuration results in the isolation of: 1) the slow growing nitrifying bacteria in a zone high in ammonia and dissolved oxygen, maximizing conversion of ammonia to nitrate, and 2) the denitrifying bacteria in a zone of low dissolved oxygen and high nitrate.

The objective of this portion of the study is to provide evidence of the ability of the SAB to treat soluble wastewaters by combined nitrification, heterotrophic oxidation and denitrification.

Operating Conditions

Biochemistry

The reduction of nitrate to nitrogen gas proceeds through a number of steps as follows:

$$NO_3^- \text{ --> } NO_2^- \text{ --> } NO \text{ --> } N_2O \text{ --> } N_2 \qquad \text{(VI-1)}$$

Most denitrifying bacteria can perform the process from nitrate to the end product nitrogen gas. Where acetate is used for the organic carbon source, the energy reaction is as follows McCarty (1971):

$$\frac{1}{8}CH_3COO^- + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ =$$

$$\frac{1}{10}N_2 + \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + \frac{9}{40}H_2O \qquad \text{(VI-2)}$$

and the synthesis reaction:

$$\frac{1}{8}CH_3COO^- + \frac{3}{40}CO_2 + \frac{1}{20}NH_4^+ =$$

$$\frac{1}{20}C_5H_7O_2N + \frac{3}{40}H_2O + \frac{3}{40}HCO_3^- \qquad \text{(VI-3)}$$

The overall metabolic reaction for acetate becomes:

$$4.38\ CH_3COO^- + 3.01\ NO_3^- + 3.01\ H^+ + NH_4^+ = C_5H_7O_2N +$$

$$1.505\ N_2 + 0.38\ CO_2 + 3.38\ HCO_3^- + 4.89\ H_2O \qquad \text{(VI-4)}$$

Since bicarbonate is produced in this reaction, an increase in bicarbonate alkalinity is expected during

denitrification. Equation (VI-4) gives maximum yield of 0.44 g cell/g CH_3COO^-, nitrate consumption of 0.16 g NO_3^--N/g CH_3COO^-, ammonia utilization of 0.054 g NH_4^+-N/g CH_3COO^-, and alkalinity production of 39.89 g $CaCo_3$/g CH_3COO^-.

The biochemistry of nitrification and acetate oxidation are discussed in Chapters IV and V respectively, and the stoichiometry of growth is in Appendix B.

Nutrients

The macronutrient requirements for denitrifying bacteria are an organic carbon source and nitrate. Apart from these macronutrients, other micronutrients are required for optimum growth conditions. Mineral nutrients such as sulfate, phosphate, chloride, sodium, potassium, magnesium, and calcium are reported in literature to be necessary. Also trace amounts of metals (molybdenum, iron, copper, and manganese) are reported to be required for successive enzymic steps. Most of the micronutrient reported to be required for bacterial growth are listed in Appendix I with the range of concentration reported to be stimulatory.

Oxygen

There is much evidence in literature that oxygen presence has a profound effect on denitrification. Painter

(1970) concluded that denitrification can occur under aerobic conditions, although soluble oxygen concentrations were not given. Drews and Greeff (1973) observed denitrification in activated sludge plants at dissolved oxygen level of 0-0.3 mg/1. Christensen and Harremoes (1977) indicated that the critical factor governing whether aerobic or anaerobic metabolism occurs at a definite site is the soluble oxygen concentration at the micro-environment around the bacteria and not the oxygen concentration within the macro-environment. Payne (1973) indicated that oxygen inhibits denitrification by either repressing the formation of nitrate reductase or act as an electron acceptor. Skerman and MacRae (1957a, b) concluded that nitrate reduction ceased at dissolved oxygen concentration above 0.2-0.4 mg/1 in the reaction vessels. He suggested that the occurrence of dentrification may be the result of an oxygen gradient in the culture that some cells at zero dissolved oxygen level and are able to reduce nitrate.

Denitrification is easier to achieve within biofilms in the presence of dissolved oxygen in the bulk liquid. Strand and McDonnel (1985) and Strand et al. (1985) provided evidence that the reactor dissolved oxygen (0.1-14 mg/1) had no significant effect on denitrification in deep microbial film with aerobic surface if the film is sufficiently deep for an anoxic layer to develop. He

suggested that denitrification started when oxygen level in the film falls below 0.045 mg/1.

Temperature

The SAB reactor temperature was kept constant during the run at 25 °C with a water bath. Dawson and Murphy (1972) studied the effect of temperature (5-27 °C) on the denitrification rate of Pseudomonas denitrificans in a mixed batch reactor. He illustrated that the temperature dependency can be approximated by the standard Arrhenius equation. Stensel et al. (1973) studied denitrification in suspended-growth reactor reported that temperature has no significant effect on the process from 20 to 30 °C, but when temperature is decreased to 10 °C the biological activity decreases significantly.

pH

The reactor pH was controlled at pH 7.5 by the addition of 12 mg/1 of potassium bicarbonate. Delwiche (1956) studied the effect of pH on denitrification rate and the product distribution of the reaction using Pseudomonas aeruginosa. He observed that denitrification proceeds from pH 5.8 to 9.2 with an optimum value between pH 7.0 and 8.2. He also observed that above pH 7.0 nitrogen gas is the end product while this level the nitrous oxide production tends to increase.

Theory of SAB Process

The biofilm in these reactor can be described schematically as three layers of deep biofilms on top of each other. The nearest layer to the semi-permeable membrane will be rich in nitrifying bacteria, where oxygen is the electron-acceptor and ammonia is the electron-donor. Oxygen is supplied in excess of ammonia. The second layer is rich in heterotrophic bacteria, where oxygen is the primary electron-acceptor and acetate is the primary electron-donor. The third layer which is closest to the bulk liquid, is rich in denitrifying bacteria, where nitrate is the primary electron-acceptor and acetate is the primary electron-donor. Figure (VI-1) shows a schematic diagram for nitrification, heterotrophic oxidation, and denitrification in the biofilm.

In the SAB acetate, ammonia and the micronutrient have to diffuse from the bulk liquid into the biofilm in which reaction takes place. Oxygen diffuses through the semi-permeable membrane into the biofilm. All products (nitrite, nitrate, carbon dioxide, nitrogen, and hydrogen) likewise have to be removed by molecular diffusion out through the biofilm and into the bulk liquid.

Oxygen diffuses through the semi-permeable membrane into the biofilm along a concentration gradient formed by the reduction of oxygen, due to nitrification in the first

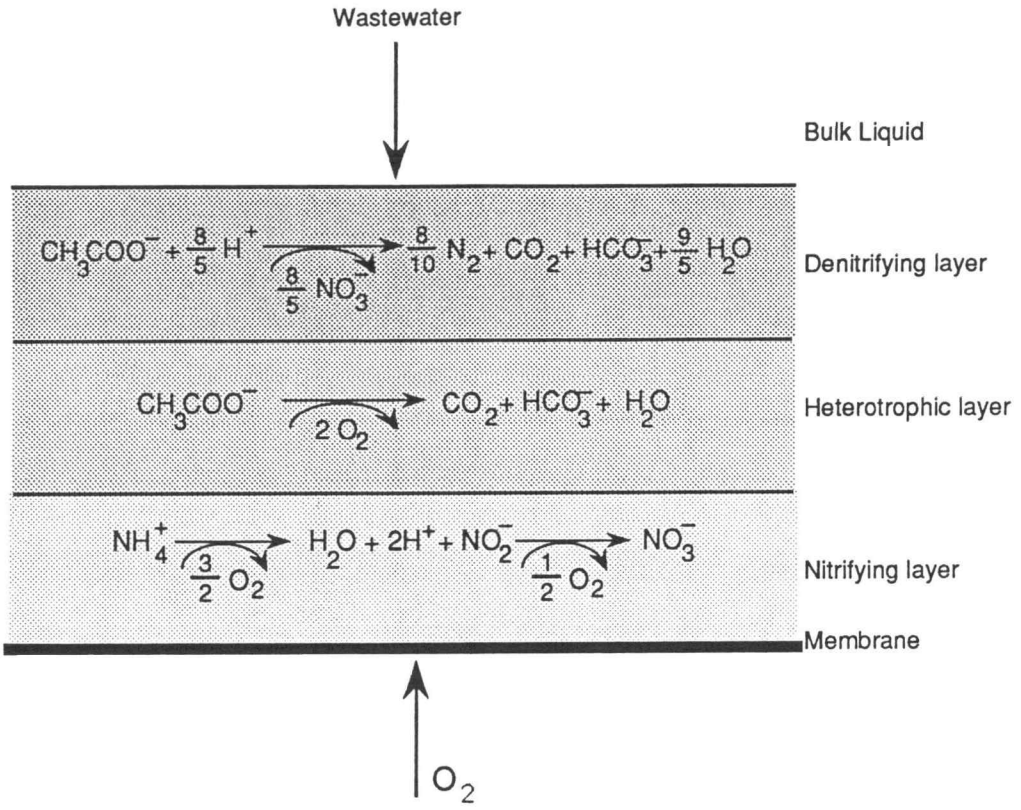


Figure VI-1. Schematic diagram for nitrification, aerobic heterotrophic oxidation, and denitrification processes in a biofilm.

layer and carbon oxidation in the second layer, in the biofilm.

Ammonia ions diffuse from the bulk liquid into the biofilm along a concentration gradient formed by the uptake in biomass synthesis in the denitrifying and carbon oxidation layers, and oxidation of ammonia in the nitrifying layer. Nitrite is produced and quickly transformed to nitrate in the nitrifying layer. Both nitrite and nitrate diffuse from the biofilm along concentration formed by their production in the nitrifying layer and their reduction in the denitrifying layer. Since nitrite is rapidly converted to nitrate, the amount of nitrate diffusing out of the biofilm must be larger in comparison to nitrite diffusion.

Acetate ions diffuse from the bulk liquid into the biofilm along a concentration gradient formed by the oxidation of acetate with nitrate in the denitrifying layer and by the oxidation with oxygen in the heterotrophic layer.

The hydrogen ions produced during the oxidation of ammonia diffuse out of the biofilm to the bulk liquid along their concentration gradient and are eventually neutralized by the bicarbonate ions in the bulk liquid.

Nitrogen and carbon dioxide produced in denitrifying layer and heterotrophic oxidation diffuse out of the biofilm into the liquid. The bicarbonate ions diffuse into

the biofilm along a concentration gradient formed by their neutralization in the biofilm by hydrogen ions.

The diffusion of non-electrolytes as oxygen, carbon dioxide, and nitrogen are not influenced by the presence or absence of other materials. However, the diffusion of electrolytes such as ammonia, nitrite, nitrate, acetate, hydrogen, and bicarbonate must be balanced by either a concurrent flow of opposite charge or a counter-current flow of the same charge. Figure (VI-2) shows the concentration profile in the biofilm.

Results and Discussion

The Phase III experiments started two weeks after the nitrification experiment reached steady-state. Before feeding acetate, the SABs were inoculated with denitrifying bacteria that were grown in a 2-liter batch reactor in the laboratory. One-half liter from the batch reactor was pumped into each SAB and allowed to settle to the bottom. After 4 hours, the SABs were emptied and cleaned, then feed and mixing were started and maintained continuously. SAB #3 was fed 20 mg/l acetate during this experiment, while SAB #4 was fed 40 mg/l until steady-state was achieved, then acetate was increased to 100 mg/l acetate.

In SAB #3 acetate concentration dropped to less than 1 mg/l two days after acetate feed was begun (Figure VI-3). During this time ammonium increased from 0.8 to 5.2 mg/l,

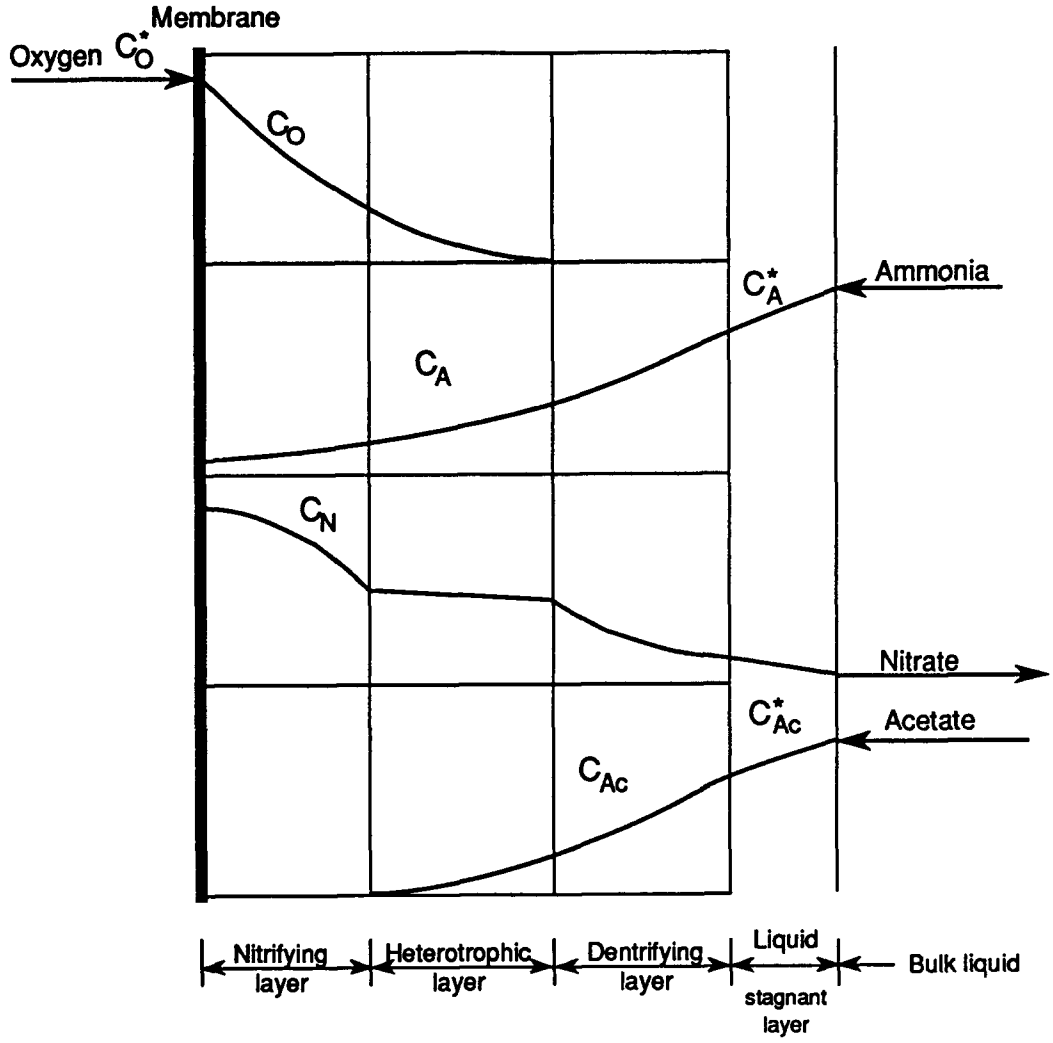


Figure VI-2. Conceptual concentration profile in a biofilm illustrating nitrification, aerobic heterotrophic oxidation, and denitrification.

