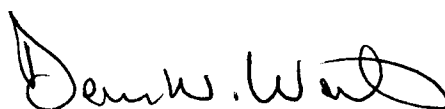


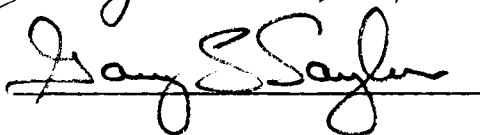
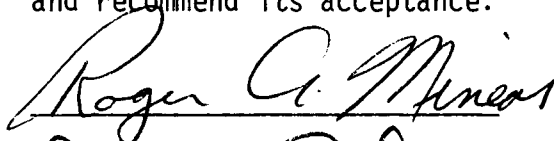
To the Graduate Council:

I am submitting herewith a thesis written by Debby Hines entitled "Production of Ammonia in a Packed-Bed Anaerobic Upflow (ANFLOW) Bioreactor." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.



Dennis W. Weeter, Major Professor

We have read this thesis
and recommend its acceptance:



Accepted for the Council:



Vice Chancellor
Graduate Studies and Research

PRODUCTION OF AMMONIA IN A PACKED-BED ANAEROBIC
UPFLOW (ANFLOW) BIOREACTOR

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Debby Hines

June 1981

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ABSTRACT

A study was conducted to examine the production of ammonia in a packed-bed anaerobic upflow (ANFLOW) bioreactor which uses fixed-films of microorganisms. ANFLOW bioreactors have been under development as innovative alternative wastewater treatment systems. Domestic wastewater contains proteins from the wastes of humans and animals; as the amino acids from the proteins break down, there is formation of ammonia, volatile fatty acids, and other products. Under anaerobic conditions, proteinaceous material is converted to volatile fatty acids by a complex group of bacteria; the volatile acids are then reduced to methane by specialized methanogenic bacteria which are very sensitive to environmental conditions. The methane gas may be collected and utilized for energy. Some of the ammonia is used in cell synthesis but there is a net accumulation of ammonia in the system because the rate of cell growth of the methanogens is slow.

A synthetic organic waste feed was used containing glucose (as a carbon source), nutrient broth (as a protein and trace mineral source), and sodium bicarbonate (for alkalinity). Three concentrations of soluble chemical oxygen demand (SCOD) and two hydraulic loading rates (HLR) were used resulting in a three-by-two experimental matrix design.

Gas production, percentage methane of the off-gas, pH, alkalinity, volatile acids, and SCOD were used to monitor perturbations of steady-state conditions. Nitrogen mass balances were determined by analyzing total Kjeldahl nitrogen (TKN), nitrate+nitrite-nitrogen, and ammonia-nitrogen.

Removal rates of SCOD ranged from 87-95% within the ANFLOW bioreactor. The most efficient SCOD removal occurred at the highest organic loading rate (OLR). Profiles of SCOD and volatile acids concentrations showed distinct regions of complex organics breakdown and wastes stabilization. Hydraulic flow within the ANFLOW bioreactor behaved approximately as in a plug-flow reactor (PFR).

The effluent ammonia concentration appeared to be a function of HLR. For a given OLR or influent SCOD, effluent ammonia concentrations were less at the higher flow rate. These results suggest that at the shorter hydraulic residence time (HRT), there was less time for ammonia accumulation and conversion of organic-nitrogen to ammonia-nitrogen could not be fully completed. However, overall ammonia-nitrogen formulation (in terms of grams per day produced) was found to be linear as a function of OLR. The figures presented could be used for predicting effluent ammonia concentration for a given OLR, HLR, or SCOD, or ammonia formation for a given OLR.

It was demonstrated that the ammonia can be nitrified using an aerobic, upflow packed-bed bioreactor with only 5% volume of the ANFLOW bioreactor and a hydraulic residence time of only a few hours. A series of packed-bed bioreactors was recommended for carbon removal/waste stabilization--ammonia removal--denitrification. The methane gas produced could be used for power requirements.

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I. INTRODUCTION

Anaerobic-upflow (ANFLOW) bioreactors which use fixed-films of microorganisms are under development as innovative alternative wastewater treatment systems which are more economical in terms of costs and energy utilization than conventional systems (1-3). One economic advantage of the process is the elimination of operating energy requirements associated with the aeration of aerobic-based processes. A significant decrease in the sludge-handling required with conventional activated sludge treatment systems will also lower costs. Methane gas which is produced in these systems may be recovered and utilized for power generation, thus further reducing operating costs. Also, these fixed-film systems are presently assumed to be advantageous since outer layers of films exposed to shock loadings of toxic materials may slough off leaving lower levels of film viable for continued wastewater treatment.

Conventional anaerobic treatment occurs in digestors or continuously stirred-tank-reactors (CSTR) for waste stabilization. These systems are more sensitive to shock loadings or toxic substances since the whole volume of microorganisms are exposed. The ANFLOW bioreactors perform similar to plug-flow reactors (PFR) and thus the phases of methanogenesis can be observed. When shock loadings occur, microorganisms near the influent absorb the bulk of any toxic material. The acid-formers (precursors to methane-formers) are present in this area and are less sensitive than methane-formers to stresses in environmental conditions.

Ammonia is formed during anaerobic processes from the degradation of proteins occurring in wastewater. Breakdown of proteins occur at a higher rate than cell synthesis, thus resulting in ammonia accumulation. Ammonia is inherent in anaerobic systems and is actually beneficial at certain concentrations as it increases the bicarbonate alkalinity.

From discharge regulation considerations, it must be noted that ammonia released to receiving waters causes a high nitrogenous oxygen demand. Ammonia is also toxic to certain biota at very low concentrations and enhances eutrophication. Thus ammonia in the effluent of wastewater treatment systems must be regulated. Most wastewater treatment plants employ advanced treatment methods to remove ammonia.

The objective of this study was to monitor effluent ammonia concentrations and ammonia production rates within the ANFLOW bioreactor as influent soluble chemical oxygen demand (SCOD) concentrations and flow rates were varied. Correlations between influent organic loadings and effluent ammonia concentrations and ammonia formation were sought. Subsequent ammonia removal methods were proposed. An aerobic, upflow packed-bed bioreactor was tested for nitrification of effluent ammonia from the ANFLOW bioreactor.

II. LITERATURE REVIEW

Anaerobic Processes

Anaerobic digestion has been used since the middle 1800's for wastewater solids treatment. Methane gas produced from anaerobic treatment was first utilized in the early 1900's (4). Methane as a substitute natural gas (SNG) has received more attention with acknowledgement of the energy crisis. Conventional anaerobic treatment occurs in digestors or continuously stirred-tank-reactors (CSTR). Extensive research by microbiologists and engineers has focused on types of microorganisms present and design criteria for optimal biological growth.

The stepwise conversion of organic wastes to methane during anaerobic stabilization has been well established (4-10). Two or three major groups of bacteria decompose organic matter during methanogenesis. The first is a complex group of saprophytic species which hydrolyzes organic substrates such as cellulose, lipids, fats, carbohydrates, and proteins and then ferments these products to acetate and other volatile fatty acids, alcohols, hydrogen, carbon dioxide, ammonia, and sulfides. The second group, mainly hypothetical, could oxidize fatty acids other than acetic acid to produce acetic acid and hydrogen (8). These acid-formers include several genera: Clostridium, Peptococcus, Bifidobacterium, Desulphovibrio, Corynebacterium, Lactobacillus, Actinomyces, Staphylococcus, and Escherichia (4).

The third group includes a variety of methanogenic bacteria that would utilize hydrogen, carbon dioxide, and acetic acid for growth forming methane and carbon dioxide. Several genera are responsible

for methanogenesis including Methanobacterium, Methanosarcina, Methanococcus, and Methanobacillus (4, 6). Ciliated protozoa and amoeba are also present (11). Photosynthetic bacteria and blue-greens are found in anaerobic systems, for example, anaerobic lagoons, but these groups utilize other end products than those used by the methanogens (12). A flow chart of methanogenesis is shown in Figure 1.

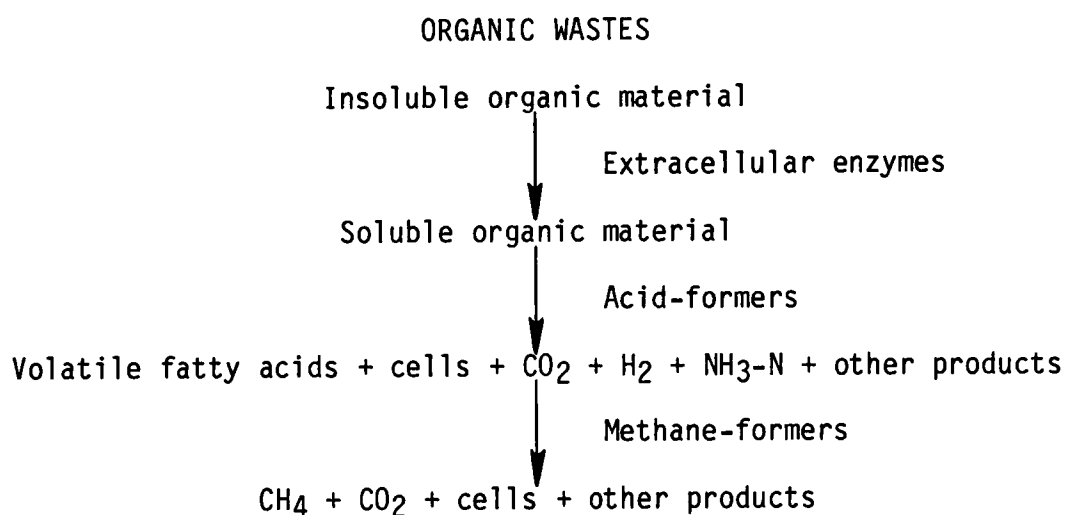


FIGURE 1. Methanogenesis (5).

Little energy is available to the methane-formers during conversion, thus their growth rate is slow and only a small portion of wastes is converted to new cell material. The major portion of degradable wastes is converted to methane gas. This conversion represents waste stabilization since the gas is relatively insoluble and can be burned to carbon dioxide and water. As much as 80-90% of the degradable organic portion of waste can be stabilized even at high loading rates. In aerobic

systems, only about 50% of wastes is actually stabilized even at conventional loadings (9). It should be possible to maintain higher organism concentration in anaerobic systems than in aerobic since concentration is not limited by oxygen transfer rate (not considering available nutrients required for cell growth) (5). Rates of oxygen transfer are crucial in the design of activated sludge units. Overall treatment rates in anaerobic units are not limited by oxygen transfer (9). Sludge disposal is minimized since only a small portion of wastes is converted to cells.