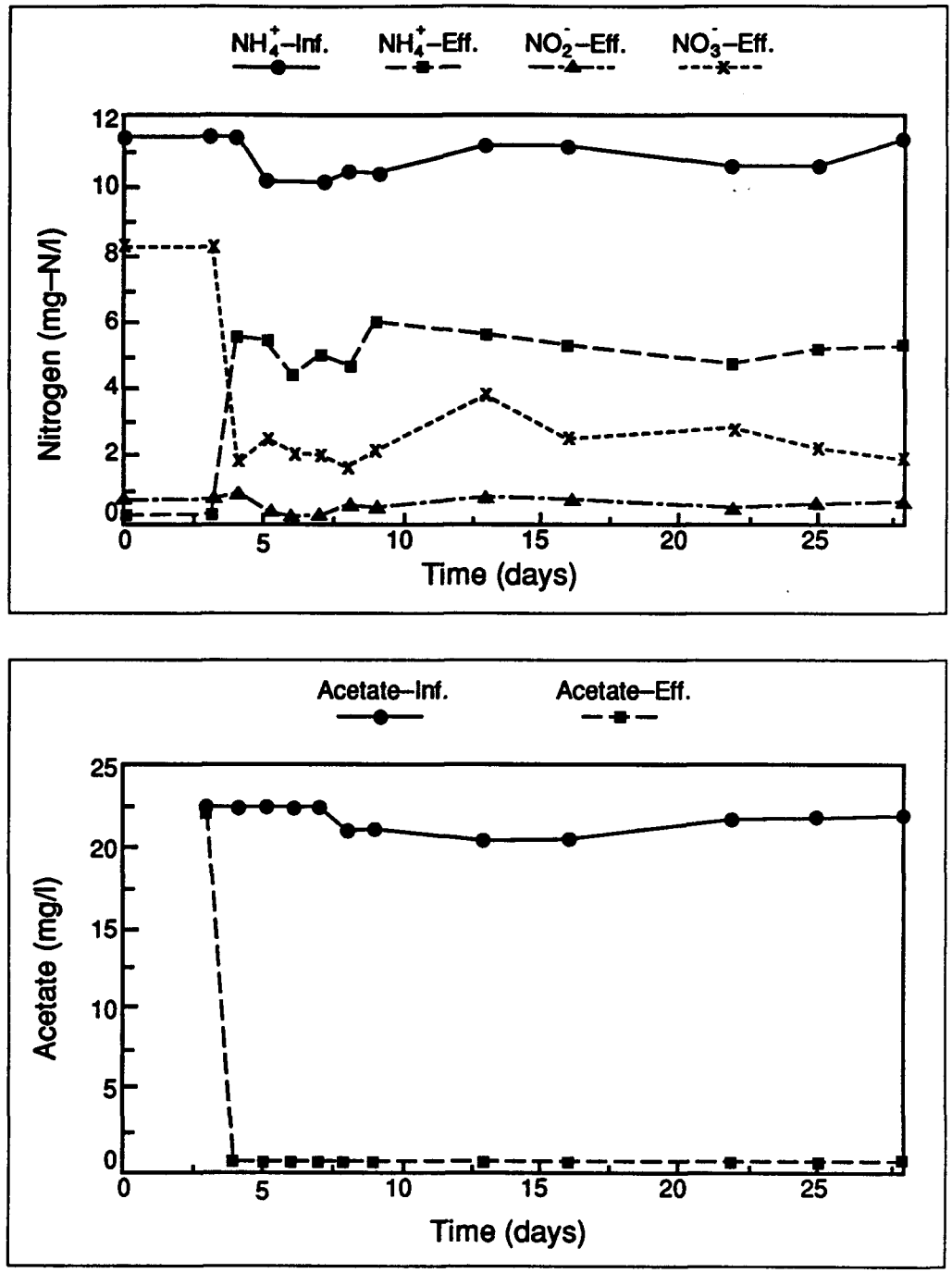


Figure VI-3. Nitrogen and acetate influent and effluent concentration, SAB #3 (influent concentration = 10 mg/l NH_4^+-N/l and 20 mg acetate/l).

and nitrate decreased from 8.3 to 2.2 mg/l. In SAB #4 acetate concentration dropped to 2 mg/l two days after acetate feed was begun (Figure VI-4). During this time ammonium increased from 1.3 to 5.9 mg/l, and nitrate decreased from 7.3 to 0.3 mg/l. Acetate in the feed for SAB #4 was then increased to 100 mg/l (Figure VI-5). Acetate in the effluent immediately increased to 30 mg/l. Ammonia and nitrate levels did not change. The steady-state results of the SABs experiments are summarized in Table (VI-1).

The average total suspended solids in the effluent was less than 3 mg/l. No sloughing of the biofilm was observed, and cell growth in the reactor continued to accumulate during the experimental runs.

The average pH value for the SABs was 7.9. Potassium bicarbonate was used as a buffer and also to insure that sufficient inorganic carbon would be available for nitrification. The reactor temperature of the SABs was kept constant at 25°C through the run.

The acetate loading rates applied to the SABs were 1.46, 2.79, and 6.89 mg acetate/cm^2.d with 20, 40, 100 mg/l acetate influent concentrations, the efficiency of acetate removal ranged from 62 to 95% at a hydraulic detention time of 8 hours. The ammonia mass loading was 0.71 mg NH_4^+-N/cm^2.d with 10 mg NH_4^+-N/l influent concentration. The efficiency of ammonia removal ranged from 45 to 50%.

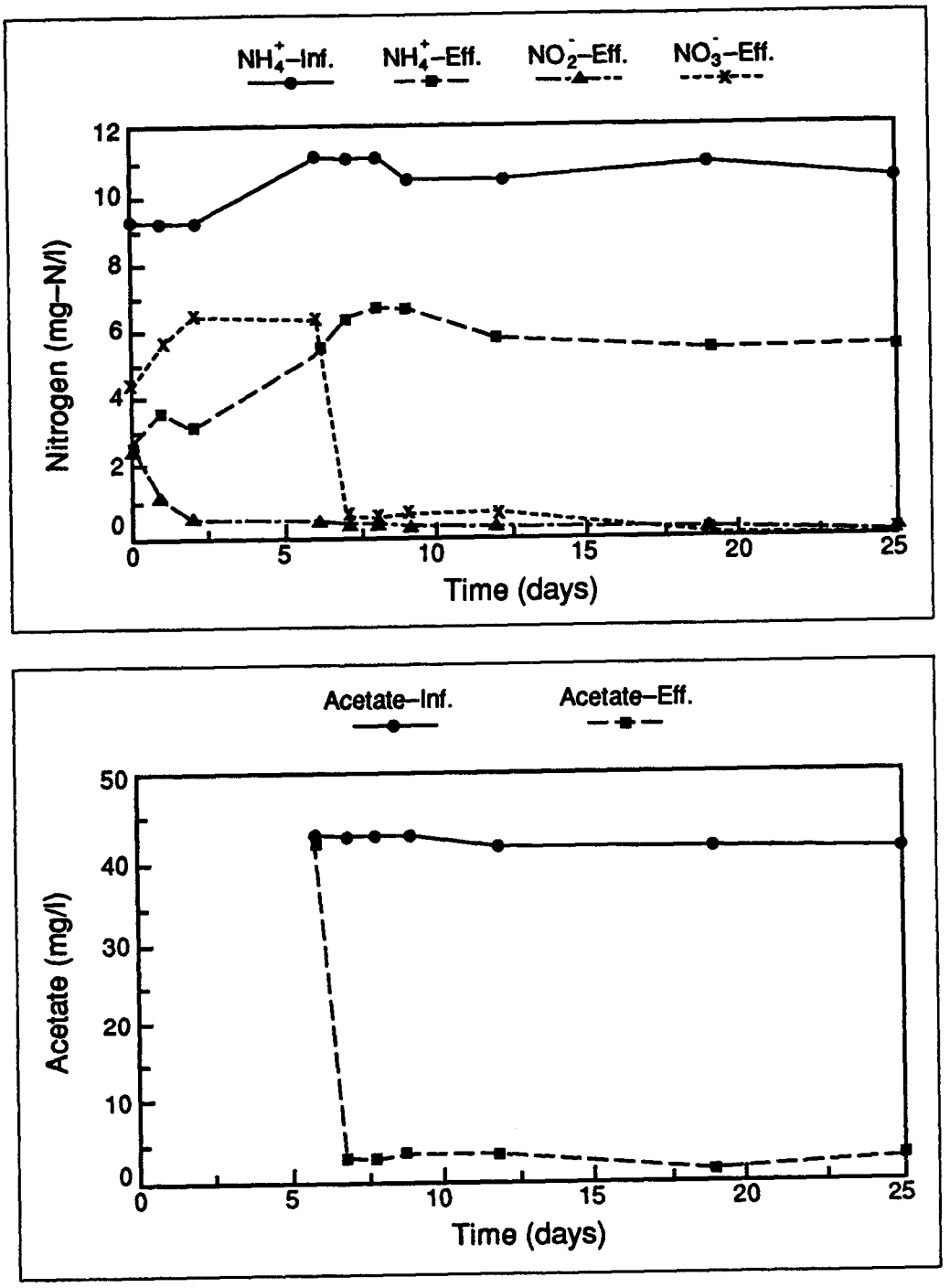


Figure VI-4. Nitrogen and acetate influent and effluent concentration, SAB #4 (influent concentration = 10 mg/l NH_4^+-N/l and 40 mg acetate/l).

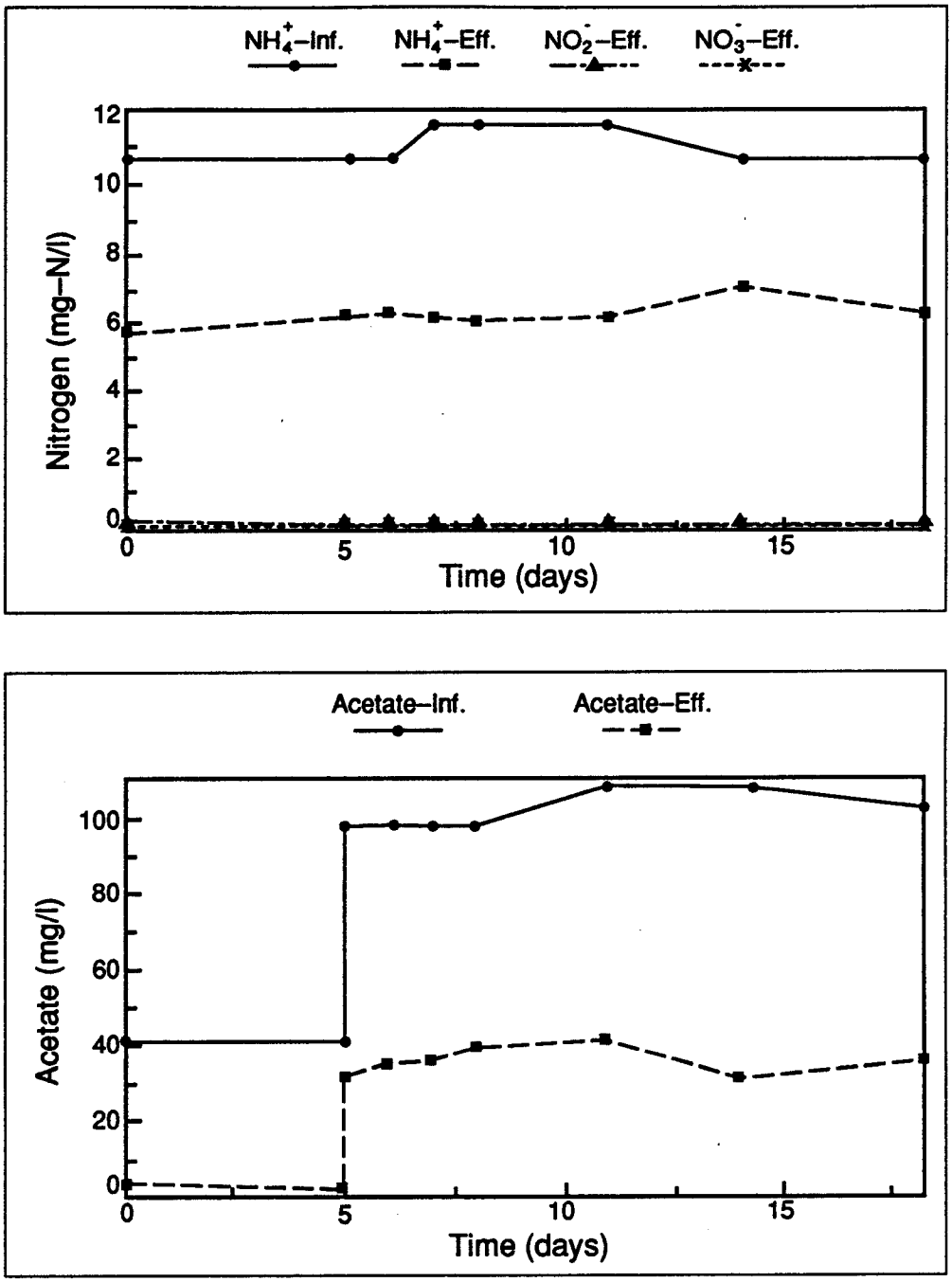


Figure VI-5. Nitrogen and acetate influent and effluent concentration, SAB #4 (influent concentration = 10 mg/l NH_4^+-N/l and 100 mg acetate/l).

Table VI-1. SAB Steady-State Data for Phase III

SAB number	#3	#4	#4
NH_4^+-N mg/l inf.	10.5±0.54	10.8±0.74	11.2±0.47
NH_4^+-N mg/l eff.	5.2±0.25	5.9±0.51	6.1±0.35
conversion, %	50.5	45.4	45.5
NO_2^--N mg/l inf.	0.46±0.1	0.23±0.1	0.0
NO_3^--N mg/l eff.	2.2±0.36	0.26±0.23	0.0
Acetate mg/l inf.	21.9±0.77	41.9±0.6	103.3±4.52
Acetate mg/l eff.	<1.0	2.0±0.58	38.5±3.16
Conversion, %	>95	95	62.7
pH	7.92	7.8	7.6

A series of mass balances based on growth thermodynamics and energetics model (McCarty, 1971) were developed to follow the fate of the nitrogen compounds and acetate in the SABs. A sample calculation is in Appendix C The total acetate removal fluxes were 1.4, 2.7, and 4.3 mg acetate/cm^2.d, respectively (Figure VI-6). Nitrification fluxes were 0.28, 0.18, and 0.1 mg-N/cm^2.d, while assimilation fluxes were 0.08, 0.15, and 0.24 mg-N/cm^2.d., respectively (Figure VI-7). Denitrification fluxes were 0.1, 0.15 and 0.1 mg-N/cm^2.d. The measured flux values are significantly higher than those reported by Timberlake et al. (1988) and predicted by Kissel et al. (1984) for mixed-culture biofilm. They reported nitrification and denitrification fluxes from 0.01 to 0.062 mg-N/cm^2.d, and carbon oxidation fluxes from 0.2 to 0.4 mg/cm^2.d. The higher rates in Phase III are probably due to 1) high concentration of nitrifying bacteria, in a zone rich of oxygen, which enhance the conversion of ammonia to nitrate, and 2) isolation of the denitrifying bacteria so that all nitrate from nitrification reaction must diffuse through the denitrifying layer.