The methane-formers that use acetic and propionic acids are slow growers; some methane-formers can use methanol and formic acid and grow rapidly (9). Methane formation is usually the rate limiting step. The methane carbon is derived from the methyl carbon of acetate (5, 6, 9). Exponential growth has been observed on acetate in the absence of hydrogen and diauxic growth was observed in favor of methanol during growth in the presence of methanol and acetate; however, hydrogen and carbon dioxide were favored over acetate by a strain of Methanosarcina (6). Propionic acid is utilized at an even slower rate than acetic acid, thus large amounts may indicate failure (5). Other volatile fatty acids found include butyric, valeric, caproic, and isovaleric. Environmental conditions such as pH, available minerals, and redox potential will exert influence on types and amounts of volatile acids produced in mixed cultures through selection of species or groups best adapted to existing conditions (5).

Acetic acid is the source of most methane (5, 6, 9, 13). Assuming a complex municipal type waste (primarily carbohydrates, proteins, and fats) of 100% COD, only about 20% of the waste is converted directly to

acetic acid (9). A larger portion (about 52%) is formed from the action of various methane producing bacteria which ferment propionic acid and other intermediates to acetic acid and methane. Approximately 72% of the methane is produced from the acetic acid (9). Some of the remainder is formed from the reduction of carbon dioxide. Hydrogen, which is removed from organic compounds by enzymes, reduces carbon dioxide to methane. Carbon dioxide functions as a hydrogen (or electron) acceptor just as oxygen does in aerobic systems (9). Thus for wastewater feeds, acetic acid appears to be the preferred intermediate to methane formation.

In fermentation of many substances such as glucose, cellulose, glycerol, and amino acids, pyruvic acid is a key intermediate from which acetic and propionic acids are then formed. However, volatile acids may also be formed directly from amino acids with subsequent ammonia formation, as shown in Figure 2.

Volatile acids can also be produced from fats which are hydrolyzed to glycerol and long-chain fatty acids such as stearic (18 carbons), and palmitic acids (16 carbons). The glycerol is formed from pyruvic acid through glycolysis and fermented to volatile acids; acetic acid is then formed from stearic acid and other long chain acids by beta-oxidation (5).

Methane formers require low oxidation-reduction potential (negative numbers), a pH in the range of 6.4-7.2, and bicarbonate alkalinity ranging from about 1000-5000 mg/liter (as CaCO_3) (5). Anaerobic treatment usually requires high temperatures; optimum temperature is 29-38°C (mesophilic) and 49-57°C (thermophilic). Vitamin B₁₂ and derivatives are required in methanogenesis and are usually abundant; cultures may often appear pink in color due to these vitamins.

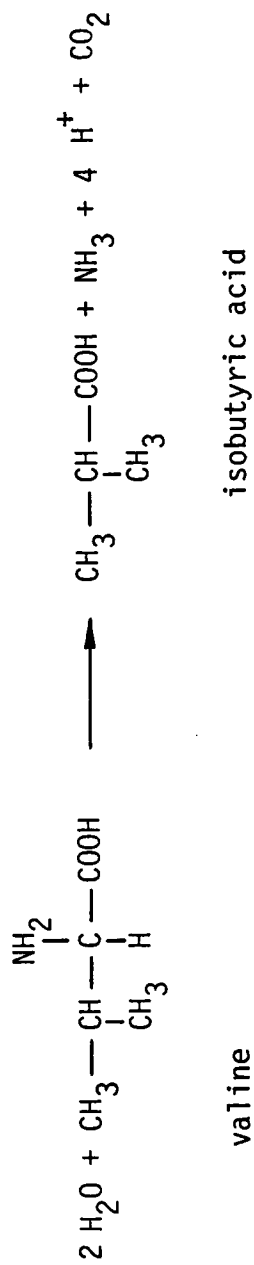
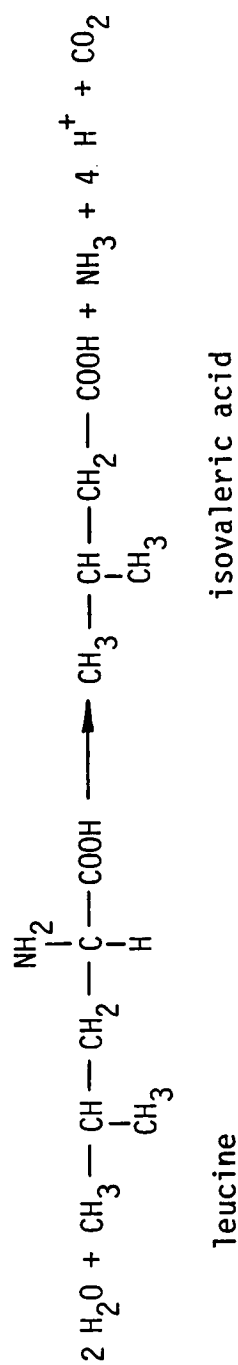


FIGURE 2. Volatile acids and ammonia formation from amino acids (5).

Methanogens are very sensitive to heavy metals and some salts; for example, sodium, potassium, magnesium, calcium, and ammonium salts, and sulfides can inhibit growth in high concentrations (9). Excessive concentrations of volatile acids and ammonia may be harmful (7). Inhibition is usually best indicated by a decrease in total gas production (in liters per day) and an increase in volatile acids concentration.

Volatile acids may be toxic in excess of 2000 mg/liter as acetic acid. The unionized form is more toxic even in the range of 30-50 mg/liter (5, 7). The pH will determine the degree of ionization of the acid. The charge may influence the ability of an acid to pass through cell membranes. Propionic acid is more toxic than acetic acid; propionic acid is used at a slower rate than acetic acid thus accumulation would indicate problems in steady state conditions. Effluent concentrations of volatile acids in anaerobic treatment range from 140-2500 mg/liter as acetic acid (5, 7, 14).

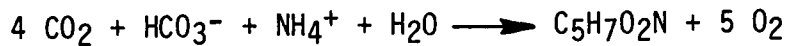
Theoretical methane production is estimated to be 5.62 ft³ of CH₄/lb BOD (0.35 m³ CH₄/kg BOD) converted as glucose (4). The heat value of 1 ft³ (0.028 m³) of methane is 960 Btu (1013 kJ) (9). If methane was used in pipelines as a substitute natural gas (SNG), it could contribute up to 20% of the annual natural gas consumption in the United States (7).

Anaerobic digestion stabilizes large volumes of organic slurries, kills pathogens, and the residue may be suitable as a soil conditioner (unless high in heavy metals) (14). Degradation of chlorinated hydrocarbons has been known to occur in anaerobic systems (15, 16).

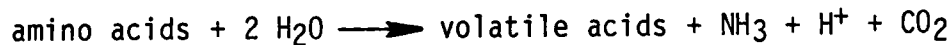
Ammonia Formation

As mentioned, ammonia is formed from the conversion of protein, or rather amino acids, to volatile acids, ammonia, and other products. Thus a waste high in protein, for example, wastewater containing human and animal wastes, would yield high ammonia. Some ammonia is used in cell synthesis but due to slow growth rates of methane-formers, a net accumulation of ammonia results.

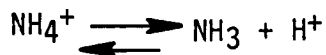
Cell synthesis:



Breakdown of protein:



Thus ammonia is inherent to anaerobic processes when organic-nitrogen containing material is available. Ammonia is usually found as an ion at pH normal to wastewater stabilization:



This equation shifts to the left at low pH and to the right at high pH. Free ammonia (NH_3) is inhibitory at a much lower concentration than the ammonium ion (NH_4^+). Ammonia-nitrogen has been found to be beneficial in the range of 50-200 mg/liter, no adverse effect in the range of 200-1000 mg/liter, inhibitory at higher pH in the range of 1500-3000 mg/liter, and toxic when greater than 3000 mg/liter (9). Another study found that a digester functioned when ammonia-nitrogen was as high as 3600 mg/liter in a pH range of 8.0-8.3 with a resulting free ammonia concentration of 350 mg/liter (7). As the ammonia-nitrogen concentration increased to greater than 5000 mg/liter, inhibition

occurred as noted by a decrease in gas production; as the ammonia-nitrogen concentration reached 6700 mg/liter, gas production ceased. Volatile acids ranged between 2000 and 6000 mg/liter.

Ammonia-nitrogen combines with carbon dioxide and water to form ammonium bicarbonate alkalinity. This extra alkalinity helps to buffer the anerobic system and to withstand transient phenomena. The resulting high pH will offset the low pH due to volatile acid formation.

Ammonia-associated alkalinities were found to be 80% or more of the total alkalinity indicating the importance of ammonia formation during organic-nitrogen catabolism for the production of necessary buffer. A decrease in organic-nitrogen conversion to ammonia results in a decrease of alkalinity production of buffering. A chemical addition did not cause any toxic effects, but reduced the biodegradability of certain organic materials, especially those with nitrogen. This reduction decreased ammonia production which decreased alkalinity and reduced pH buffering capacity (17).

The production of ammonia-nitrogen was shown to be decreased with the addition of alum, ferric chloride, or organic coagulants which were added to make the wastes more digestible (compared to wastes without the additives) (6).

Ammonia-nitrogen in the effluent of anaerobic treatment systems has been found to range from 50-6000 mg/liter (as $\text{NH}_3\text{-N}$) (5, 7, 9, 10, 14, 17, 18). High concentrations of ammonia usually indicate digester failure, for example, in the range of 1200-1400 mg/liter $\text{NH}_3\text{-N}$, but this was a result due to high solids loadings and subsequent excessive volatile acids (19-20). A recent study indicated that acclimation occurred when

ammonia-nitrogen ranged from around 600 to greater than 3000 mg/liter ($\text{NH}_3\text{-N}$) as indicated by a slow decrease in methane production rate (10).

When volatile acids are greater than 2000-6000 mg/liter, toxicity occurs associated with the salt, sodium acetate, rather than the straight acetic acid. Although ammonium bicarbonate alkalinity may buffer the subsequent low pH, a high concentration may create ammonium ion toxicity. Ammonium acetate may form which results in a decreased rate of acetic acid utilization by methane-formers. Toxicity at high volatile acids concentrations is associated with salt toxicity and dependent on the cations rather than volatile acid anions. But these high concentrations are usually a result rather than a cause of digester failure (18).

Anaerobic Filter

The upflow anaerobic filter was first introduced in 1969 by Young and McCarty for treatment of low strength soluble wastes (11). The mechanism is similar to aerobic trickling filters. Some of the advantages of the anaerobic filter include its suitability for soluble wastes, no effluent or solids recycle is required because the biological solids remain in the filter, the accumulation of high concentrations of active solids in the filter permit treatment of dilute wastes without heating, and very low volumes of sludge are produced, thus the effluent is essentially free of suspended solids.

The synthetic organic waste feed used by Young and McCarty consisted of glucose (carbon source) and nutrient broth (almost pure protein) in equal COD amounts; sodium bicarbonate was added for alkalinity. The organic loadings ranged from 26.5-212 lbs COD/1000 ft³/d

(.425-3.4 kg/m³/d) of total filter volume (including volume of stone). This is about the maximum range used with other biological processes such as activated sludge, trickling filter, and other anaerobic contact processes. The hydraulic retention time (HRT) varies from 4.5-72 hours. Table 1 gives a summary of the experimental design and results of the study by Young and McCarty.

Profiles showed COD removal within 2-2.5 ft (0.61-0.76 m) in the six-foot (1.83 m) column for runs 1 through 4. At the lower loadings, volatile acids were converted to methane gas almost as rapidly as they were formed. At higher loadings, up to 600 mg/liter volatile acids were observed at the lower portion of the column. Efficiencies of treatment decreased with an increase in OLR.

It was observed that solids did not become attached readily to surfaces but lay loosely in interstitial spaces. Low biological synthesis permitted long periods of operation before sludge wasting.

The anaerobic filter compared favorable with other treatment processes. Loadings reported for activated sludge ranged from 20-150 lbs BOD/1000 ft³/d (0.32-2.5 kg/m³/d) with 60-90% BOD removal. Trickling filter loadings ranged from 10-200 lbs BOD/1000 ft³/d (0.16-3.2 kg/m³/d) of filter volume. BOD loadings to anaerobic processes often do not exceed 200 lbs BOD₅/1000 ft³/d (3.2 kg/m³/d).

The anaerobic filter readily accepted shock loads, especially of short duration; effluent quality did not change appreciably. The filter showed suitability for treatment of intermittent wastes since active solids remain in the future. Soluble wastes are best treated since solids may clog the column. The slow rate of anaerobic growth at low COD concentrations may limit use of anaerobic filters for treating

Table 1. Experimental Design and Results of Original Anaerobic Filter Study (11)

Phase No.	1	2	3	4	5	6
Chemical Oxygen Demand (COD) COD influent mg/liter	3000	3000	1500	1500	1500	1500
Organic Loading Rate (OLR) lbs COD/1000 ft ³ /d (kg/m ³ /d)	26.5 .42	53 .85	26.5 .42	53 .85	106 1.7	212 3.4
Gas Production liter/d	5	--	5	10-12	20	25
COD Effluent mg/liter	170	280	112	110	315	600
COD Removal Percent	93	89	92	91	79	60
Hydraulic Retention Time (HRT) hours	72	36	36	18	9	--
Suspended Solids mg/liter	24	48	9	6	--	4.5
Volatiles Acids mg/liter	18	78	15	22	--	--

very low strength wastes; the anaerobic filter appeared to operated best at strengths under 1000 mg/liter of COD.

Other studies have shown anaerobic filters to be effective for low strength wastes of COD less than 600 mg/liter at a high OLR of 500 lbs COD/1000 ft³/d (8 kg COD/m³/d) and a HRT of several hours (21).

In another study, high COD concentrations of 3800-9400 mg/liter were used with OLRs ranging between 27-142 lbs COD/1000 ft³/d (0.43-2.27 kg COD/m³/d); HRT was 10.5-21.1 hours. COD ranged between 68-99%. Effluent volatile acids were 10-60 mg/liter as acetic acid. Gas production was 4.0-7.3 ft³/lb COD (250-455 liters/kg COD) with 65-80% CH₄. With the ammonium bicarbonate alkalinity formed, no additional buffers were needed; effluent alkalinity varied from 335-850 mg/liter (as CaCO₃). It was concluded that anaerobic filters are more cost effective at high strength wastes of BOD greater than 1000-1500 mg/liter (22).

Anaerobic filters have been used for extremely high COD concentrations of 37,000-2,000,000 mg/liter with HRT of 120-840 hours resulting in OLR of 108 lbs COD/1000 ft³/d (1.7 kg COD/m³/d). COD removal ranged from 75-83% (8).

A completely mixed anaerobic filter has been studied in contrast to packed-bed reactors. Vertical plates were used for bio-film attachment. This mechanism would apparently reduce head loss and clogging. This column used the entire length for substrate removal. It was thought that less buffering would be needed in this system. OLR was 440 lbs COD/1000 ft³/d (7 kg COD/m³/d). Percent removal of COD was said to be a function of HLR and depth of filters at a constant

influent concentration. This would indicate first-order reaction kinetics. Percent removal of COD was higher at the higher OLR, but effluent COD was also higher (23).

Ammonia Removal

Ammonia discharged into receiving waters will cause a high oxygen demand as the ammonia is converted into nitrate. The depletion in oxygen is detrimental to aquatic systems; low concentrations of ammonia are toxic to certain fish. Wastewater with 20 mg/liter of $\text{NH}_3\text{-N}$ has a nitrogenous oxygen demand (NOD) of 87 mg/liter (4, 24). When nitrogen is combined with phosphorus, eutrophication is enhanced. Therefore, it is necessary to remove ammonia from wastewater treatment effluents. Industrial processes may result in extremely high concentrations of ammonia which would be suitable for recovery.

Various treatment processes are used for conventional ammonia removal: biological nitrification (and nitrification-denitrification), bacterial assimilation and harvesting, breakpoint chlorination, selective ion exchange for ammonia, ammonia stripping, reverse osmosis, and land application (4). Biological processes and land application are more economical than chemical processes and physical operations; 60-90% removal of nitrogen can be obtained with these methods.

Some of the ammonia formed is used in cell synthesis with the rest being converted to nitrate:

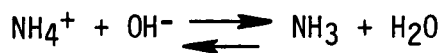


Nitrification occurs in two steps by nitrifying autotrophic bacteria: Nitrosomonas sp. convert ammonium ion to nitrite and Nitrobacter sp.

convert nitrite to nitrate. Carbon dioxide or bicarbonate ion is used as the carbon source and oxygen must be supplied as the electron acceptor.

Denitrification requires anaerobic conditions and an external carbon source; nitrate is reduced since it is used as an electron acceptor. The principal genera responsible for denitrification include Pseudomonas, Micrococcus, Achromobacter, and Bacillus (4).

In high concentrations, recovery and recycle of ammonia may be worthwhile. Desorption by lime addition causes ammonia gas formation:



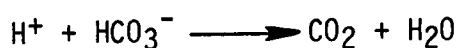
Subsequent absorption in phosphoric acid will form an ammonium salt. This solution of ammonia and phosphoric acid at a neutral pH is believed to be valuable as fertilizer (25).

Biological nitrification-denitrification can occur during suspended-growth or attached-growth processes. Attached film mechanisms include fluidized-bed reactors (FBR) using sand or fine particles of coal as the support material, rotating biological contactors (RBC), and packed-bed reactors (PBR) using plastic or ceramic materials or rocks as support for attachment (4, 6, 10). Attached growth allows long cell retention times and less cell washout.

Nitrification with submerged filters was patterned after the original anaerobic filter and later used for denitrification (11, 26, 27). The upward flow allows control of hydraulic retention time. Nitrifying bacteria grow on the surfaces of the stones, and long solids retention times (θ_c) are possible. In the original study, the submerged filter was able to achieve rapid and stable nitrification at temperatures as low as 1°C (26). During nitrification, cells would contain about 2% of

the original ammonium ion. The nitrifiers have long generation times (10-30 hours) which are hard to achieve in activated sludge or trickling filters; also HRT is hard to control in trickling filters. The rate of ammonia oxidized is greatest near the influent of the upflow submerged filter where ammonia concentration is greatest. The sum of $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$ was constant because no significant sources or sinks of nitrogen occurred within the filter (26).

Hydrogen produced during nitrification will be neutralized by bicarbonate:



This decrease in bicarbonate alkalinity and increase in carbon dioxide lowers the pH. To neutralize hydrogen ions produced during oxidation of 1 mg of $\text{NH}_3\text{-N}$, 7.13 mg of bicarbonate alkalinity (as CaCO_3) is required. About 4.6 mg of oxygen is used for each milligram of $\text{NH}_3\text{-N}$ oxidized to nitrate. The submerged filter demonstrated rapid restarting after short periods of inoperation and proved to be effective for secondary effluents (ammonia-nitrogen concentrations were around 30 mg/liter) (26).

Other studies of PBRs showed successful ammonia removal greater than 90% (influent $\text{NH}_3\text{-N}$ was around 15 mg/liter). Hydraulic surges did not cause extreme loss of organisms. Sand, crushed coal, and loosely packed plastic rings performed at about the same removal rates (28).

Studies of single-stage nitrification, similar to activated sludge, showed 90% ammonia-nitrogen removal with HRT of 10 hours and solids retention time (SRT) of 10 days (29).

FBRs have been used for both BOD and nitrogen removal. Sand was used for media growth which creates enormous surface area. The need for

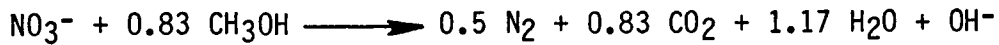
secondary clarifiers is eliminated when attached growth mechanisms are used. Ninety-nine percent ammonia-nitrogen removal was achieved (influent concentration was 20 mg/liter $\text{NH}_3\text{-N}$). Savings in land usage and time required were obtained (30).

Nitrification-denitrification can also be accomplished in looped channels similar to oxidation ditches or stages series of activated sludge-type reactors. SRT is controlled by recycling of effluent (31).

Nitrite formed during nitrification enhances eutrophication and may also cause methemoglobinemia in infants. Regulations are imposed for nitrate discharge into receiving waters. Denitrification reduces nitrate to nitrogen gas which is considered harmless. Nitrification-denitrification is most commonly employed for ammonia removal. Many studies have focused on operation and processes of denitrification (4, 6, 8, 10, 27, 30, 32-34, 40). Numerous other investigations have sought a suitable carbon source other than methanol which is widely used in denitrification because of its low cost compared to other carbon sources, for example, acetic acid (8, 35-39, 41).