Antonie (1974), Mueller et al. (1980), Strand (1986), and Weng and Molof (1974) reported organic oxidation flux ranges which compare well with the Phase III values ranging from 0.01 to 5 mg COD/cm^2.d. They reported nitrification flux ranged from 0.01 to 0.9 mg-N/cm^2.d which is higher

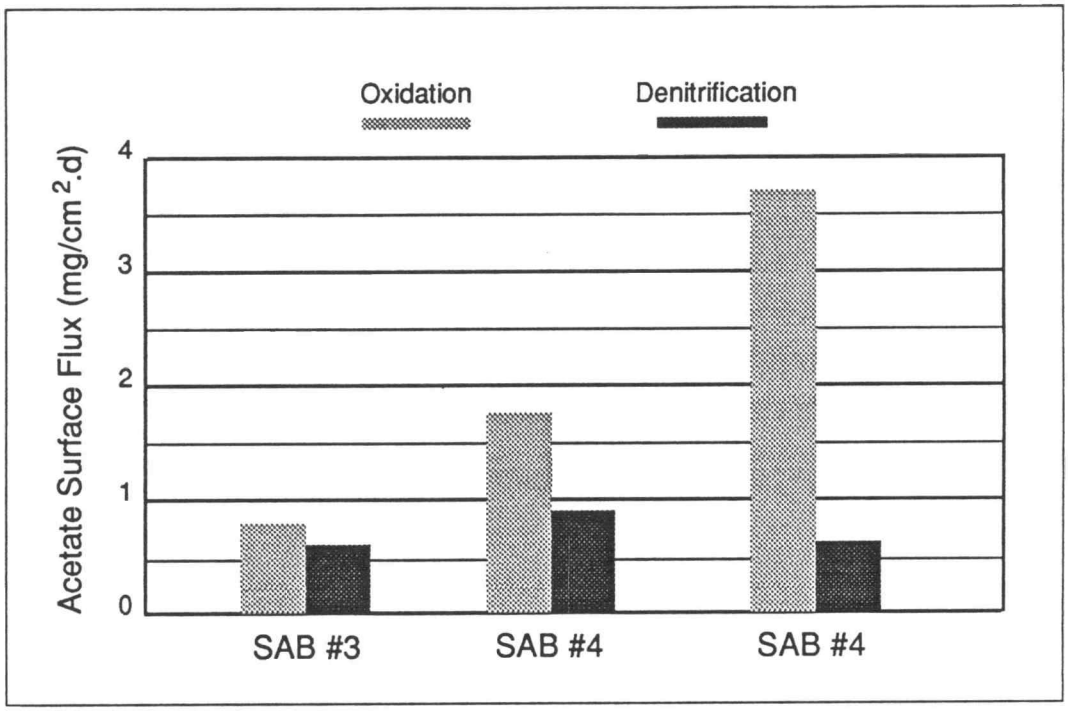


Figure VI-6. Acetate flux for SABs #3 and #4 calculated from mass balances.

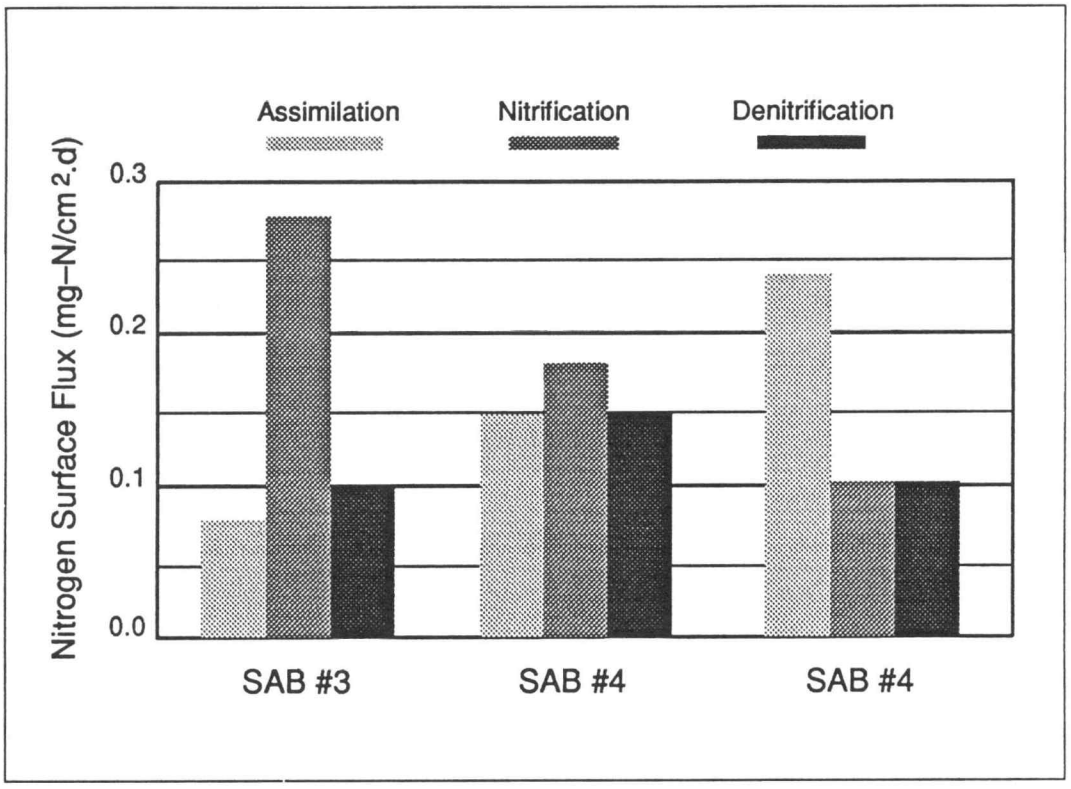


Figure VI-7. Nitrogen flux for SABs #3 and #4 calculated from mass balances.

than the SAB. They reported that nitrification took place when organic carbon level was below 15 mg/l, which means almost all the RBCs devoted entirely to nitrification. In comparison, ammonia and carbon oxidation occur simultaneously in the SAB with carbon level up to 100 mg/l.

Strand et al. (1985) observed an average denitrification flux of 1.49 mg-N/cm^2.d. Blanc et al. (1980), Davies and Pretorius (1975), and Jansen (1983) reported denitrification flux ranged from 0.1 to 0.6 mg-N/cm^2.d. In the SAB, denitrification rates are totally dependent upon the rates of nitrification since nitrification process is the only source for nitrate in the system. Thus the maximum rate of denitrification equals the maximum rate of nitrification.

VII. HETEROTROPHIC OXIDATION AND FERMENTATION

Background

Anaerobic treatment processes have been used for biological stabilization of concentrated organic waste primarily wastewater sludge. Their low operating costs, low production of microbial cells, high degree of waste stabilization, and methane production are the contributing factors favoring the use of anaerobic treatment over the aerobic treatment. The hesitation to install anaerobic system is based on presumed lack of stability, low loading rates, slow recovery after failure, specific requirements for waste composition, and ineffectiveness for treatment of low-strength waste.

The anaerobic process involves a complex mixture of interacting microbial species, most of which do not produce methane. The process proceeds in an strict anaerobic environment, where organic waste is stabilized by conversion to methane, carbon dioxide, and inorganic products.

Methane fermentation is a three-stage process, each of which requires a unique group of bacteria (Bryant, 1979; McInerney et al., 1971). The efficient metabolism of each group is dependent on the others. In the first stage, complex organic compounds, such as fats, protein, and carbohydrates are hydrolyzed and fermented to volatile

acids, alcohols, carbon dioxide, and hydrogen by fermentative bacteria to obtain energy for growth. In the second stage, the products of the first-stage, volatile acids and alcohols, are oxidized to acetic acid, hydrogen and carbon dioxide. The second stage is mediated by a group of bacteria, composed of many species each having different energy source, called hydrogen-producing acetogenic bacteria. The third stage, acetate and hydrogen are converted to methane by the methanogenic bacteria.

Methanogens consists of two physiologically different groups of methane-forming bacterial. Acetate-utilizing bacteria which uses acetate as a carbon source for cell synthesis as well as for energy. Hydrogen-utilizing bacteria that use hydrogen for energy and carbon dioxide, acetate, or some other carbon source for cell synthesis. Figure (VII-1) shows McCarty (1981) quantification of the three-stage process. During the first two stages little waste stabilization occurs and the COD of the organic acids is almost equivalent to the COD content of the original organic matter. Bacteria mediate these stages to obtain energy for growth and to convert some organic compounds into cells.

It is in the third-stage that waste stabilization occurs where methanogens ferment acetate to methane and carbon dioxide. While the overall conversion of organic matter to methane requires the synergistic action of all

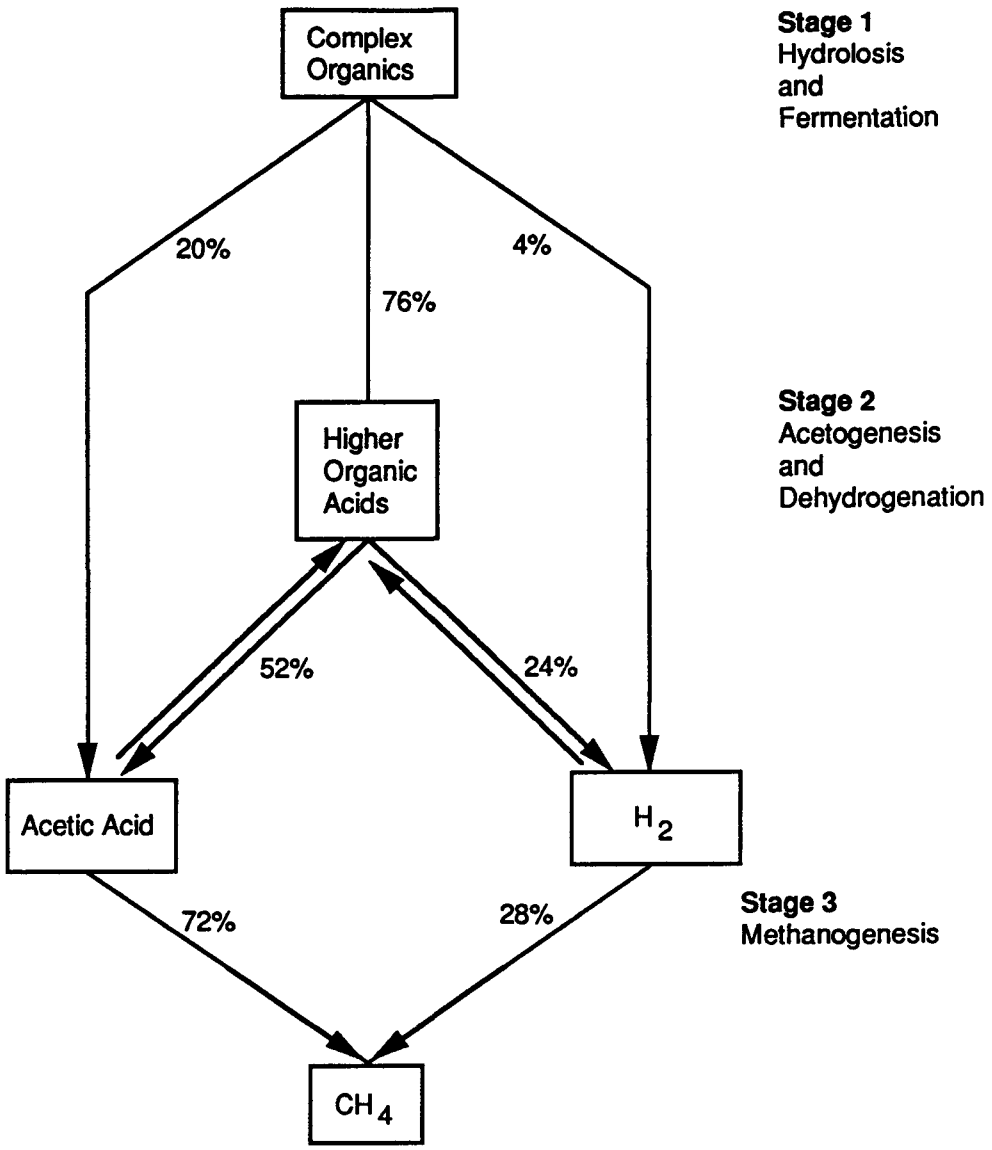


Figure VII-1. Three stages of methane fermentation, McCarty (1981).

three groups of bacteria, the process is governed by the kinetics of the slowest step. McCarty (1971a) concluded from research on the fermentation of primary municipal sludge that the degradation of fatty acids is the rate-limiting step in methane fermentation. In contrast Kaspar and Wuhrmann (1978) concluded that acetate degradation was the rate-limiting reaction in methane fermentation. Bryant et al. (1967) showed that the symbiotic association between the hydrogen-producing bacteria and the hydrogen-utilizing bacteria can result in proper hydrogen partial pressure for the oxidation of volatile acids. He showed that hydrogen partial pressure must be maintained at low level to enable favorable thermodynamic conditions for the conversation of volatile acids and alcohols to acetate. Figure (VII-2) illustrates the relationship that exists between hydrogen partial pressure and free energy available to the hydrogen-producing and hydrogen-utilizing bacteria (McCarty, 1981). In order for energy to be available for the conversion of ethanol and propionate to acetate and hydrogen, the hydrogen partial pressure must be below 10^{-1} and 10^{-4} atmosphere, respectively.