FBRs have shown 99% removal of nitrate (30). As previously mentioned, anaerobic PBRs have been used for denitrification of nitrate concentrations around 20 mg/liter. It was concluded that the media must be greater than one inch (2.54 cm) to reduce head losses; also backwashing was not needed (27). Upflow reactors without a medium for attachment have shown around 60% removal (32). Other studies have indicated preference of FBR over PBR stating that PBRs have lead loss, clogging problems, and backwashing required. The methanol to nitrate ratio can be hard to control (33). RBCs have also shown high removal rates of nitrate (6, 10).

Methanol is used as a carbon source because it is less expensive than other carbon sources. Methanol requirements can be determined from equations of reactions such as:



When 1 mg of $\text{NO}_3\text{-N}$ is reduced to N_2 , 3.57 mg of alkalinity (as CaCO_3) is produced; this helps restore the alkalinity lost in nitrification (34).

McCarty's equation is used for determining required methanol (4):

$$C_m = 2.47 N_0 + 1.53 N_i + 0.87 \text{ DO}$$

where C_m equals milligram per liter of methanol; N_0 equals nitrate concentration; N_i equals nitrite concentration; and DO equals dissolved oxygen concentration (all in milligrams per liter). Other findings state that three times as much organic carbon is needed per nitrate, and that denitrification occurs as a first order reaction (6).

Denitrification is a respiratory mechanism in which nitrate replaces oxygen as an electron acceptor. This is in contrast to nitrate assimilation where nitrate is reduced to ammonia and used in cell protein synthesis. Acetic acid showed the highest percent nitrogen removal at a shorter time when used as a carbon source (41). Acetic acid is the preferred carbon source of *Pseudomonads* (42) but is the most expensive (41).

Methane as a carbon source has been investigated. Several bacteria have been found to be capable of growth and denitrification with methane as sole carbon source, including *Pseudomonas* and *Bacillus*. However, *Micrococcus*, did not grow. Fifty percent denitrification was achieved (35). It was generally concluded that use of methane as a carbon source was not feasible because of the very slow rate of denitrification (8, 36, 38).

III. EXPERIMENTAL DESIGN AND METHODS

The ANFLOW bioreactor and feed composition used in this study were patterned after the original anaerobic filter research by Young and McCarty (11). The ANFLOW bioreactor was a six-foot (1.8) plexiglass column, eight inches (20.3 cm) in diameter. Sample ports were placed at one-half foot (15.24 cm) intervals along the length of the column. The packing materials used were one-inch (2.54 cm) unglazed, ceramic Raschig rings. Hydraulic behavior in the ANFLOW bioreactor was approximately plug flow. Diagrams of the ANFLOW and aerobic (used for nitrification) bioreactors are shown in Figure 3.

The synthetic feed used contained nutrient broth as a source of protein and trace minerals, glucose as a carbon source, and sodium bicarbonate for buffering. The nutrient broth and glucose each contributed one-half of the total soluble chemical oxygen demand (SCOD). Evaluation of feed composition was based on SCOD. It was found that 1.68 mg/liter of nutrient broth and 1.39 mg/liter of glucose resulted in a SCOD of approximately 3000 mg/liter; 3.36 mg/liter of sodium bicarbonate gave alkalinity of approximately 2000 mg/liter (as CaCO_3).

Initially feed was made once a week in a large stainless steel tank kept in a 4°C cold room. This feed was pumped to a small tank containing a heat coil to warm the feed to approximately 25°C; the warmed feed was then pumped into the ANFLOW bioreactor. During the latter phases of the project, and as summer months approached, feed was made everyday and pumped directly into the ANFLOW bioreactor.

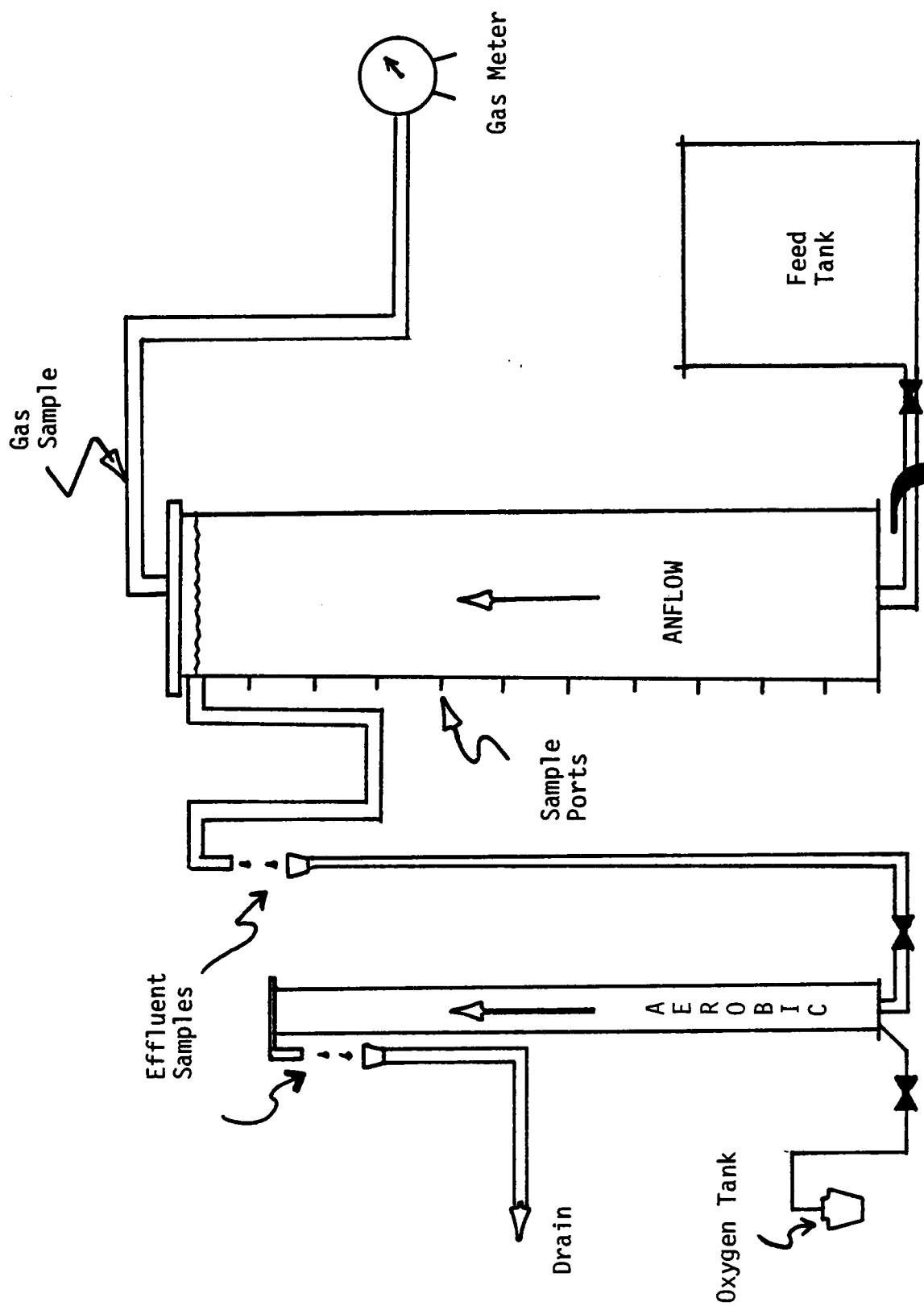


FIGURE 3. Diagram of ANFLOW and Aerobic bioreactors.

Ambient temperatures often exceeded 30°C enhancing cell growth in the feed container and promoting treatment. Thus, it was necessary to make fresh feed daily.

Gas production, percentage methane of the off-gas, pH alkalinity, volatile acids, and SCOD were used to monitor perturbations of steady-state conditions. Mass balances of nitrogen were determined by analyzing ammonia-nitrogen, nitrate+nitrite-nitrogen, and total Kjeldahl nitrogen (TKN).

Axial ammonia profiles, gas production rates and composition, pH and alkalinity, and the SCOD of influent and effluent were measured almost daily. The SCOD profiles, volatile acid profiles, TKN, and nitrate+nitrite-nitrogen were usually measured twice during each experimental phase.

Ammonia was measured with the Orion selective ion electrode (44). Nitrate+nitrite-nitrogen was measured by the cadmium reduction method (44). TKN was analyzed by digestion and steam distillation and measurement of ammonia (43).

The pH was measured with an Orion electrode. Bicarbonate alkalinity (as CaCO_3) was measured according to Standard Methods (43) using the pH electrode.

SCOD was determined using the USEPA approved standard ampule method from Oceanography International Corporation. The samples were centrifuged and the soluble fraction was then analyzed.

Volatile fatty acids were analyzed using a Shimadzu mini-gas chromatograph with a flame-ionization detector (FID). Samples were

also centrifuged and a portion of the soluble fraction was injected directly into the gas chromatograph. Aqueous samples without extraction can be used with the FID. Column packing used for volatile fatty acids was Supelco (brand) 3% Carbowax 20 M/0.5% phosphoric acid on 60/80 Carbopack B.

Composition of the off-gas was determined using a Bendix portable gas chromatograph with a thermal conductivity detector. Gas samples were taken in the rubber tubing leading from the top of the ANFLOW bioreactor to the wet gas meter. A syringe and needle was used to collect the sample and then injected directly into the gas chromatograph. A column packed with Supelco (brand) porapak Q was used routinely to analyze methane and carbon dioxide. Three peaks were resolved using this column: methane, carbon dioxide, and one other component which consisted of any other gases (notably nitrogen and any oxygen). This other component was termed "other" gases. Occasionally a column packed with Supelco (brand) molecular seive was used which separated only oxygen, nitrogen, and methane. Thus the "other" gases was found to contain only nitrogen and small amounts of oxygen.

Dissolved oxygen (DO) was measured occasionally using a YSI oxygen meter. Samples from the ANFLOW column were checked to observe anaerobic conditions, and samples from the aerobic bioreactor were checked for aerobic conditions.

Two hydraulic loading rates, 0.01 and 0.02 gpm/ft² (HLR = 0.0068 and 0.0136 liter/m²·s), and three SCOD concentrations (3000, 1500, and 750 mg/liter) were used resulting in a three-by-two experimental design matrix. The resulting OLRs in this experimental program ranged from

27-216 lbs COD/1000 ft³/d (0.43-3.50 kg/m³·d). Typical CODs of untreated domestic wastewater range from 250-1000 mg/liter (4). The experimental design matrix including OLRs is given in Table 2.

Data were collected for each phase over a period of about four weeks allowing about one week in between phases to reach steady-state conditions. When gas production rates and SCOD concentrations of the ANFLOW bioreactor effluent were constant, it was assumed that steady-state had been reached. The chronological order of study actually occurred as phase 1, phase 3, phase 4, phase 6, phase 5, and finally phase 2.

Pulse tests using fluorescein dye were run on a control ANFLOW bioreactor at HLR = 0.01 gpm/ft² (0.0068 liter/m²·s) to determine a hydraulic residence time (HRT). The void fraction was found to be 0.42. This was used to calculate HRT in the experimental ANFLOW bioreactor used in this study.

The aerobic bioreactor was a five-foot (1.52 m) plexiglass column, 1.75 inches (4.45 cm) in diameter, packed with 3/4 inch (1.91 cm) plastic Pall rings. This part of the study was patterned after the research by Haug and McCarty (26). Effluent from the ANFLOW bioreactor fed directly into the aerobic bioreactor. Pure oxygen entered through spargers at the influent. The void fraction was found to be 0.89 by measuring liquid volume with and without plastic ring packing (before biological film growth). Ammonia effluent concentrations from the aerobic bioreactor were measured daily; nitrate+nitrite-nitrogen, TKN, and DO were measured occasionally. Only effluent samples were taken. This nitrification study was initiated during phase 3.

Table 2. Experimental Design Matrix

		Hydraulic Loading Rate gpm/ft ² (liter/m ² ·s)	
		.01 (.007)	.02 (.014)
Chemical Oxygen Demand mg/liter	3000	Phase 1 108 (1.7)	Phase 2 216 (3.5)
		Phase 3 54 (.86)	Phase 4 108 (1.7)
	1500	Phase 5 27 (.43)	Phase 6 54 (.86)
	750		

Numbers inside boxes are organic loading rates in pounds of COD/1000 ft³/d (kg/m³·d).

The aerobic bioreactor was seeded with activated sludge from a nearby wastewater treatment plant. Fixed-films in the ANFLOW bioreactor had been established with heavy seeding prior to the initiation of this study.

IV. RESULTS AND DISCUSSION

The actual SCOD, HLR, and OLR values differed from the proposed conditions due to experimental variations in flow rate and in composition of weekly and/or daily batches of synthetic feed. The actual average loading rates and influent SCOD concentrations are given in Table 3 which includes a summary of all results. Appendixes A through G contain data from all six experimental phases. Calculations for certain parameters are given in Appendix H. Routine analyses ($\text{NH}_3\text{-N}$, influent and effluent SCOD, gas production and composition, pH, alkalinity, and flow rate) are given in the Appendixes with statistical information of average standard deviation and range. Non-routine analyses [SCOD profiles, volatile acids profiles, total Kjeldahl nitrogen (TKN), nitrate+nitrite-nitrogen] were performed usually on consecutive days (twice per phase). Values were found to be within 5% of the previous days results. These values were averaged but statistical analyses were not performed. Duplicates were analyzed for TKN and were always within 1%.

Feed was initially kept in a large feed tank inside a 4°C cold room and pumped to a small container with a water-heated coil (25°C) and from here pumped to the ANFLOW bioreactor. This feed was made weekly; during the maintenance period, the pump line and feed lines were cleaned with a caustic solution. Biological treatment was occurring in the heated feed as was observed by a decrease in SCOD from the cold room feed tank to the heated feed; pH also decreased as volatile acids increased. During phase 4 and for the remainder of the phases

Table 3. Summary of Average Results of ANFLOW and Aerobic Bioreactors*

Phase No.	1	2	3	4	5	6
Organic Loading Rate (OLR) lbs COD/1000 ft ³ /d (kg/m ³ ·d)	84.5 (1.35)	189 (3.03)	49.2 (0.79)	86.9 (1.39)	23.5 (0.38)	45.7 (0.73)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² ·s)	0.007 (0.005)	0.015 (0.010)	0.008 (0.005)	0.016 (0.011)	0.008 (0.005)	0.015 (0.010)
Aerobic HLR, gpm/ft ² (liter/m ² ·s)	0.147 (0.100)	0.316 (0.215)	0.164 (0.112)	0.340 (0.231)	0.163 (0.111)	0.317 (0.215)
Hydraulic Residence Time (HRT) Hours	≈41	≈20	≈41	≈20	≈41	≈20
Aerobic HRT, hours	≈3	≈1.5	≈3	≈1.5	≈3	≈1.5
Soluble Chemical Oxygen Demand (SCOD)	2500	2620	1310	1236	631	632
Influent, mg/liter	167	156	121	106	70	78
Effluent, mg/liter	93	94	87.5	91	89	88
Percent Removal	31.4	70.9	17.8	35.0	8.32	16.0
Removal Rate, grams/day	--	167	166	128	64	58
Aerobic Effluent, mg/liter						

Table 3. Continued

Phase No.	1	2	3	4	5	6
Ammonia-nitrogen Influent, mg/liter	14	2	10	13	3	10
Effluent, mg/liter	217	179	136	101	76	65
(range)	(160-341)	(141-250)	(104-183)	(63-122)	(56-90)	(54-90)
Formation, grams/day	2.9	5.2	2.2	3.1	1.1	1.9
Aerobic Effluent, mg/liter	168	35	11	18	3	9
ANFLOW to Aerobic Percent Reduction	23	80	92	82	96	86
Gas Production, liters/day	19.9	43.4	9.6	15.9	4.41	8.32
Percent Methane	71.7	66.2	78.5	76.7	78.0	78.6
Percent Carbon Dioxide	25.7	31.9	17.4	17.2	14.8	14.1
Percent Other Gases	2.6	1.8	4.1	6.1	7.2	7.3
ft ³ CH ₄ /1g COD Removed (m ³ CH ₄ /kg COD removed)	7.3 (0.46)	6.5 (0.41)	6.8 (0.42)	5.6 (0.35)	6.6 (0.41)	6.6 (0.41)
pH--Influent	6.5	6.7	7.3	7.0	7.5	7.5
Effluent	7.2	7.3	7.2	7.1	7.5	7.4
Aerobic Effluent	8.0	7.2	7.2	7.2	7.6	7.8

Table 3. Continued

Phase No.	1	2	3	4	5	6
Bicarbonate Alkalinity						
mg/liter as CaCO ₃						
Influent	1850	1865	2000	1350	1960	2400
Effluent	3200	2930	3000	1750	2370	2500
Amount Increase	1350	1065	1000	400	410	100
Theoretical Increase From Literature (17)	775	639	486	361	271	232
Aerobic Effluent	--	2000	1800	1000	1925	2210
Amount Decrease	--	930	1200	750	445	290
Theoretical Decrease From Literature (4)	--	1023	814	592	520	399

*Results pertain to ANFLOW bioreactor except where Aerobic (bioreactor) is noted.

(6, 5, and 2), feed was made every day and fed directly to the ANFLOW bioreactor without heat exchange. During phase 1, the detention time in the heat exchange unit was very long; a level control was used to pump fresh feed from the cold room and only small amounts were pumped. During phase 3, the level control was adjusted to empty the heat exchange unit before fresh feed was pumped. This shortened the detention time and reduced the amount of treatment occurring in the heat exchange feed unit and feed lines. The pH values for the influent, effluent, and aerobic bioreactor effluent are given in Table 3; the low influent pH during phase 1 reflects the treatment occurring in the feed system.

Bicarbonate alkalinity. Alkalinity was measured in the feed to ensure proper buffering. Alkalinity was found to increase in the ANFLOW bioreactor due to ammonium bicarbonate alkalinity. The highest increase occurred during phase 1 where ammonia concentration was highest. These results are given in Table 3. Each subsequent phase resulted in decreasing ammonia concentration and decreasing alkalinity percentage increase. As mentioned in the literature review (17), one mole of ammonia (14 g $\text{NH}_3\text{-N}$) will form one equivalent (50 g) of bicarbonate alkalinity. These calculated values are also given in Table 3 with the resulting bicarbonate alkalinity found in this study. The values from this study were higher except for phase 6.

During nitrification, alkalinity (as CaCO_3) is decreased as stated in the literature (4). The requirement is 7.13 mg of alkalinity (as CaCO_3) to neutralize oxidation of 1 mg of $\text{NH}_3\text{-N}$. These calculated values are given in Table 3 with the actual values found in this study. Wide differences were observed during phases 2 and 3.

Overall, bicarbonate alkalinity (as CaCO_3) was always found to increase in the ANFLOW bioreactor due to formation of ammonium alkalinity. A decrease in bicarbonate alkalinity (as CaCO_3) was always observed in the aerobic bioreactor due to required neutralization of ammonia oxidation. As mentioned in the literature review, hydrogen is produced during nitrification which is neutralized by bicarbonate, thus reducing the alkalinity.

Soluble chemical oxygen demand (SCOD). The removal of SCOD ranged between 87.5 and 94%, as given in Table 3. Best removal occurred at the highest OLR. ANFLOW bioreactor effluent SCOD concentrations were lowest at the lower OLRs, but overall efficiency was reduced.

The effect of influent SCOD on SCOD removal (grams/day) is illustrated in Figure 4. Removal rates are higher at the higher flow rate. This effect was enhanced as influent SCOD concentration increased. The effect of OLR on effluent SCOD concentration is shown in Figure 5; at the higher flow rate, the rate of increase of effluent SCOD was less as the OLR increased. Figure 5 is of particular interest if effluent quality of a certain concentration is desired, that is, at the higher flow rate, a lesser concentration of SCOD is predicted. However, if overall bioreactor efficiency is observed, the removal of SCOD (grams/day) is linear as a function of OLR, as shown in Figure 6. The removal rate, in terms of grams per day, versus the ORL becomes normalized as the flow rate becomes canceled in both terms, thus the function becomes linearized.