Fermentation Systems

Several designs of attached and suspended growth reactors have been used to achieve fermentation (McCarty,

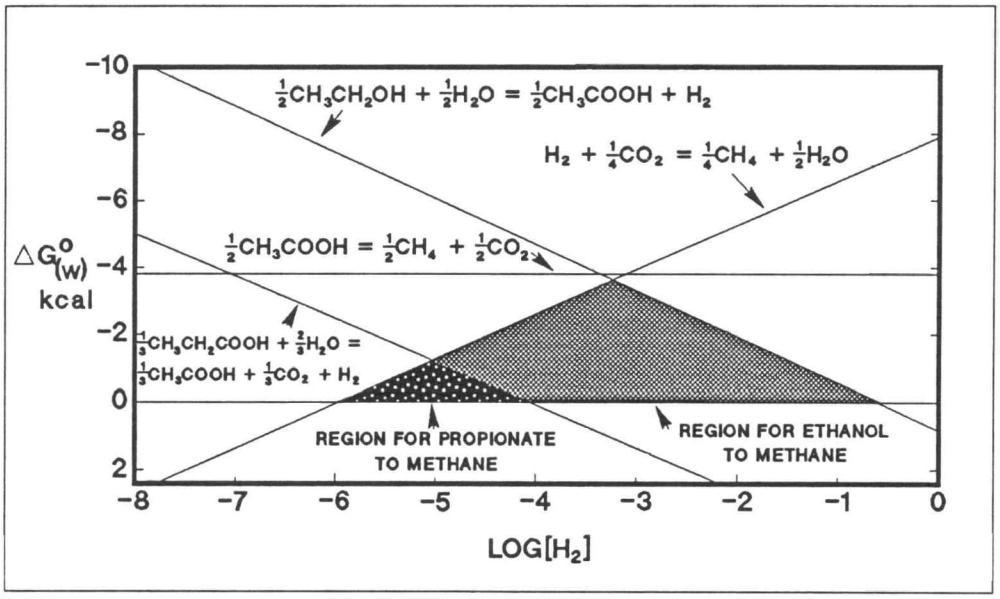


Figure VII-2. Effect of hydrogen partial pressure on the free energy of conversion of ethanol, propionate, acetate, and hydrogen during fermentation, McCarty (1981).

1981; and Speece, 1983). First generation reactors involved anoxic tanks for treatment of municipal sludge. They resulted in a stratified digester with biomass at the bottom and scum at the top. Mixing was introduced which removed the scum layer and produced high-rate of digestion. Subsequently, solid recycle as the anaerobic contact process was introduced to increase the solid retention time (SRT) and to increase the efficiency of the process.

McCarty (1964a) pointed out the significant of SRT to the efficiency of the anaerobic process. He stated that a SRT of at least 10 days was required for proper operation of the process. Lawrence and McCarty (1969) concluded that wash-out of methanogens occurred with SRT of about 4 days at 35°C.

The long SRT required for proper operation of anaerobic system rationalized the attempt to develop and use biofilm reactors. Young and McCarty (1969) introduced the "anaerobic filter" in which waste passed in an upward direction through a rock-filled bed to which the bacteria attached. He observed that much of cells did not attach to the packing, instead they retained a loose structure in the packing interstices. The anaerobic filter was used for the treatment of intermediate strength (1500 to 6000 mg/l COD) synthetic protein-carbohydrates and volatile acid wastes. The anaerobic filters were operated at a constant 25°C and hydraulic detention times were varied between 4.5 and 72

hours while COD loading varied between 0.425 to 3.4 $kg/m^3/day$, respectively.

Switzenbaum and Jewel (1980) developed the anaerobic attached-film expanded bed reactor, in which wastes pass in an upward direction through a bed of suspended media to which the bacteria attached. The large surface-area-to volume ratio enabled a large active mass of attached microorganisms to remain in the reactor, and yet maintain freedom from clogging. A major drawback is the high rate of recycle generally required to keep the media to which bacteria are attached in suspension. The process was used to treat low strength (200 to 600 mg/l COD) synthetic organic waste. The experiments were conducted at varied temperature between 10 to 30°C, organic loading between 0.8 to 4.8 Kg $COD/m^3/day$, and hydraulic retention time between 0.33 to 6 hours.

Van Den Berg and Lentz (1979) presented results of comparative studies between upflow and downflow fixed film reactors. The bacterial films were allowed to grow on the inside wall as no media was contained in the reactor. Bean blanching waste was used in this study and the reactors were fed high strength waste at volatile suspended solids loading varying between 3.1 to 15.3 $kg/m^3/day$ at liquid detention time between 13 hours and 2.7 days. The experiments were conducted at 35°C. Overall performance results between upflow and downflow reactor were almost

identical. The downflow reactors were exclusively biofilm reactor, while upflow reactors operated more as fluidized or expanded bed reactors.

The objective of this experiment is to determine the ability of the SAB reactor to treat high strength soluble wastewater by combined nitrification, heterotrophic oxidation, denitrification, and fermentation.

Operating Conditions

Biochemistry

Methane fermentation process occurs in environment in which the main electron acceptor is carbon dioxide. Methanogens require a strict anaerobic environment, absence of electron-acceptor such as nitrogen oxides (NO_3^-, NO_2^-) and/or sulfur oxides (SO_3^{2-}, SO_4^{2-}), and redox potential below -300mv (Balderston and Payne, 1976; Bryant et al. 1977; Winfrey and Zeikus, 1977).

There are two major mechanisms of methane formation (McCarty 1964a). First, acetate is fermented with formation of methane and carbon dioxide:

$$\overset{*}{C}H\dot{C}OO^- + H_2O = \overset{*}{C}H_4 + H\dot{C}O_3^- \qquad \text{(VII-1)}$$

The methyl carbon of acetate, marked with an asterisk, is reduced to methane and the carboxyl carbon, marked with a circle, is oxidized to bicarbonate. Second, carbon dioxide

is reduced to methane and hydrogen is oxidized to water.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{(VII-2)}$$

Buswell and Sollo (1948), and stadtman and Baker (1951) provided evidence, using C^{14} tracer, that the majority of methane is derived from the methyl group of acetate and not from carbon dioxide reduction. Jeris and McCarty (1965), and Smith and Mah (1966) reported that about 70% of the methane produced in anaerobic sewage digester resulted from the degradation of acetate.

Methanogens have been observed to utilize acetate as a sole carbon source for cell synthesis as well as for energy (Smith and Mah, 1980). The energy reaction is a follows (McCarty, 1971):

$$\tfrac{1}{8}\ CH_3COO^- + \tfrac{1}{8}\ H_2O = \tfrac{1}{8}\ CH_4 + \tfrac{1}{8}\ HCO_3^- \qquad \text{(VII-3)}$$

and the synthesis reaction is:

$$\tfrac{1}{8}\ CH_3COO^- + \tfrac{3}{40}\ CO_2 + \tfrac{1}{20}\ NH_4^+ = \tfrac{1}{20}\ C_5H_7O_2N + \tfrac{3}{40}\ H_2O + \tfrac{3}{40}\ HCO_3^- \qquad \text{(VII-4)}$$

The overall metabolic reaction becomes:

$$55.32\ CH_3COO^- + 51.32\ H_2O + 1.5\ CO_2 + NH_4^+ = C_5H_7O_2N + 52.82\ CH_4 + 54.32\ HCO_3^- \qquad \text{(VII-5)}$$

As acetate is fermented bicarbonate ions are produced, which may increase the pH and adversely affect the rate of

fermentation. Equation (VII-5) shows that bicarbonate is produced at the rate 50.76 mg $CaCo_3$/mg acetate, methane at 0.259 mg CH_4/mg acetate, and ammonia is consumed at 0.0043 mg NH_4^+-N/mg acetate. The biochemistry of carbon oxidation is discussed in Chapter V, and stoichiometry of growth is in Appendix B.

Nutrients

The macronutrient requirement for methane fermentation are acetate and carbon dioxide. Apart from these macronutrients, other micronutrient are required for optimum growth conditions. Speece and McCarty (1964) reported that cobalt and iron were required by the methanogens. Speece (1983) showed that the addition of nickel to acetate-utilizing methanogens has stimulated the production of methane. Schonheit et al. (1979) reported that the growth of methanogens was dependent on nickel, cobalt, and molybdenum. Mountford and Asher (1979), and Ronnow and Gunnarsson (1981) demonstrated the requirement for sulfide. However, the sulfide may precipitate essential trace metals such as nickel, iron, and cobalt. Most of the micronutrients reported to be required for bacterial growth are listed in Appendix A with the range of concentration reported to be stimulatory.

Temperature

The most common temperature range for anaerobic process operation is 30 to 40°C, however the anaerobic process can operate in a temperature range of 10 to 45°C. Van Den Berg (1977) reported an optimum growth temperature of 35°C with only small differences for other temperatures in 30-40°C range. Temperature outside the 30-40°C caused substantial reduction in growth rate. Jewell and Morris (1981) examined the effect of temperature shock on expanded bed reactors, and demonstrated that very loaded processes is not very sensitive to temperature shocks. Kennedy and Van Den Berg et al. (1981) demonstrated that decreasing temperature decreased the maximum steady state loading rate for a downflow biofilm reactor operating at 35°C.

pH

The SAB reactor pH was controlled at pH 7.2 with potassium bicarbonate. Van Den Berg et al. (1976) observed that methane production proceeded from pH 5.8 to 7.6 with an optimum value between pH 6.5 to 7.1

Theory of SAB Process

The biofilm in this reactor can be described schematically as two layers of deep biofilms. The biofilm nearest layer to the membrane will be dominated by the

aerobic heterotrophic bacteria with oxygen as the electron-acceptor and acetate as the electron-donor. The biofilm nearest the bulk liquid is dominated by fermentative bacteria with carbon dioxide as the electron-acceptor and acetate as the electron-donor. Figure (VII-3) shows a schematic diagram for heterotrophic oxidation and fermentation in the SAB reactor.

In the SAB, acetate and the micronutrients diffuse from the bulk liquid into the biofilm in which reaction takes place. Oxygen diffuses through the membrane into the biofilm. All products (carbon dioxide and methane) likewise have to be removed by molecular diffusion out through the biofilm and into the bulk liquid.

Oxygen diffuses through the membrane into the biofilm along a concentration gradient formed by the reduction of oxygen due to carbon oxidation in the first layer.

Acetate ions diffuse from the bulk liquid into the biofilm along a concentration gradient formed by the oxidation of acetate with oxygen in the aerobic layer and by the fermentation of acetate with carbon dioxide in the anaerobic layer. Methane produced during the fermentation of acetate diffuses from the biofilm to the bulk liquid along a concentration gradient formed by its production in the fermentation layer.

The diffusion of non-electrolytes as oxygen, carbon dioxide, and methane are not influenced by the presence or

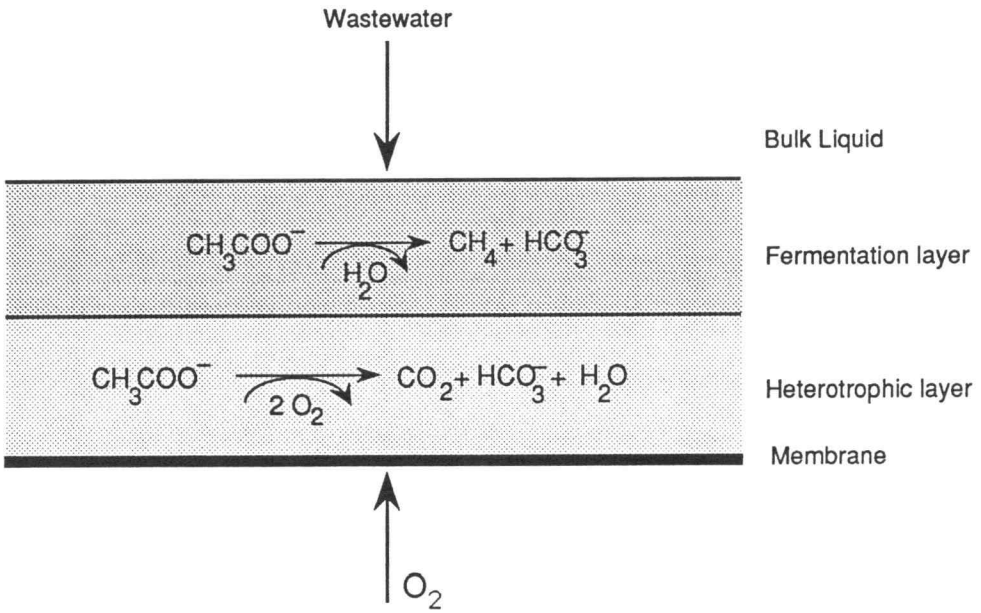


Figure VII-3. Schematic diagram for aerobic heterotrophic oxidation and fermentation processes in a biofilm.

absence of other materials. However, the diffusion of electrolytes such as ammonium, acetate and bicarbonate must be balanced by either a concurrent flow of opposite charge or a counter-current flow of the same charge. Figure (VII-4) shows the concentration profiles in the biofilm.

Results and Discussion

The Phase IV experiments started after the Phase III denitrification experiment reached steady state. SAB #2 was seeded with a 0.5 L supernatant of anaerobic digester from Waste Water Reclamation Plant in Corvallis, Oregon. The seed was allowed to settle for four hours, then mixing were started and maintained continuously. The influent concentration of acetate was increased from 100 mg/l to 800 mg/l, while ammonia concentration remained at 10 mg/l. The SAB was operated at constant 25°C temperature and 8 hours hydraulic detention time. Operation of the SAB involved analysis of the influent and effluent for acetate, ammonia, volatile suspended solids, and total suspended solids. Methane production was monitored to provide a check on acetate utilization. Continuous operation was carried out until the SAB reached steady-state as indicated by consistent methane production and effluent acetate concentration. The steady-state results of the SAB experiment are summarized in Table (VII-1).

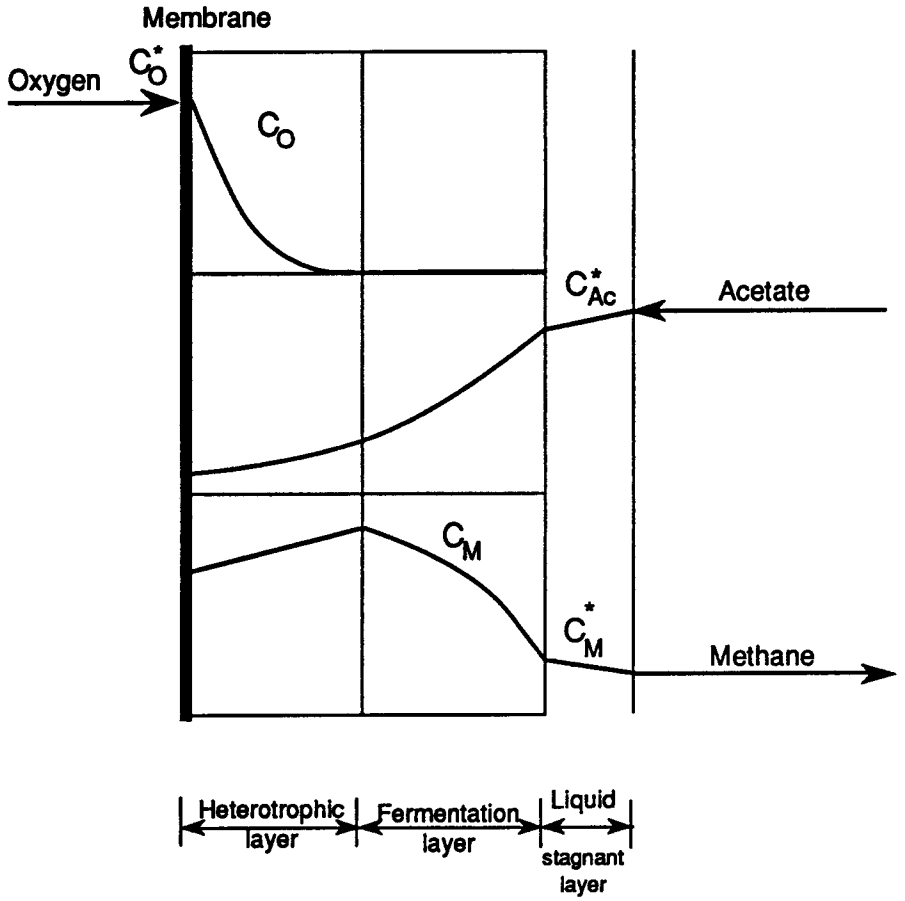


Figure VII-4. Conceptual concentration profile in a biofilm illustrating aerobic heterotrophic oxidation and fermentation.