The SCOD removal in the aerobic bioreactor was reduced only slightly during phase numbers 5 and 6, given in Table 3. During the

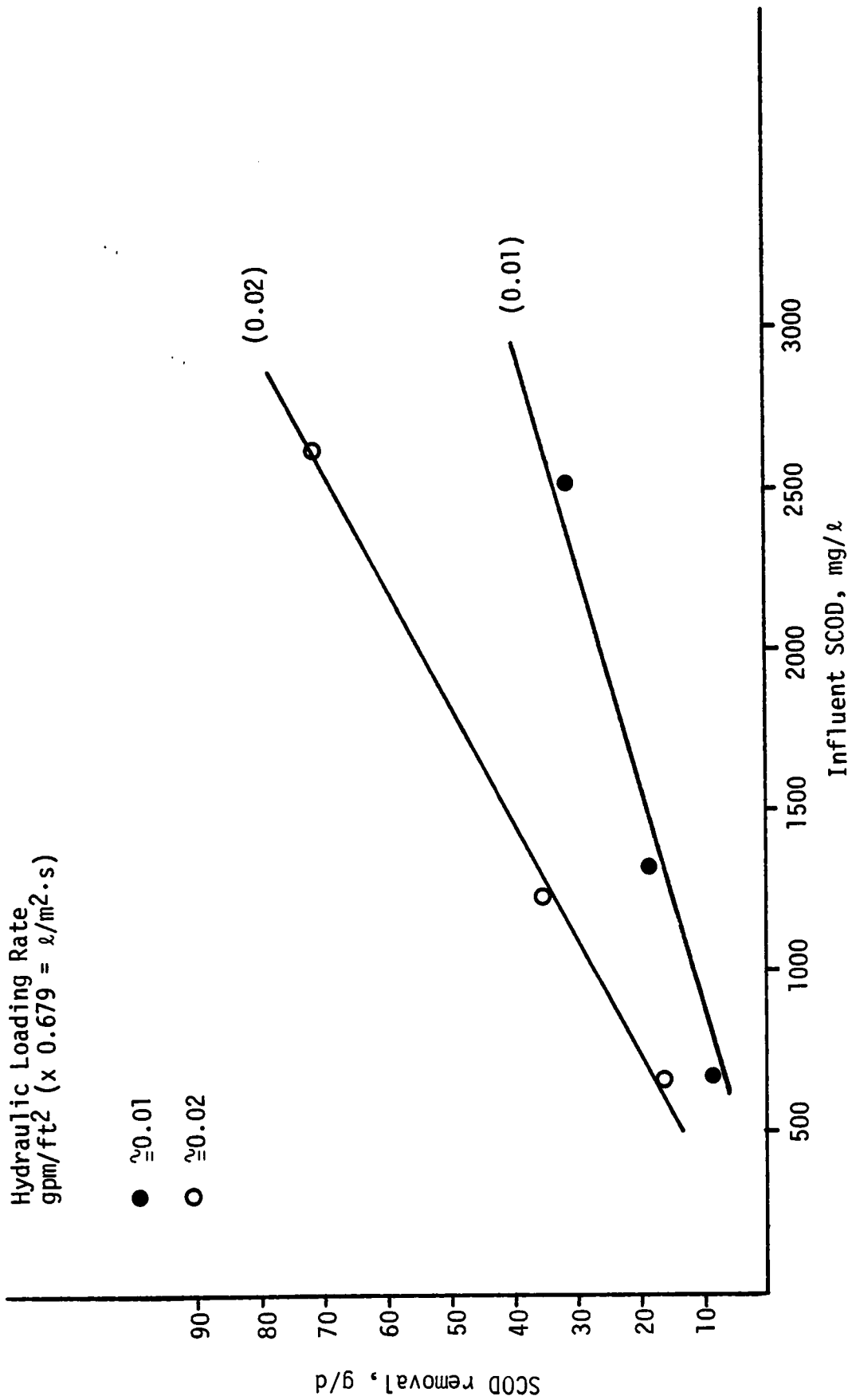


FIGURE 4. Effect of influent soluble chemical oxygen demand (SCOD) on ANFLOW bioreactor SCOD removal.

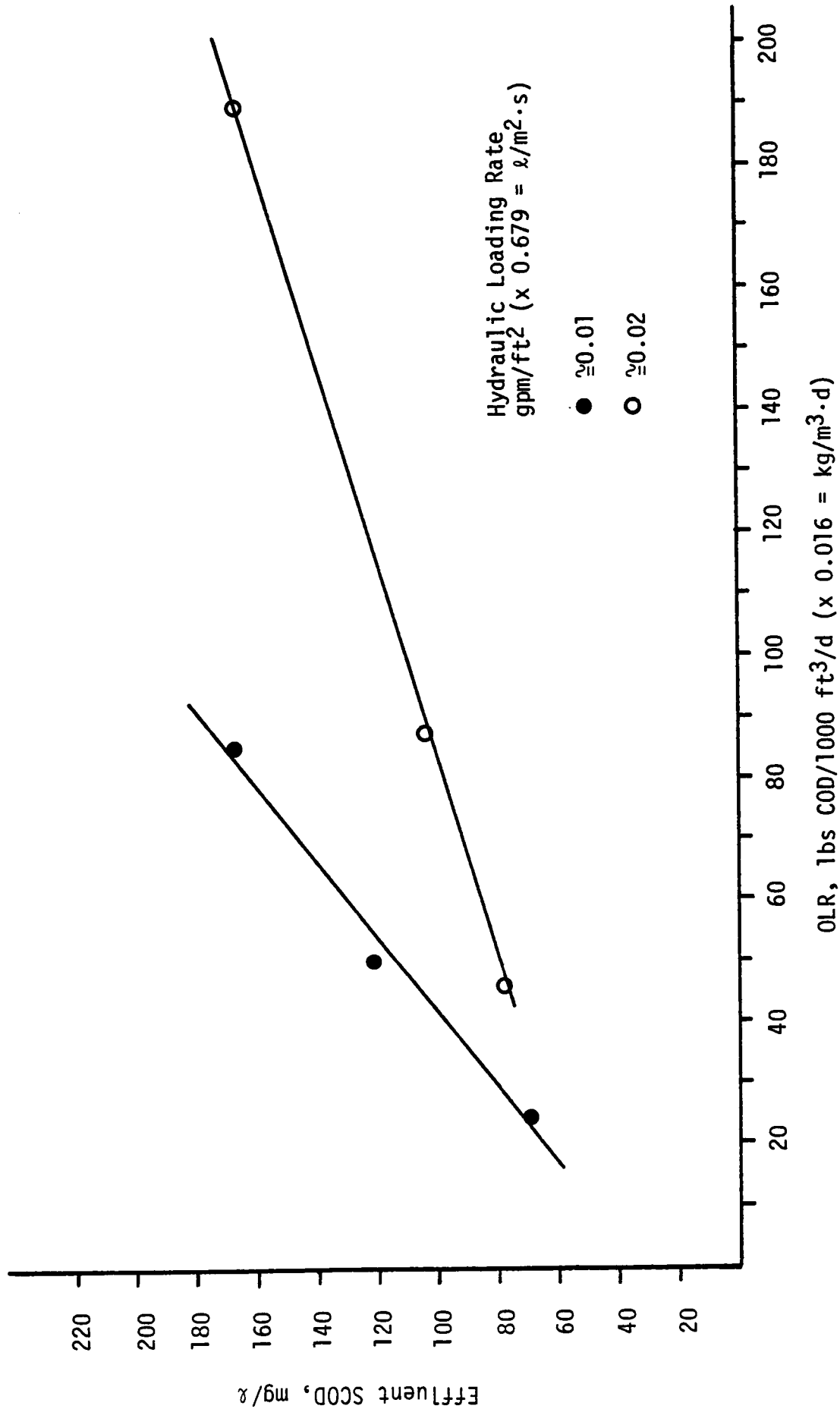


FIGURE 5. Effect of organic loading rate (OLR) on effluent soluble chemical oxygen demand (SCOD).

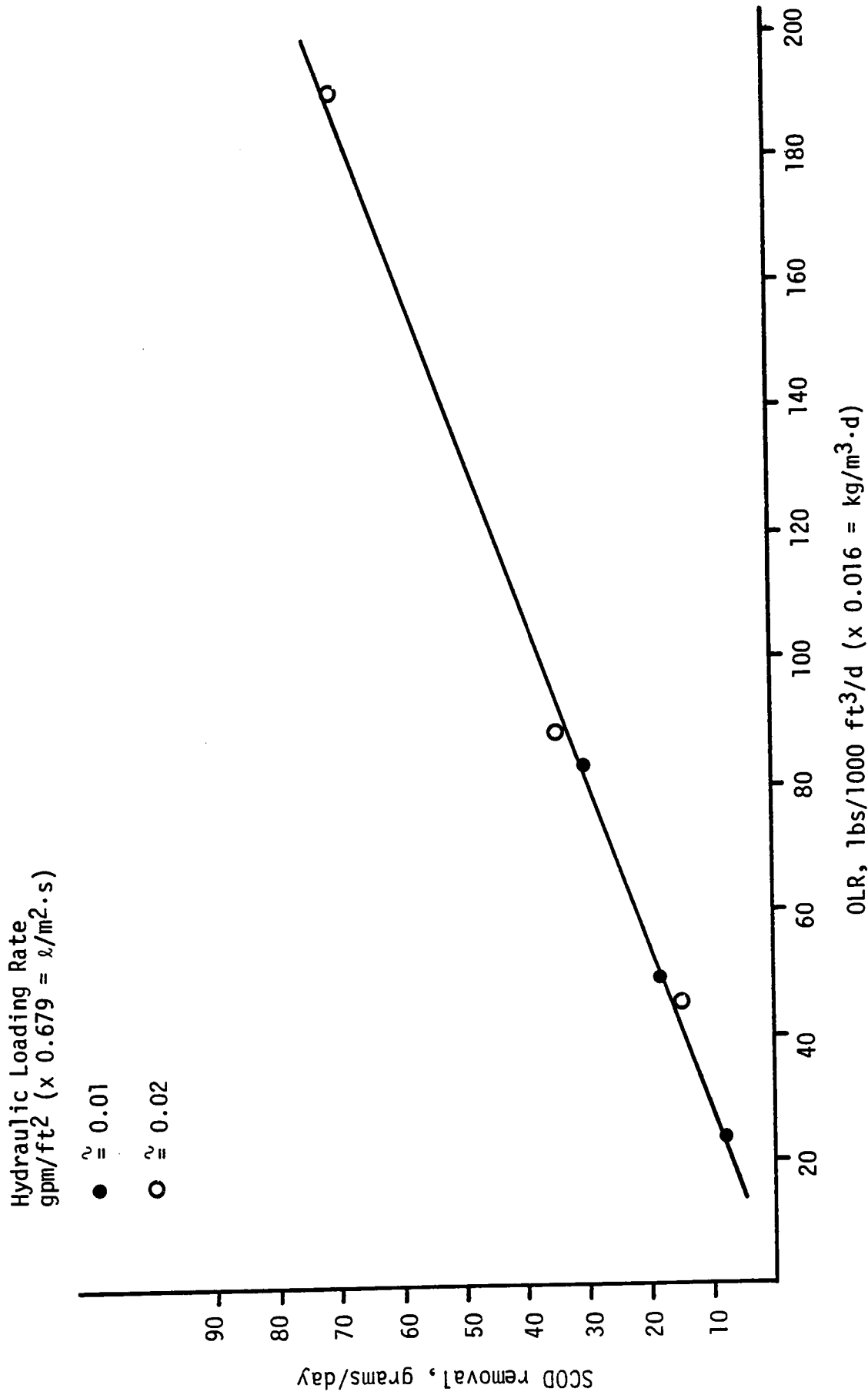


FIGURE 6. Effect of organic loading rate (OLR) on ANFLOW bioreactor soluble chemical oxygen demand (SCOD) removal.

other phases, SCOD actually increased within the aerobic bioreactor. The aerobic bioreactor was shown not to be significant in carbon removal.