Table VII-1. SAB Steady-State Data for Phase IV

SAB number	#2
NH_4^+-N, mg/l inf.	10.59±0.59
NH_4^+-N, mg/l eff.	5.44±0.64
Acetate mg/l inf.	820.94±14.71
Acetate mg/l eff.	175.15±12.67
conversion, %	78.68
Acetate Oxidation, mg/l	84.94±10.45
Acetate Fermentation, mg/l	565.23±15.3
Methane Production, mg/day (STP)	313.01±5.85
Biofilm Depth, mm	2

The increase of the acetate concentration from 100 to 800 mg/l inhibited the nitrification activity and, consequently, the denitrification activity in the SAB. Therefore, the only remaining sink for ammonia was bacterial assimilation. If there is no nitrification activity, then all of the oxygen will be used for acetate oxidation by aerobic heterotrophs at the bottom of the biofilm. This accounted for an observed average acetate removal of 85 mg/l. Figure (VII-5) shows the effect of increasing acetate concentration on nitrification within the SAB. The acetate flux due to the aerobic activity was 5.76 $mg/cm^2.d$ which compares well with the higher range values of carbon oxidation flux observed for rotating biological contactors (0.01-5 mg $COD/cm^2.d$; Antonie, 1974; Mueller et al., 1980; Strand, 1986; Weng and Molof, 1974).

After 4.5 months of an average acetate removal of 85 mg/l, methane production began. Methane in the off-gas stabilized at an average value of 313 mg/day after 25 days. More than five months were required to reach 70% acetate removal. Around 565 mg/l acetate were removed due to the fermentation activity in the top layer of the biofilm (Figure VII-6). The measured acetate flux due to fermentation and the corresponding methane production rate are 37.71 $mg/cm^2.d$ and 7.86 $cm^3/cm^2.d$ respectively. These values are higher than those reported by Switzenbaum and Jewell (1980) for the anaerobic expanded bed reactor, Young

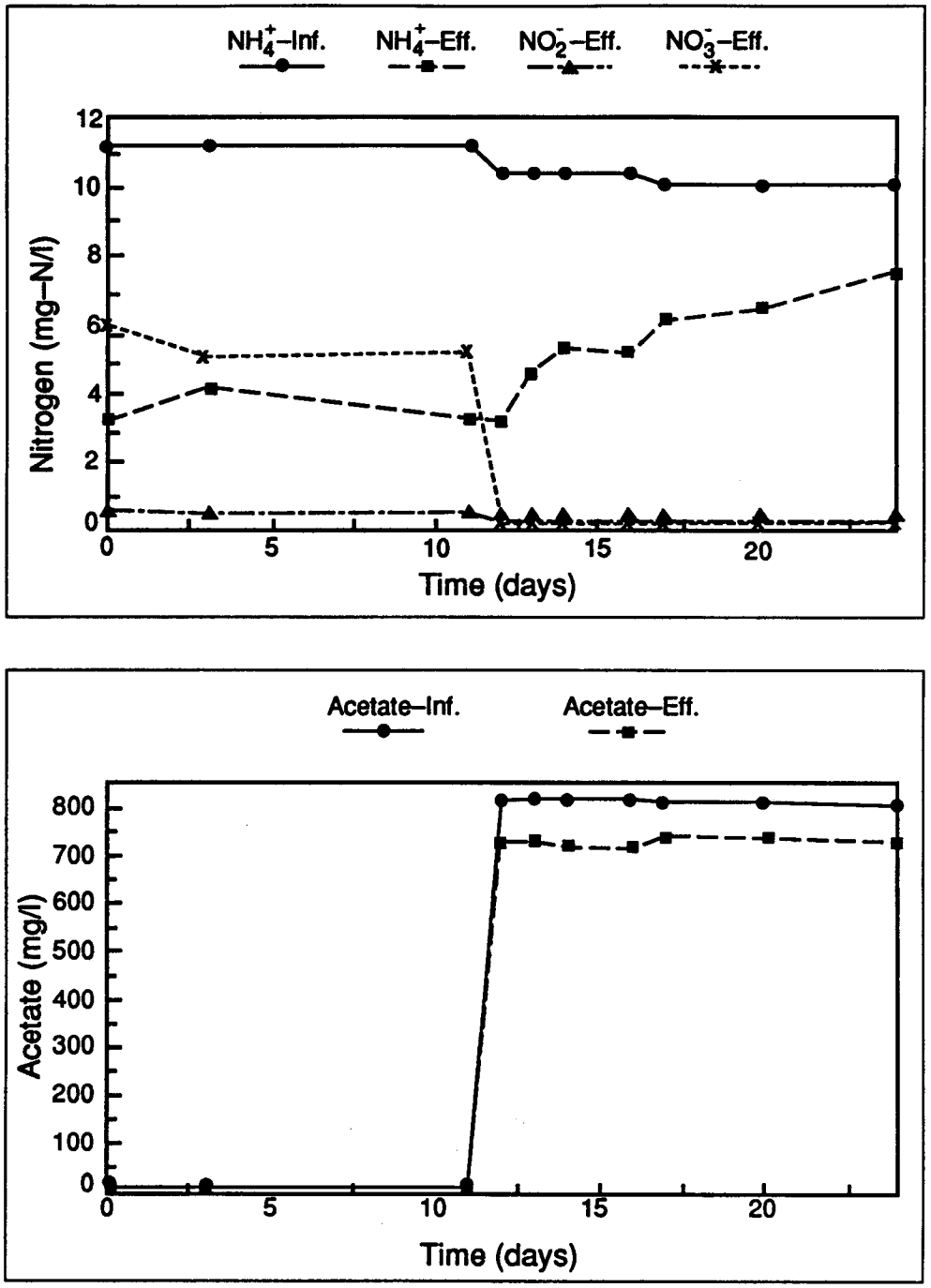


Figure VII-5. Effect of increasing acetate concentration on nitrification.

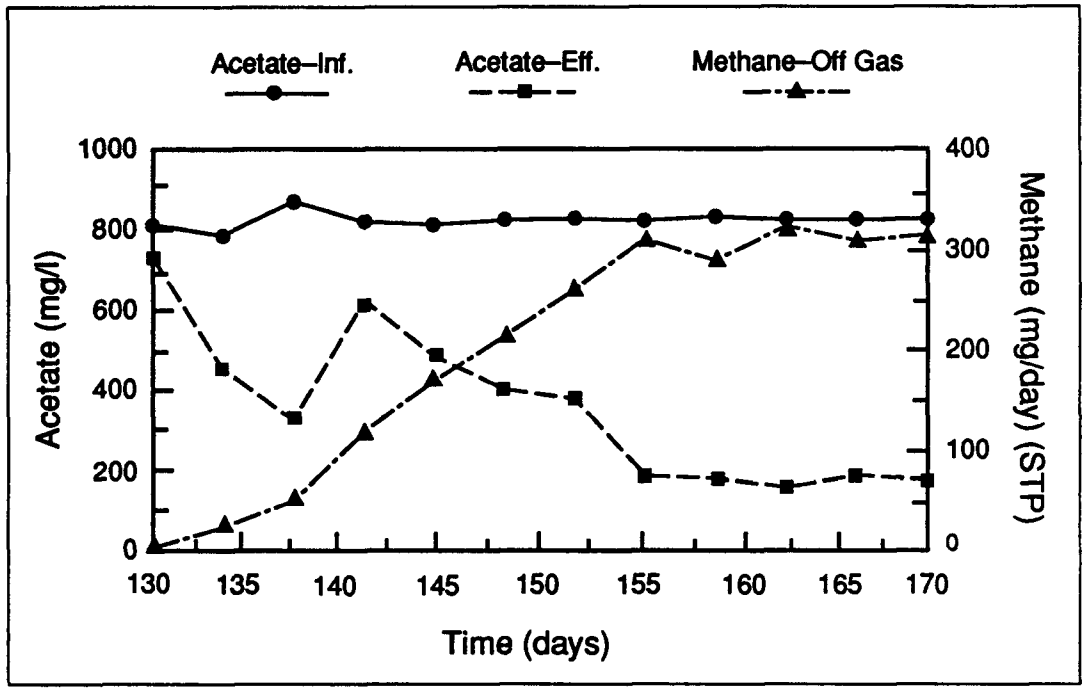


Figure VII-6. Acetate influent and effluent concentration and rate of methane production (influent concentration = 800 mg/l).

and McCarty (1969) for the anaerobic filter, Van Den Berg and Lentz (1979) for the upflow and downflow anaerobic fixed film and Van Den Berg et al. (1981) for fixed film fermentor. Table (VII-2) presents a comparison of the reactor types and performance data.

The higher acetate flux values for the SAB may have been due to large biomass concentration and the high SRT values achieved with a thick biofilm. Another important reason is the well balanced nutrient composition in the feed solution. The sulfur source was sulfate, which was continually reduced providing a continual source of sulfide and preventing precipitation of other metal ions essential for growth, such as nickel, iron and cobalt.

The presence of adequate nickel may have been another factor that contributed to the high flux values. Speece (1983) showed that the addition of nickel to acetate-utilizing methanogens has resulted in methane production rate of 50 gm/l.d, which is higher than any reported values. However, this value was for a suspended-growth system.

The average total suspended solids in the effluent was less than 15 mg/l with no significant sloughing of the biofilm. The biofilm continue to grow throughout the duration of the experiment. The biofilm observed was thick and dense, and adhered strongly. The biofilm thickness observed was around 2 mm at the end of the experiment.

Table VII-2. Comparison of Reactor Types and Performance Data

Reactor Type	Loading ($mg/cm^2.d$)	% Removal	COD Flux ($mg/cm^2.d$)	Methane Production ($cm^3/cm^2.d$) (STP)	Temperature °C
SAB	54.8±0.98	78.68	*37.71±1.1	7.86±0.15	25
Anaerobic Filter	0.39-3.11	68.4-98.6	0.38-2.7	1.93	25
Down-Flow Filter	3.2-8.5	81-90	2.88-7.23	1.1-2.7	35
Up-Flow Filter	2.1-12.7	83-93	1.91-11.56	0.76-4.3	35
Expanded-bed	0.07-0.4	80	0.05-0.32	0.01-0.16	30
Fixed Film Fermentor	9.6	86	8.31	2.75	-

*acetate flux ($mg/cm^2.d$)
1 mg acetate=1.085 mg COD

VIII. ENGINEERING SIGNIFICANCE

The engineering significant of this work rests in the ability of the SAB to combine several bacterial types in a single reactor.

In combined carbon oxidation/nitrification biofilm reactors, nitrification is often limited due to the faster growing heterotrophic bacteria excluding the nitrifiers from the oxygenated portion of the biofilm. As a result, carbon oxidation and nitrification in biofilm reactors occur sequentially with nitrification limited to the effluent end of the reactor where carbon levels are low. In the SAB, nitrification can occur simultaneously with carbon oxidation. This process occurs mainly because the slower growing nitrifying bacteria are isolated on the inside of the biofilm in a zone of high ammonia and dissolved oxygen concentrations. Furthermore, isolation prevents significant losses of the nitrifiers when sloughing occurs.

Combined nitrification/denitrification can occur in biofilm reactors if the biofilm is deep enough for oxygen to be depleted, if sufficient organic carbon exists to allow significant denitrification, and if oxygen transfer is adequate to allow conversion of ammonia to nitrate. In competing technologies, such as rotating biological contactors, nitrification has been limited by oxygen

transfer. As a result, the concentrations of nitrate do not reach high enough levels to allow significant denitrification. In the SAB, combined nitrification/denitrification can occur simultaneously. This process occurs mainly because of 1) the isolation of the nitrifying bacteria on the inside of the biofilm in a zone of high ammonia and dissolved oxygen concentrations, and 2) the isolation of the denitrifying bacteria on the outside of the biofilm so that all nitrate from nitrification reaction must diffuse through the denitrifying layer, enhancing the removal of organic carbon and nitrate.

The SAB reactor, unlike competing technologies, supports a combined carbon oxidation/fermentation process, in which the fermentation bacteria are isolated on the outside of the biofilm in an anaerobic zone of high organic carbon concentration, enhancing the removal of organic carbon and production of methane. It also offers the advantages of enclosure of the oxygen supply to enhance efficiency of transfer, and allows for mixing to be controlled independent of aeration, so as to facilitate a deep biofilm, long SRT, and limited sloughing,

With the above advantages the SAB reactor could result in lower construction costs due to fewer reactors and their associated piping and pumping, elimination of recycling streams, and increased oxygen transfer efficiencies. In

addition the SAB reactor offers increased operational flexibility due to the high adaptability to a wide variety of wastewaters, and high stability due to long SRTs in the deep biofilms.

IX. CONCLUSIONS

Based upon the results of this research the following conclusions were reached:

1. The SAB reactor can effectively achieve nitrification of ammonia containing wastes. The fluxes of carbon and nitrogen for the SAB are greater than the reported values for competing technologies such as rotating biological contactors.
2. The SAB reactor can effectively achieve simultaneous nitrification and aerobic heterotrophic oxidation for COD:NH^{+}_{4}-N/l ratio up to 10. Under conditions of combined heterotrophic oxidation and nitrification, carbon and nitrogen fluxes are comparable to competing technologies such as rotating biological contracts.
3. The SAB reactor can effectively achieve support simultaneous nitrification, aerobic heterotrophic oxidation, and denitrification. The fluxes of nitrification and denitrification are higher than those reported for multiple component biofilms and are within the lower range of values observed for biofilm reactors devoted entirely for either nitrification or denitrification. The carbon fluxes are higher than those reported for biofilm

reactors supported simultaneous nitrification and denitrification.

4. The SAB can support simultaneous heterotrophic oxidation and fermentation. The SAB reactor is capable of treating medium-strength soluble organic wastes (800 mg acetate/l) at ambient temperature. The fluxes of carbon under conditions of aerobic heterotrophic oxidation and fermentation are much greater than those of comparable technologies such as anaerobic filters and upflow and downflow fixed film reactors.
5. The biofilm within the SAB exhibits no significant sloughing of bacterial mass. Effluent suspended solids of soluble waste is very low, approximately only 2 to 3 mg/l.

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APPENDICIES

Appendix A

Nutrient Media Preparation and Composition

The nutrient media used was adapted from Owen (1979), and modified to suit the research needs. The media contained essential nutrients and vitamins in excess of the amounts required for microbial growth. Sodium sulfide was added to provide a reducing environment. The sulfur source was added as sulfate to prevent sulfide precipitation of nickel, iron, and cobalt to very low concentrations at equilibrium. The reduction of sulfate to sulfide provided a continual source of sulfur to bacterial growth. Nickel was added to enhance the rate of acetate utilization by methanogens (Speece, 1983b). The concentrated stock solutions of the nutrient media were prepared in 1-L volumetric flasks and stored at 4°C. Table A-1 presents the composition of the nutrient media solutions.

The feed was prepared in 25-L glass carboys and stored in 4°C to prevent bacterial growth. The carboys were intially filled up to 20L with distilled water, then 100 ml of minerals solution, 15 ml of phosphorus, iron, sulfate, and vitamins solutions, and 2 ml of sulfide solution were added. Sodium acetate, ammonium chloride, and potassium bicarbonate were added. The carboys were filled to the 25L mark. Then nitrogen gas was bubbled through a porous diffuser in the solution for 15 minutes.

Table A-1. Nutrient Media Solutios Composition

Solution	Compound	Concentration (mg/1)
Minerals	$CaCl_2$	12.6
	$MgCl_2.6H_2O$	120.0
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2.0
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
	$NiCl_2.6H_2O$	2.0
Phosphorus	Na_2HPO4	28.7
Iron	$FeCl_2.4H2O$	37.0
Sulfate	Na_2SO_4	41.0
Sulfide	$Na_2S.9H2O$	50.0
Vitamins	Biotin	0.02
	Folic acid	0.02
	Pyridoxine Hydrochloride	0.1
	Riboflavin	0.05
	Thiamin	0.05
	Nicotinic acid	0.05
	Pantothenic acid	0.05
	B_{12}	0.001
	p-aminobenzoic acid	0.05
	Thioctic acid	0.05

Appendix B

Reaction Stoichiometry

The stoichoimetries of the four mircobial reactions were calculated using the energetic model presented by McCarty (1971). The model assumes that reactants and products at unit activity and a pH of 7.

$$A = - \frac{\frac{\Delta G_p}{K^m} + \Delta G_c + \frac{\Delta G_n}{K}}{K \Delta G_r}$$

A = the ratio of electron equivalents of substrate used for energy to electron equivalent of cells synthesized.

ΔG_p = free energy gained or lost in the conversion of one electron equivalent of cell carbon source to intermediate pyruvate.

ΔG_c = free energy for the conversion of one electron equivalent of intermediate to one electron equivalent cells (7.5 Kcal/e^-).

ΔG_n = free energy per electron equivalent of cells for reduction of nitrogen source to ammonia, ΔG_n=0 if ammonia is nitrogen source.

ΔG_r = free energy per electron equivalent of substrate converted to energy.

$\Delta G°$ = standard free energy at 25°C and pH=7.

K = efficiency of energy transfer (60%).

m = constant, equal to +1 when ΔG_p>0, and -1 when ΔG_p<0.

The overall reaction equal to the synthesis reaction plus the energy reaction multiplied by the ratio A.

Autotrophic Growth

Conditions:

Electron Donor - NH_4^+, Carbon Source - CO_2, Bacteria - Nitrosomonas

Energy:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{6}\ NH_4^+ + \frac{1}{3}\ H_2O = \frac{1}{6}\ NO_2^- + \frac{4}{3}\ H^+ + e^-$	7.852
-Ra: $e^- + H^+ + \frac{1}{4}\ O_2 = \frac{1}{2}\ H_2O$	-18.675

Rd-Ra

$$\frac{1}{6}\ NH_4^+ + \frac{1}{4}\ O_2 = \frac{1}{6}\ NO_2^- + \frac{1}{3}\ H^+ + \frac{1}{6}\ H_2O$$

Synthesis:

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

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

R_w-R_c

$$\frac{1}{5}\ CO_2 + \frac{1}{20}\ HCO_3^- + \frac{1}{20}\ NH_4^+ + \frac{1}{20}\ H_2O = \frac{1}{4}\ O_2 + \frac{1}{20}\ C_5H_7O_2N$$

Overall Reaction:

Normalized to one mole of ammonia

$$NH_4^+ + 1.269\ O_2 + 0.142\ CO_2 + 0.036\ HCO_3^- = 0.036\ C_5H_7O_2N + 0.964\ NO_2^- + 1.929\ H^+ + 0.929\ H_2O$$

Conditions:

Electron Donor - NO_2^-, Electron Acceptor - O_2, Nitrogen source -NH_4^+, Carbon Source -CO_2, Bacteria-Nitrobacter

Enegery:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{2}\ NO_2^- + \frac{1}{2}\ H_2O = \frac{1}{2}\ NO_3^- + H^+ + e^-$	9.425
-Ra: $\frac{1}{4}\ O_2 + H^+ + e^- = \frac{1}{2}\ H_2O$	-18.675

Rd-Ra

$$\frac{1}{2}\ NO_2^- + \frac{1}{4}\ O_2 = \frac{1}{2}\ NO_3^-$$

Synthesis:

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

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

$R_w - R_c$

$$\frac{1}{5}\ CO_2 + \frac{1}{20}\ HCO_3^- + \frac{1}{20}\ NH_4^+ + \frac{1}{20}\ H_2O = \frac{1}{4}\ O_2 + \frac{1}{20}\ C_5H_7O_2N$$

Overall Reaction:

Normalized to one mole of nitrite

$$NO_2^- + 0.047\ O_2 + 0.042\ CO_2 + 0.01\ HCO_3^- + 0.01\ NH_4^+ + 0.01\ H_2O$$
$$= 0.01\ C_5H_7O_2N + NO_3^-$$

Heterophic Growth

Conditions:

Electron Donor-Acetate, Electron Acceptor-O_2, Nitrogen Source - NH_4^+, Carbon Source-Acctate, Bacteria-Aerobic heterotrophic.

Energy:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{8}\ CH_3COO^- + \frac{3}{8}\ H_2O = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + H^+ + e^-$	-6.609
-Ra: $\frac{1}{4}\ O_2 + H^+ + e^- = \frac{1}{2}\ H_2O$	-18.765

Rd-Ra

$$\frac{1}{8}\ CH_3COO^- + \frac{1}{4}\ O_2 = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + \frac{1}{8}\ H_2O$$

Synthesis:

Rd: $\frac{1}{8}\ CH_3COO^- + \frac{3}{8}\ H_2O = \frac{1}{8}\ O_2 + \frac{1}{8}\ HCO_3^- + H^+ + e^-$

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

Rd-Rc

$$\frac{1}{8}\ CH_3COO^- + \frac{3}{40}\ CO_2 + \frac{1}{20}\ NH_4^+ = \frac{1}{20}\ C_5H_7O_2N + \frac{3}{40}\ H_2O + \frac{3}{40}\ HCO_3^-$$

Overall Reaction:

Normalized to one mole of acetate

$$CH_3COO^- + 0.8310_2 + 0.235\ NH_4^+ = 0.235\ C_5H_7O_2N + 0.061\ CO_2 + 0.765\ H_2O + 0.765\ HCO_3^-$$

Conditions:

Electron Donor-Acetate, Electron Acceptor-NO_2^-, Nitrogen Source-NH_4^+, Carbon Source-Acetate Bacteria-Denitrifying.

Energy:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{8}\ CH_3COO^- + \frac{3}{8}\ H_2O = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + H^+ + e^-$	-6.609
-Ra: $\frac{1}{3}\ NO_2^- + \frac{4}{3}\ H^+ + e^- = \frac{1}{6}\ N_2 + \frac{2}{3}\ H_2O$	-22.263

Rd-Ra

$$\frac{1}{8}\ CH_3COO^- + \frac{1}{3}\ NO_2^- + \frac{1}{3}\ H^+ = \frac{1}{6}\ N_2 + \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + \frac{7}{24}\ H_2O$$

Synthesis:

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

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

Rd-Rc

$$\frac{1}{8}\ CH_3COO^- + \frac{3}{40}\ CO_2 + \frac{1}{20}\ NH_4^+ = \frac{1}{20}\ C_5H_7O_2N + \frac{3}{40}\ H_2O + \frac{3}{40}\ HCO_3^-$$

Overall Reaction:

Normalized to one mole of acetate

$$CH_3COO^- + 1.02\ NO_2^- + 1.02\ H^+ + 0.248\ NH_4^+ =$$

$$0.248\ C_5H_7O_2N + 0.51\ N_2 + 0.01\ CO_2 + 0.752\ HCO_3^- + 1.267\ H_2O$$

Conditions:

Electron Donor-Acetate, Electron Acceptor-NO_3^- Nitrogen Source-NH_4^+, Carbon Source-Acetate Bacteria-Dentrifying

Energy:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{8}\ CH_3COO^- + \frac{3}{8}\ H_2O = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + H^+ + e^-$	-6.609
-Ra: $\frac{1}{5}\ NO_3^- + \frac{6}{5}\ H^+ + e^- = \frac{1}{10}\ N_2 + \frac{3}{5}\ H_2O$	-17.128

Rd-Ra

$$\frac{1}{8}\ CH_3COO^- + \frac{1}{5}\ NO_3^- + \frac{1}{5}\ H^+ = \frac{1}{10}\ N_2 + \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + \frac{9}{40}\ H_2O$$

Synthesis:

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

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

Rd-Rc

$$\frac{1}{8}\ CH_3COO^- + \frac{3}{40}\ CO_2 + \frac{1}{20}\ NH_4^+ = \frac{1}{20}\ C_5H_7O_2N + \frac{3}{40}\ H_2O + \frac{3}{40}\ HCO_3^-$$

Overall Reaction:

Normalized to one mole of acetate

$$CH_3COO^- + 0.689\ NO_3^- + 0.0689H^+ + 0.228\ NH_4^+ =$$

$$0.228\ C_5H_7O_2N + 0.342\ N_2 + 0.087\ CO_2 + 0.772\ HCO_3^- +$$

$$1.114\ H_2O$$

Conditions:

Electron Donor-Acetate, Electron Acceptor-CO_2, Nitrogen Source-NH_4^+, Carbon Source-Acetate, Bacteria-Methanogens

Energy:

	ΔG° (Kcal/e^-)
Rd: $\frac{1}{8}\ CH_3COO^- + \frac{3}{8}\ H_2O = \frac{1}{8}\ CO_2 + \frac{1}{8}\ HCO_3^- + H^+ + e^-$	-6.609
-Ra: $\frac{1}{8}\ CO_2 + H^+ + e^- = \frac{1}{8}\ CH_4 + \frac{1}{4}\ H_2O$	5.763

Rd-Ra

$$\frac{1}{8}\ CH_3COO^- + \frac{1}{8}\ H_2O = \frac{1}{8}\ CH_4 + \frac{1}{8}\ HCO_3^-$$

Synthesis:

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

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

Rd-Rc

$$\frac{1}{8}\ CH_3COO^- + \frac{3}{40}\ CO_2 + \frac{1}{20}\ NH_4^+ = \frac{1}{20}\ C_5H_7O_2N + \frac{3}{40}\ H_2O + \frac{3}{40}\ HCO_3^-$$

Overall Reaction:

Normalized to one mole of acetate

$$CH_3COO^- + 0.928\ H_2O + 0.027\ CO_2 + 0.018\ NH_4^+ =$$

$$0.018\ C_5H_7O_2N + 0.955\ CH_4 + 0.982\ HCO_3^-$$

Appendix C

Sample Calculation of Mass Balances on the SAB

A solute mass balance on the SAB reactor is given in equation (C-1).

$$\frac{ds^i}{dt} = \frac{Q}{V}(S^i - S^e) - \frac{A}{V} K_{LS} (S^e - S^s) \qquad (C-1)$$

At steady-state, equation (C-I) reduces to

$$\frac{A}{V} K_{LS}(S^e - S^s) = \frac{Q}{V} (S^i - S^e) \qquad (C-2)$$

in which

$$F = \frac{A}{V} K_{LS} (S^e - S^s) \qquad (C-3)$$

under this condition

$$F = \frac{Q}{V} (S^i - S^e) \qquad (C-4)$$

Nomenclature

F=Flux into biofilm ($mg/cm^2.d$)

F(1)=Flux due to carbon oxidation

F(2)=Flux due to cell growth

F(3)=Flux due to nitrification

F(4)=Flux due to denitrification

A=Area of biofilm (cm^2)=86.6

S=Concentration of subsrate (mg/l)

Q=Flow rate (L/day)=5.78

V=Volume of reactor (cm^3)=1925.8

K_{LS}=Liquid-to-surface mass transfer coefficient (cm/day)

Superscripts

i=Influent

e=Effluent

s=At film surface

Subscripts

c=Acetate (mg/l)

m=Ammonia (mg-N/1)

i=Nitrite (mg-N/1)

a=Nitrate (mg-N/1)

h=Heterotrophic oxidation

n=Nitrification

d=denitrification

Stoichoimetric ratios

$R_h = NH_4^+/Acetate$ (mg-N/mg)=0.056

$R_d = NH_4^+/Acetate$ (mg-N/mg)=0.054

$R_d = Acetate/NO_3$ (mg/mg-N)=6.112

Flux of nitrogen for cell growth

$$F_d(2)+F_h(2) = \frac{Q}{A}(S_c^i - S_c^e) \times [(R_h(NH_4^+/Acetate) + R_d(NH_4^+/Acetate))/2] \qquad (C\text{-}5)$$

$$F_d(2)+F_h(2) = \frac{5.78}{86.6}(21.9-1.0) \times \left(\frac{0.056+0.054}{2}\right)$$

$$= 0.077 \text{ mg-N/cm}^2\text{.d}$$

Flux of nitrogen due to denitrification

$$F_d(4) = \frac{Q}{A}(S_m^i + S_a^i + S_i^i - S_m^e - S_a^e - S_i^e) - [F_d(2)+F_h(2)] \qquad (C\text{-}6)$$

$$= \frac{5.78}{86.6} (10.5+0+0-5.2-2.2-0.46)-0.077$$

$$=0.099 \text{ mg-N/cm}^2\text{.d}$$

Flux of carbon oxidation due to denitrification

$$F_d(1)=R_d \text{ (Acetate/NO}_3\text{-N) X } F_d(4) \qquad \text{(C-7)}$$

$$=6.112\text{x}0.099$$

$$=0.606 \text{ mg Acetate/cm}^2\text{.d}$$

Flux of carbon oxidation due to heterotrophic

$$F_h(1) = \frac{Q}{A}(S_c^i-S_c^e)-F_d(1) \qquad \text{(C-8)}$$

$$= \frac{5.78}{86.6}(21.9-1.0)-0.606$$

$$= 0.789 \text{ mg Acetate/cm}^2\text{.d}$$

Flux of ammonia due to nitrification

$$F_n(3)= \frac{Q}{A}(S_m^i-S_m^e)-[F_d(2)+F_h(2)] \qquad \text{(C-9)}$$

$$= \frac{5.78}{86.6}(10.5-5.2)-0.077$$

$$= 0.277 \text{ mg-N/cm}^2\text{.d}$$

Appendix D

Oxygen Mass Transfer

The procedure for evaluating the oxygen mass transfer coefficient across the Gortex Membrane involved transferring 1L of distilled water to the SAB and purging with nitrogen to decrease the concentration of dissolved oxygen to ≤1.0 mg/1. After purging stopped pure oxygen was supplied and dissolved oxygen concentration was monitored by the probe. The SAB was in batch mode and temperature was controlled at
25°C.