Twice per phase, SCOD was measured at sample ports along the ANFLOW bioreactor to observe regions of SCOD removal. These profiles are illustrated in Figure 7. Actual values are given in Appendix A. It was observed that most SCOD removal occurs by 1.5 to 2.0 feet within the ANFLOW bioreactor. During phase 2, the highest OLR used in this study, the profile was shifted so that removal was not essentially completed until a height of 3.0 feet; after this point, removal was essentially the same. These profiles show that the ANFLOW bioreactor has not exhausted its capacity for SCOD removal at the OLRs used in this study.

Gas production. Overall gas production ranged from 4.4 liters/day (the lowest OLR) to 43.4 liters/day (the highest OLR), as seen in Table 3, page 28. When observing similar influent SCOD concentrations, gas production was approximately doubled as the flow rate doubled, as shown in Figure 8. However, when observing total bioreactor efficiency, gas production was linear as a function of OLR as illustrated in Figure 9.

The cubic feet of methane produced per pound of SCOD removed ranged from 5.6 to 7.3 (0.35 to $0.46 \text{ m}^3 \text{ CH}_4/\text{kg SCOD removed}$). The theoretical amount of methane produced per pound of ultimate biochemical oxygen demand (BOD_L) stabilized is 5.6 cubic feet ($0.35 \text{ m}^3 \text{ CH}_4/\text{kg BOD}_L$) (4). The conversion of BOD_L to methane gas is determined by calculating the amount of methane produced per pound of BOD_L stabilized, assuming that the starting compound is glucose ($\text{C}_6\text{H}_{12}\text{O}_6$). Under anaerobic conditions, glucose is converted to carbon dioxide and methane; the methane has an oxygen requirement for complete conversion to carbon dioxide and water. Then using molecular ratios, the ratio of the amount

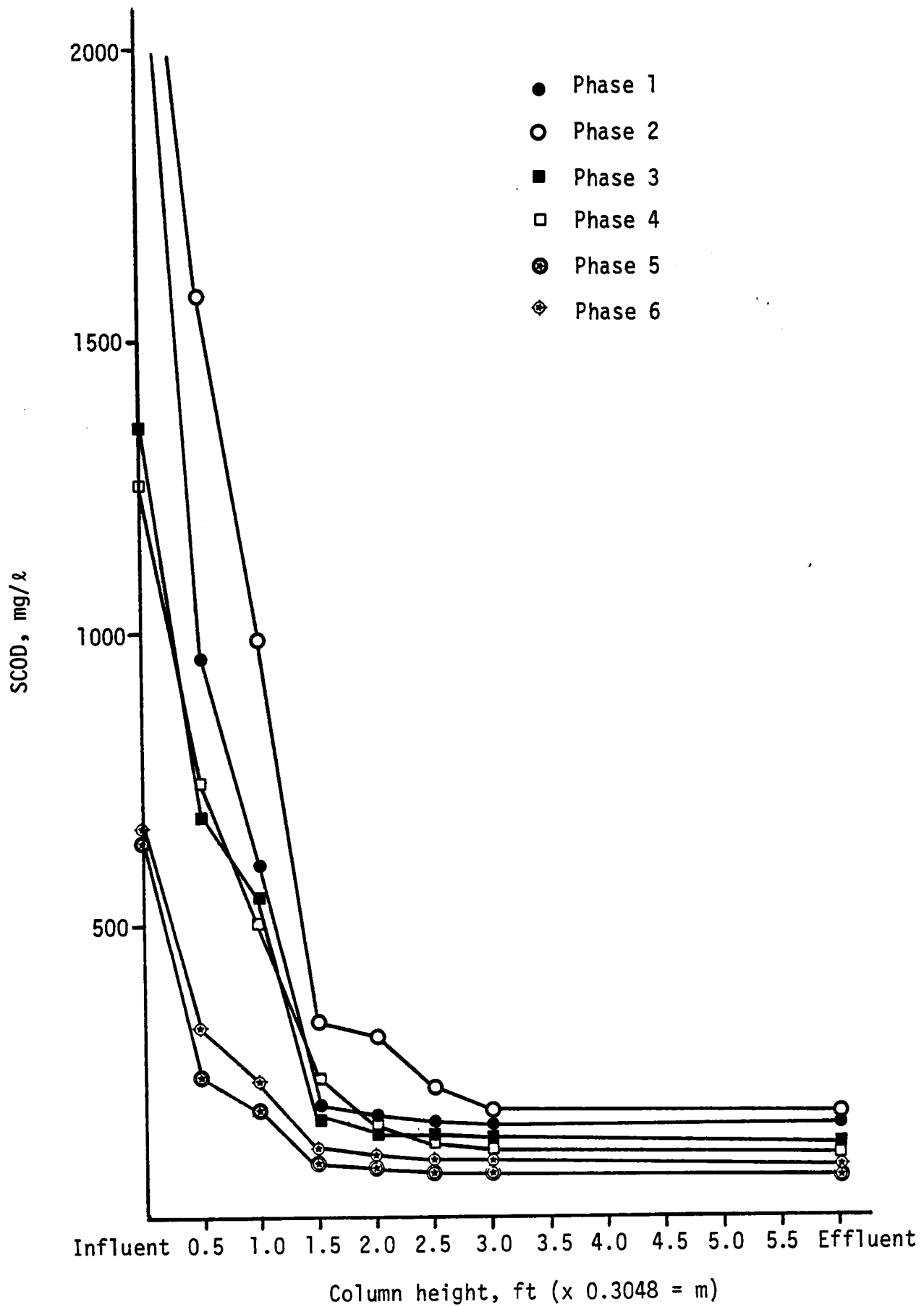


FIGURE 7. Profile of soluble chemical oxygen demand (SCOD).

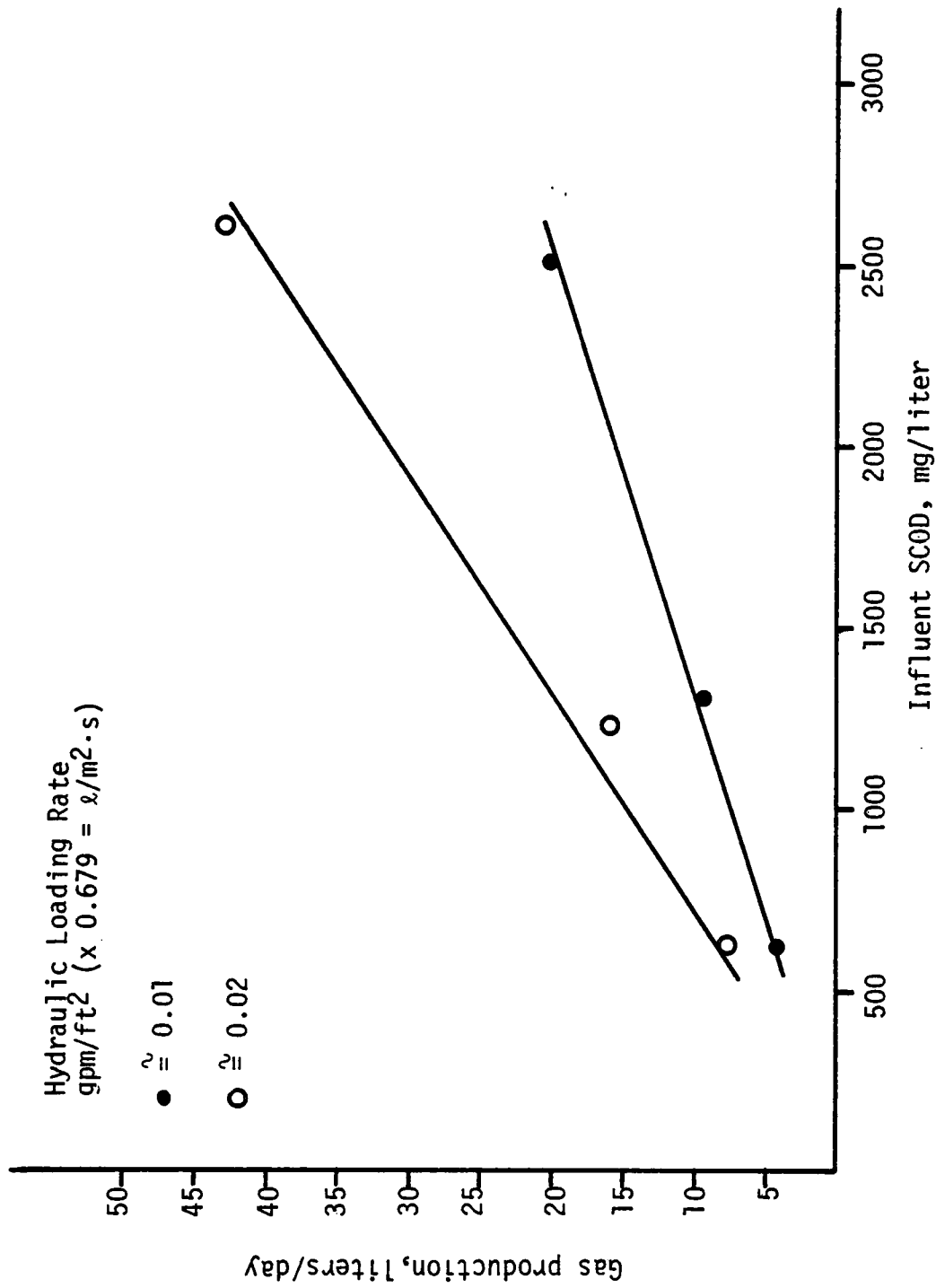


FIGURE 8. Effect of influent soluble chemical oxygen demand (SCOD) on gas production.