A mass balance on dissolved oxygen in the SAB can be written as

$$\frac{dc}{dt} = K_L a(C_s - C) \qquad \text{(D-1)}$$

where

K_L = Overall oxygen mass transfer coefficient

$$a = \frac{A}{V}$$

A = Gortex surface area

V = Volume of water

C_s = Saturation concentration of oxygen in water

C = Concentration of oxygen in the water

Equation D-1 can be rewritten

$$\frac{dc}{(C_s - C)} = K_L a dt \qquad \text{(D-2)}$$

Integrating from t = o gives

$$\text{Ln } (C_s - C) = K_L a t \qquad \text{(D-3)}$$

Oxygen mass transfer coefficients, $K_L a$, were obtained

from the slope of the least squares linear regression line for data plotted as Ln (C_s-C) versus t, as shown in figures (D-1) to (D-4). The values of $K_l a$ and the square of the correlation coefficient are summarized in table (D-1).

Table D-1. Oxygen Mass Transfer Coefficient Values (Temperature 25°C, C_s=8.4 mg/1)

Reactor	$K_l a$ (1/min)	r^2
1	0.0045	0.99
2	0.0045	0.99
3	0.0046	0.99
4	0.0042	0.99

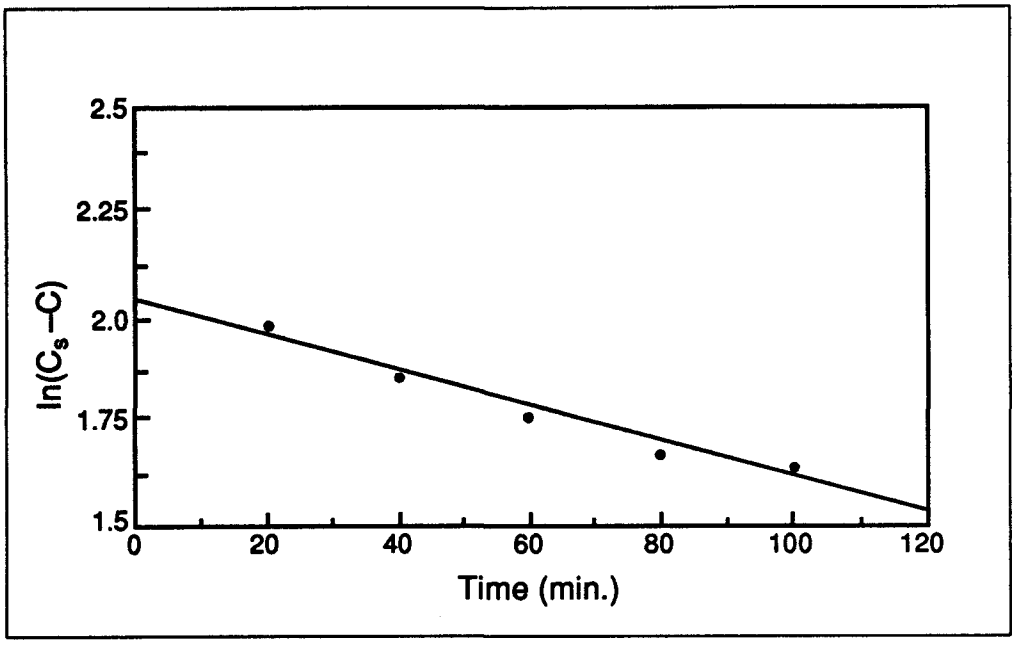

Figure D-1. Plot for determination of oxygen mass transfer coefficient for SAB #1.

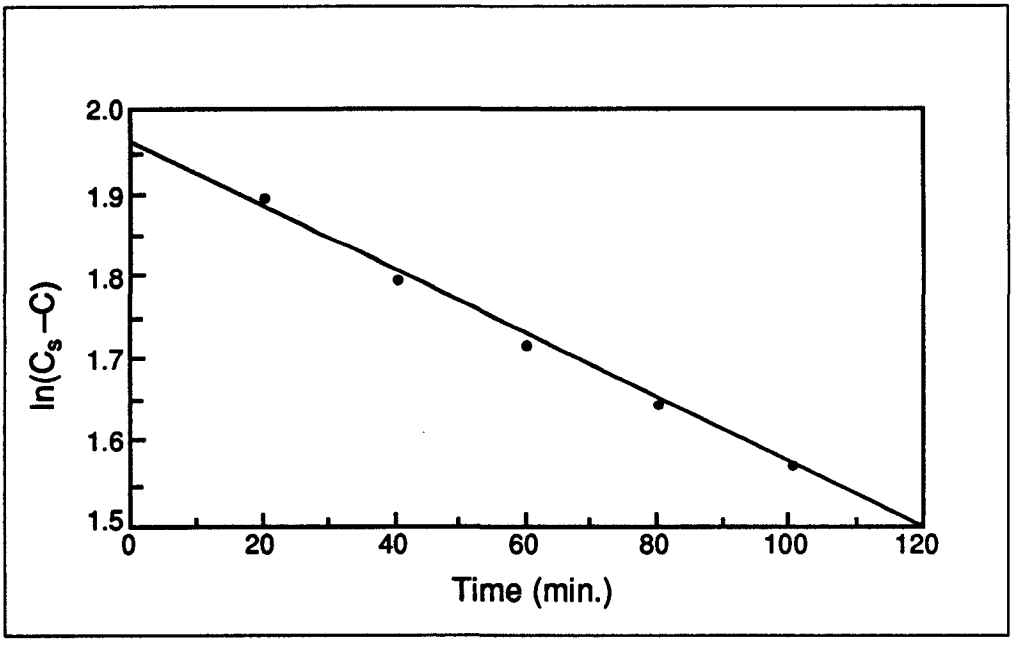

Figure D-2. Plot for determination of oxygen mass transfer coefficient for SAB #2.

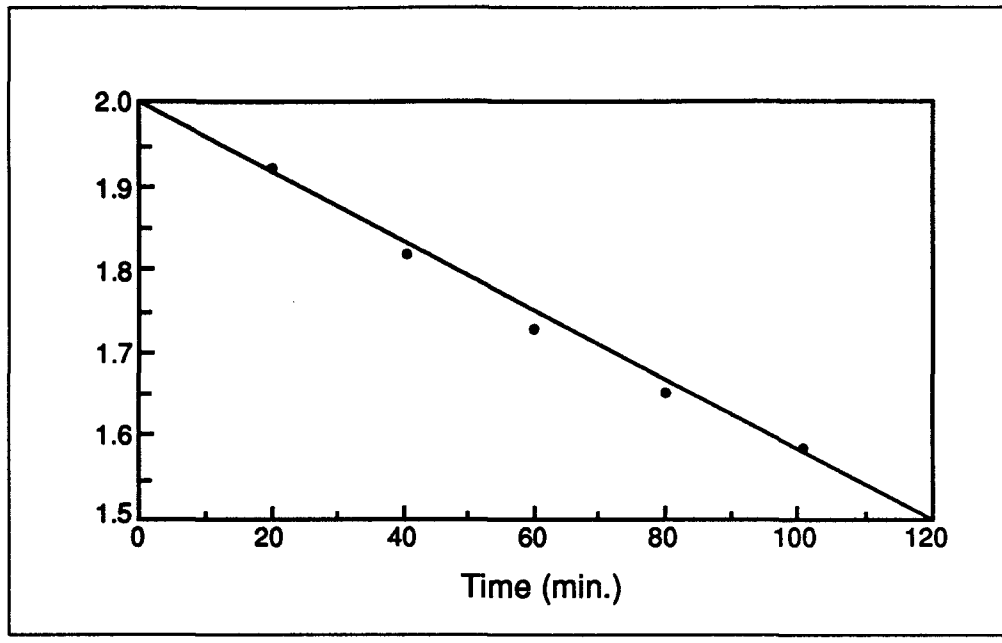

Figure D-3. Plot for determination of oxygen mass transfer coefficient for SAB #3.

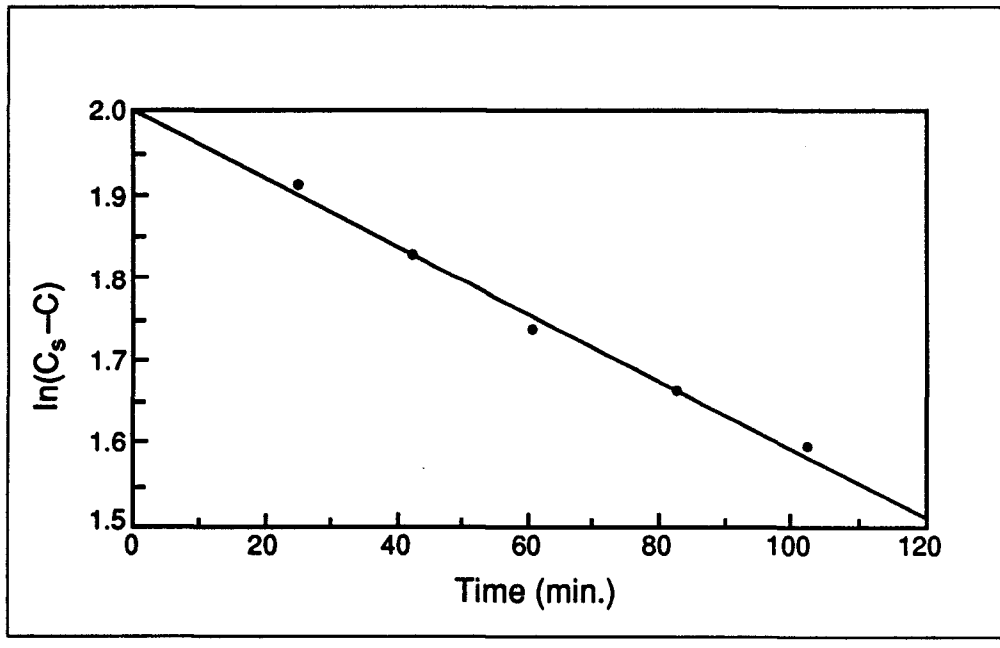

Figure D-4. Plot for determination of oxygen mass transfer coefficient for SAB #4.

Appendix E

Nitrogen, Acetate, Methane and Oxygen Experimental Data

Table E-1. Nitrogen Data for SAB #2 (influent concentration = 10 mg NH_4^+-N/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.
Nov-1 Int.	11.200	11.200	0.000	0.000
Nov-17	11.148	10.789	0.000	0.000
Nov-21	11.148	8.617	1.749	0.000
Nov-24	11.148	9.124	2.243	0.000
Nov-28	10.420	4.649	5.605	0.000
Dec-1	10.420	3.339	6.503	1.158
Dec-5	11.430	1.128	8.286	2.261
Dec-8	11.430	1.037	6.355	2.469
Dec-12	11.152	1.288	7.073	3.536
Dec-15	11.152	0.834	5.965	4.919
Dec-19	10.697	1.252	8.716	4.252
Dec-26	10.697	0.048	1.937	6.523
Dec-29	10.564	0.054	1.932	8.454
Jan-3	10.564	0.017	1.152	8.713
Jan-6	10.130	0.067	1.987	7.648

Table E-2. Nitrogen Data for SAB #3 (influent concentration 10 mg NH_4^+-N/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.
Nov-1 Int.	11.480	11.480	0.000	0.000
Nov-17	11.440	11.260	0.000	0.000
Nov-21	11.440	6.699	4.773	0.000
Nov-24	11.220	6.410	5.142	0.000
Nov-28	11.220	4.162	8.308	0.000
Dec-1	11.740	2.268	7.284	2.495
Dec-5	11.740	1.042	6.834	4.039
Dec-8	10.720	2.042	5.695	4.795
Dec-12	10.720	1.261	5.903	4.158
Dec-15	10.930	1.153	5.021	4.843
Dec-19	10.930	1.936	0.510	7.727
Dec-26	11.230	1.331	0.834	8.693
Dec-29	11.230	1.528	0.846	8.299
Jan-3	11.430	0.232	0.546	8.152
Jan-6	11.430	0.209	0.587	8.099

Table E-3. Nitrogen Data for SAB #4 (influent concentration = 10 mg NH_4^+-N/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.
Nov-1	10.80	10.80	0.00	0.00
Nov-17	10.80	10.80	0.00	0.00
Nov-21	10.72	8.39	2.19	0.00
Nov-24	10.72	7.06	4.78	0.00
Nov-28	11.08	4.70	7.69	0.00
Dec-1	11.08	3.74	5.04	1.48
Dec-5	10.86	2.45	5.40	2.59
Dec-8	10.86	3.34	5.84	1.74
Dec-12	10.54	3.21	5.29	1.35
Dec-15	10.54	3.11	5.52	1.96
Dec-19	10.69	1.94	5.91	3.37
Dec-26	10.69	1.36	3.63	4.27
Dec-29	10.41	1.42	1.20	6.89
Jan-3	10.41	1.78	1.06	7.13
Jan-6	10.17	1.07	0.94	7.41
Jan-10	10.17	0.98	0.87	7.44

Table E-4. Nitrogen and Acetate Data for SAB #1 (influent concentration = 10 mg NH_4^+-N/l and 5 mg acetate/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-5	10.16	4.243	0.832	7.671		
Apr-6 Int.	10.56	4.354	0.541	6.894	5.54	5.54
Apr-7	10.56	3.964	0.731	7.309	5.54	<1
Apr-8	10.56	3.773	0.555	7.128	5.54	<1
Apr-9	10.56	3.327	0.404	7.409	5.54	<1
Apr-10	11.24	3.579	0.865	8.011	5.23	<1
Apr-14	11.24	3.378	0.935	7.405	5.23	<1
Apr-17	10.96	4.455	1.058	7.444	5.76	<1
Apr-20	10.96	3.562	1.201	8.032	5.76	<1

Table E-5. Nitgrogen and Acetate Data for SAB #2 (influent concentratin = 10 mg NH_4^+-N/l and 10 mg acetate/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-5	11.431	3.591	0.946	6.542		
Apr-6 Int.	11.431	3.234	0.831	6.971	11.781	11.781
Apr-8	11.431	3.809	0.927	3.761	11.781	<1
Apr-9	11.431	3.724	0.305	4.733	11.781	<1
Apr-10	11.221	2.997	0.809	5.336	10.932	<1
Apr-14	11.221	3.126	0.524	5.910	10.932	<1
Apr-17	11.221	4.194	0.481	5.062	10.932	<1
Apr-25	11.221	3.272	0.459	5.248	10.932	<1

Table E-6. Nitrogen and Acetate Data for SAB #3 (infuence concentration = 10 mg NH_4^+-N/l and 20 mg acetate/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-2	11.470	0.241	0.571	8.168		
Apr-5	11.470	0.199	0.569	8.120		
Apr-6 Int.	10.100	5.531	0.832	1.757	22.567	22.567
Apr-7	10.100	5.452	0.247	2.430	22.567	<1.000
Apr-8	10.100	4.381	0.119	2.020	22.567	<1.000
Apr-9	10.100	5.009	0.133	1.985	22.567	<1.000
Apr-10	10.370	4.675	0.448	1.631	21.128	<1.000
Apr-14	10.370	5.989	0.429	2.067	21.128	<1.000
Apr-17	11.168	5.596	0.724	3.755	20.580	<1.000
Apr-20	11.168	5.276	0.619	2.414	20.580	<1.000
Apr-27	10.568	4.742	0.405	2.743	21.975	<1.000
May-1	10.568	5.168	0.533	2.181	21.975	<1.000
May-4	11.350	5.326	0.611	1.882	22.230	<1.000

Table E-7. Nitrogen and Acetate Data for SAB #4 (influent concentration = 10 mg NH_4^+-N/l and 40 mg acetate/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-6	10.26	2.71	6.31	1.98		
Apr-7	10.26	3.09	4.87	2.70		
Apr-8	9.23	2.58	2.65	4.34		
Apr-9	9.23	3.54	0.99	5.55		
Apr-10	9.23	3.12	0.35	6.40		
Apr-14 Int.	11.17	5.24	0.31	6.34	42.59	42.59
Apr-15	11.17	6.41	0.29	0.42	42.59	1.84
Apr-16	11.17	6.68	0.19	0.36	42.59	1.97
Apr-17	10.54	6.67	0.19	0.50	42.59	2.47
Apr-20	10.54	5.80	0.19	0.52	41.37	2.68
Apr-27	11.10	5.48	0.30	0.00	41.37	1.00
May-4	10.69	5.67	0.00	0.00	41.37	2.57

Table E-8. Nitrogen and Acetate Data for SAB #4 (influent cocentration = 10 mg NH_4^+-N/l and 100 mg acetate/l)

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-27	11.10	5.48	0.30	0.00	41.37	1.00
May-4	11.69	5.67	0.00	0.00	41.37	2.57
May-9 Int.	10.69	6.12	0.00	0.00	98.29	1.46
May-10	10.69	6.23	0.00	0.00	98.29	34.97
May-11	11.71	6.15	0.00	0.00	98.29	35.51
May-12	11.71	6.05	0.00	0.00	98.29	39.11
May-15	11.71	6.12	0.00	0.00	108.36	41.60
May-18	10.72	7.07	0.00	0.00	108.36	31.63
May-22	10.72	6.39	0.00	0.00	103.45	36.78

Table E-9. Effect of Increasing Acetate Concentraction on Nitrificationn

Date	NH_4^+ mg-N/l Inf.	NH_4^+ mg-N/l Eff.	NO_2^- mg-N/l Eff.	NO_3^- mg-N/l Eff.	Acetate mg/l Inf.	Acetate mg/l Eff.
Apr-26Int	11.221	3.126	0.524	5.910	10.932	0.000
Apr-27	11.221	4.194	0.481	5.062	10.932	0.000
Apr-28	11.221	3.272	0.459	5.248	10.932	0.000
Apr-30	10.422	3.256	0.000	0.000	818.285	733.188
May-1	10.422	4.619	0.000	0.000	818.285	727.844
May-4	10.422	5.344	0.000	0.000	818.285	720.393
May-8	10.422	5.276	0.000	0.000	818.285	718.204
May-11	10.110	6.191	0.000	0.000	816.697	742.792
May-18	10.110	6.505	0.000	0.000	816.697	741.497
May-22	10.110	7.613	0.000	0.000	816.697	734.005
May-26	10.121	7.611	0.000	0.000	817.057	742.854

Table E-10. Nitrogent, Acetate, and Methane Data for SAB #2 (influent concentration = 10 mg NH_4^--N/l and 800 mg acetate/l)

Date	NH_4^+ Mg-N/l		NO_2^- Mg-N/l	NO_3^- Mg-N/l	Acetate Mg/l		Methane Mg/l
	Inf.	Eff.	Eff.	Eff.	Inf.	Eff.	Off-Gas (STP)
Sep-5	9.340	5.720	0.000	0.000	812.590	732.180	0.000
Sep-8	11.430	7.680	0.000	0.000	785.230	453.040	20.576
Sep-12	11.430	6.560	0.000	0.000	869.480	322.790	46.254
Sep-15	9.640	6.540	0.000	0.000	819.680	623.680	115.567
Sep-19	9.640	4.910	0.000	0.000	812.130	484.190	166.493
Sep-22	10.810	5.410	0.000	0.000	824.550	401.930	211.731
Sep-26	10.840	5.620	0.000	0.000	827.750	377.830	258.589
Sep-29	10.950	5.540	0.000	0.000	822.110	186.110	307.565
Oct-3	10.950	5.990	0.000	0.000	832.470	179.390	286.265
Oct-6	10.320	5.360	0.000	0.000	823.070	157.450	320.619
Oct-10	10.320	5.570	0.000	0.000	823.070	187.360	306.401
Oct-13	10.210	5.380	0.000	0.000	826.450	176.380	312.017

Table E-11. Summary of Oxygen Mass Transfer Data

Time (min)	Dissolved Oxygen (mg/l)			
	SAB #1	SAB #2	SAB #3	SAB #4
0	0.58	1.28	0.86	0.89
20	1.28	1.86	1.72	1.47
40	1.99	2.56	2.42	2.05
60	2.56	3.14	2.99	2.62
80	3.08	3.59	3.43	3.07
100	3.37	3.94	3.82	3.42
120	3.78	4.23	4.07	3.84

Appendix F

Gas and Ions Chromatograms

Typical chromatograms for carbon dioxide, oxygen, nitrogen and methane are presented in Figure (F-1), using Fisher 25V gas partitioner. Figures (F-2) demonstrates typical chromatograms for acetate, chloride, nitrite, and nitrate using Dionex 4000i ion chromatograph equipped with anion separator. Figure (F-3) demonstrates typical chromatograms for sodium, ammonia, and potassium using Dionex 4000i ion chromatograph equipped with cation separator.

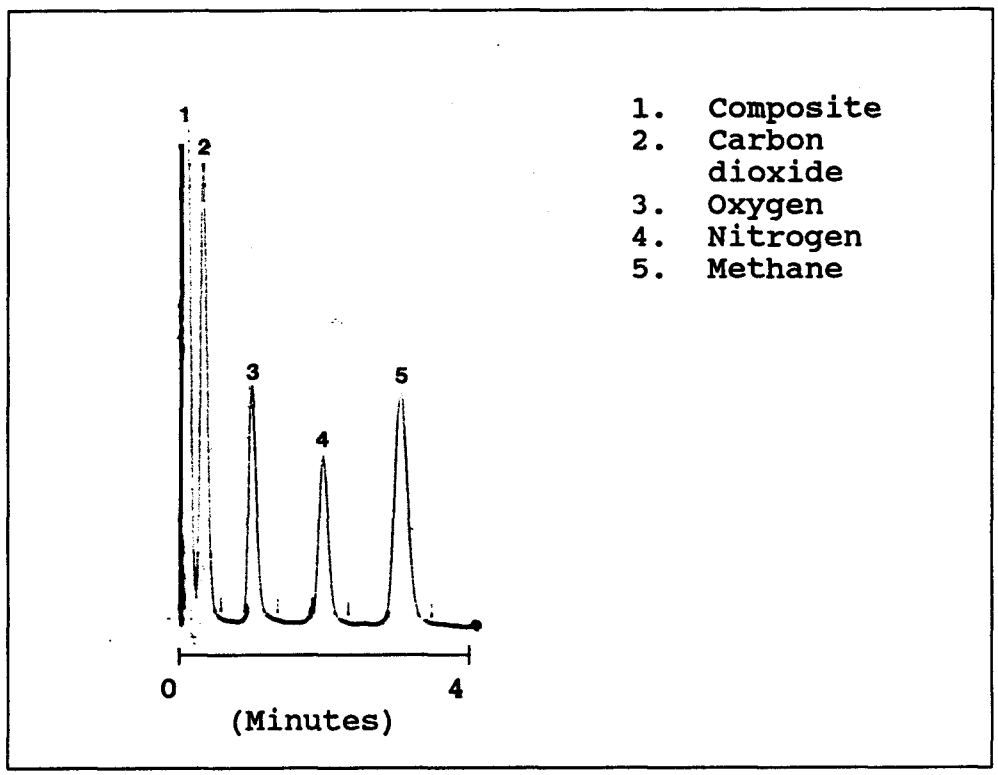

Figure F-1. Typical chromatogram from Fisher 25V gas partitioner.

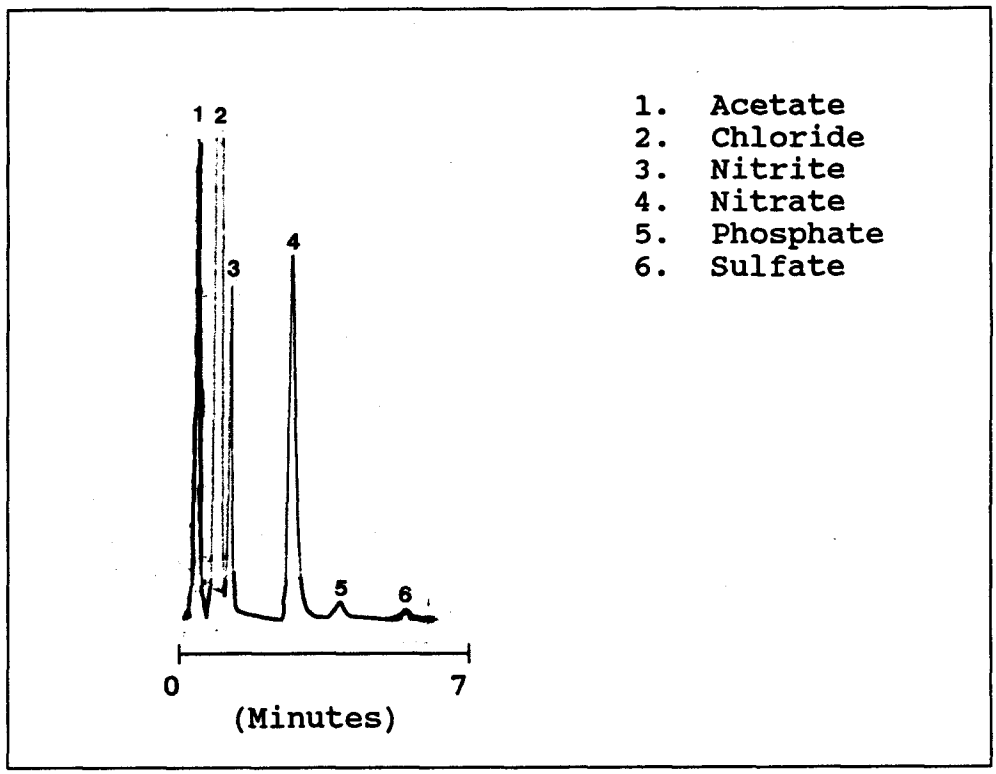

Figure F-2. Typical anion chromatogram from Dionex 4000i ion chromatograph.

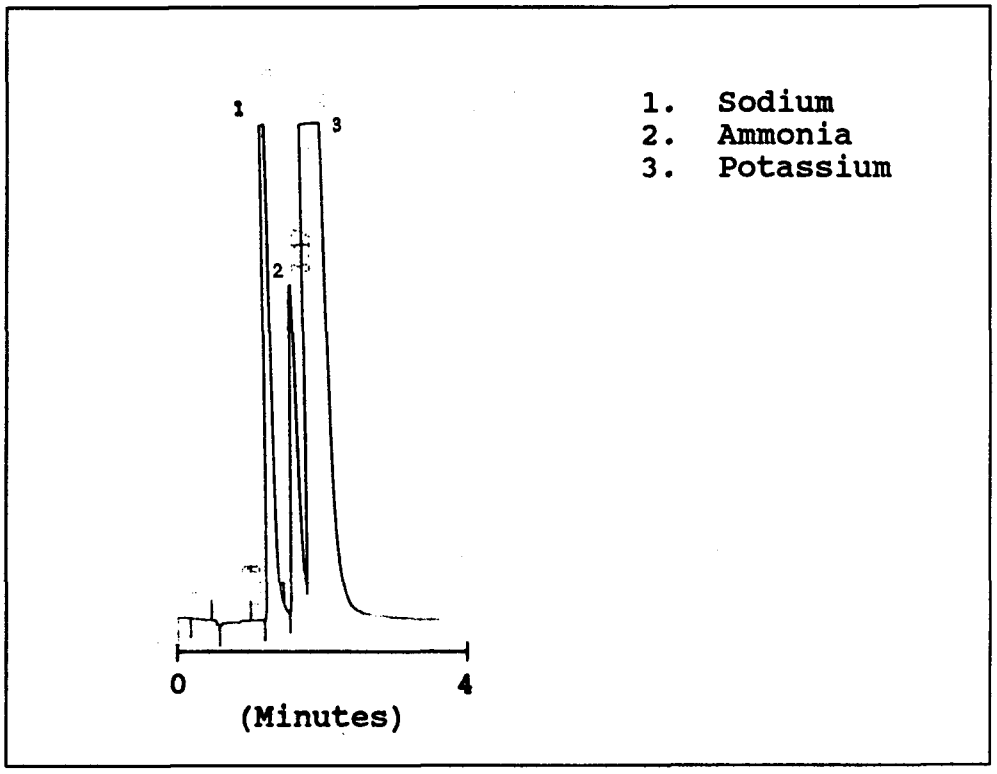

Figure F-3. Typical cation chromatogram from Dionex 4000i ion chromatograph.