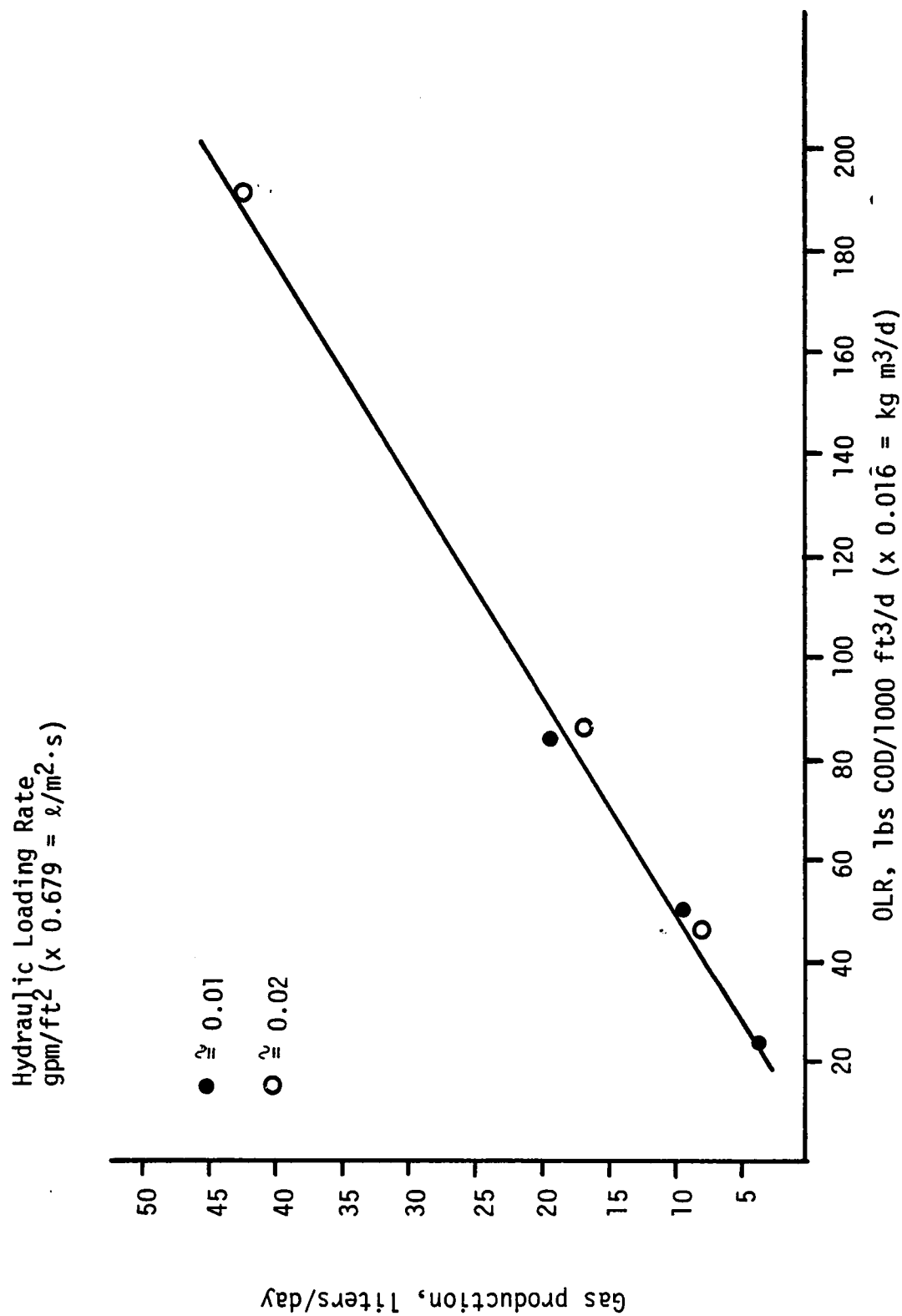


FIGURE 9. Effect of organic loading rate (OLR) on gas production.

of methane (in pounds) produced per pound of BOD converted of 0.25. The volume equivalent of the 0.25 lb of methane produced from the stabilization of 1 lb of BOD_L is 5.6 ft^3 (0.35 m^3 methane produced/kg BOD_L converted).

Percentages of gas composition are given in Table 3, page 28. Methane and carbon dioxide were the components of main interest; the "other" gases were always present at less than 8%. These other gases were found to be over 90% nitrogen and less than 1% oxygen. Hydrogen sulfide was not analyzed but it may have contributed to the remainder of the other gases since it is characteristic of anaerobic treatment; hydrogen gas would also be expected to be present in anaerobic treatment (4).

Methane gas ranged from 66.2-78.6% as shown in Table 3, page 28. The highest percentages of methane were observed at the lower OLRs; here carbon dioxide was lowest but the other gases were highest. At the lower OLRs, the methanogens appeared to be more efficient in conversion of volatile acids to methane as noted by higher percentages of methane. Total gas production was less at the lower OLRs although gas quality (in reference to percent methane) was improved. These factors become important when off-gas is to be considered as an energy source. It was of interest to note the changes in percentages of the other gases especially as it was correlated with higher percentages of methane. It was not understood why more nitrogen (the main component of the other gases) would be produced under these lower OLRs.

Volatile fatty acids. The volatile acids profile is given in Figure 10. The values represent total production of acids, that is, the summation of acetic, propionic, isobutyric, and butyric acids. Only these four acids were detected during the entire study. Acetic and propionic acids were the predominant species. Volatile acids were present in the synthetic

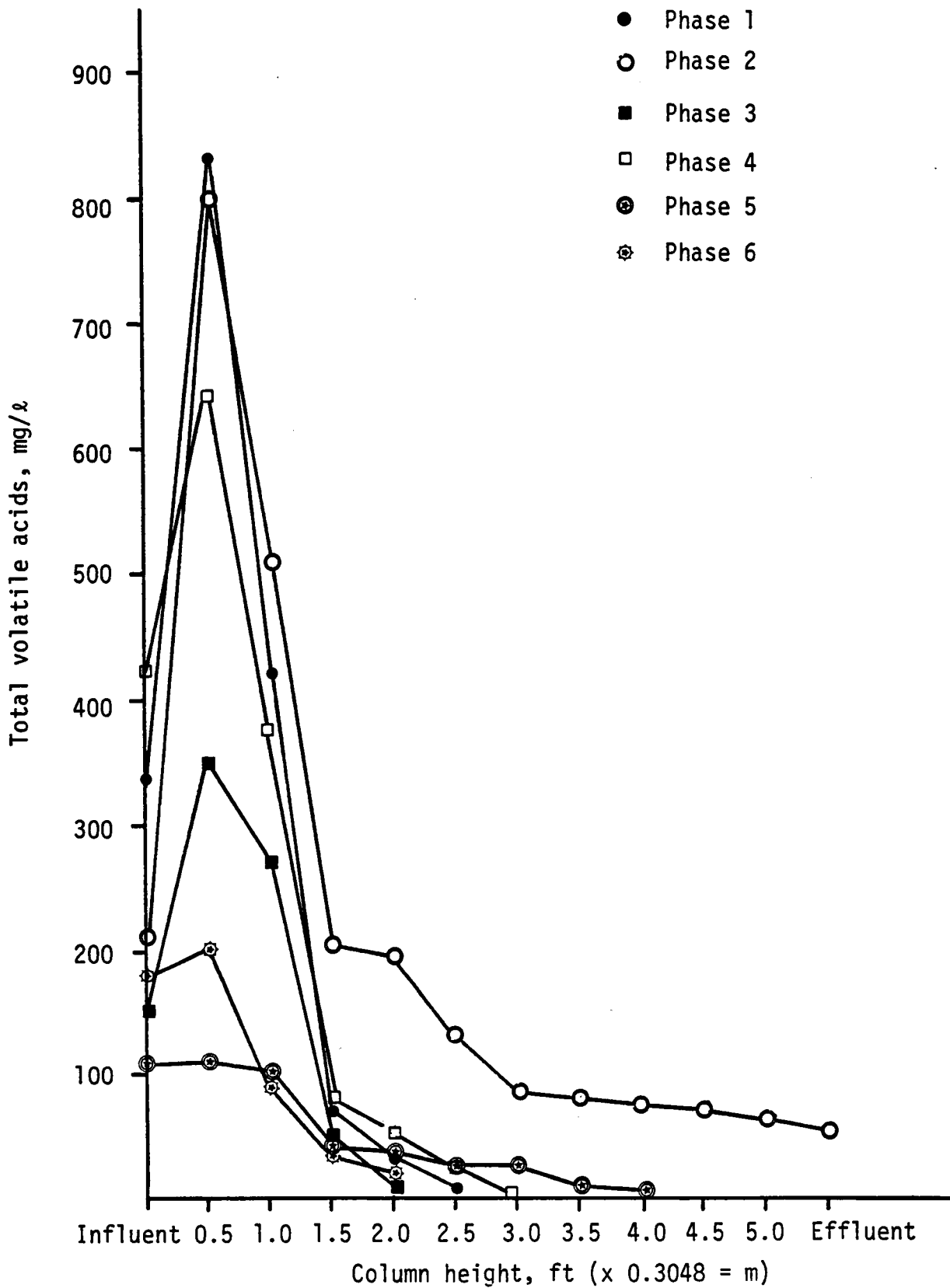


FIGURE 10. Volatile acids profile, total.

feed which, as mentioned earlier, caused the pH to be lower. Occasionally pH was measured in profile samples and found to be lowest where volatile acids were highest.

As was observed, most of the volatile acids were consumed by 2.5 ft within the ANFLOW bioreactor, except at the higher OLRs. During phase 2 (the highest OLR used in this study), volatile acids were not converted by the methanogens as efficiently and were found even in the effluent samples. At the lower OLRs, the volatile acids were consumed as soon as they were formed resulting in less net accumulation. The highest concentrations always occurred at 0.5 ft within the bioreactor. At the higher flow rates, phases 2, 4, and 6, a slight upward shift was seen in the profile. The increased flow rates appeared to carry the acids higher in the ANFLOW bioreactor.

Both the SCOD and volatile acids profiles illustrate that most waste stabilization occurs within the lower half of the ANFLOW bioreactor. Since the ANFLOW bioreactor behaves as a PFR, the biphasic stages of methanogenesis were observed, that is, production and consumption of volatile fatty acids.

Solids. Analysis of solids was determined only during phase 4. The profiles of suspended solids and volatile solids are shown in Figure 11. The actual values are given in Table E-3 of the Appendix. As was illustrated in the volatile acids profile, most biological activity appeared to occur at a height of 0.5 ft within the bioreactor. Some solids occurred in the feed. The solids were reduced by 1.5 ft in the bioreactor.

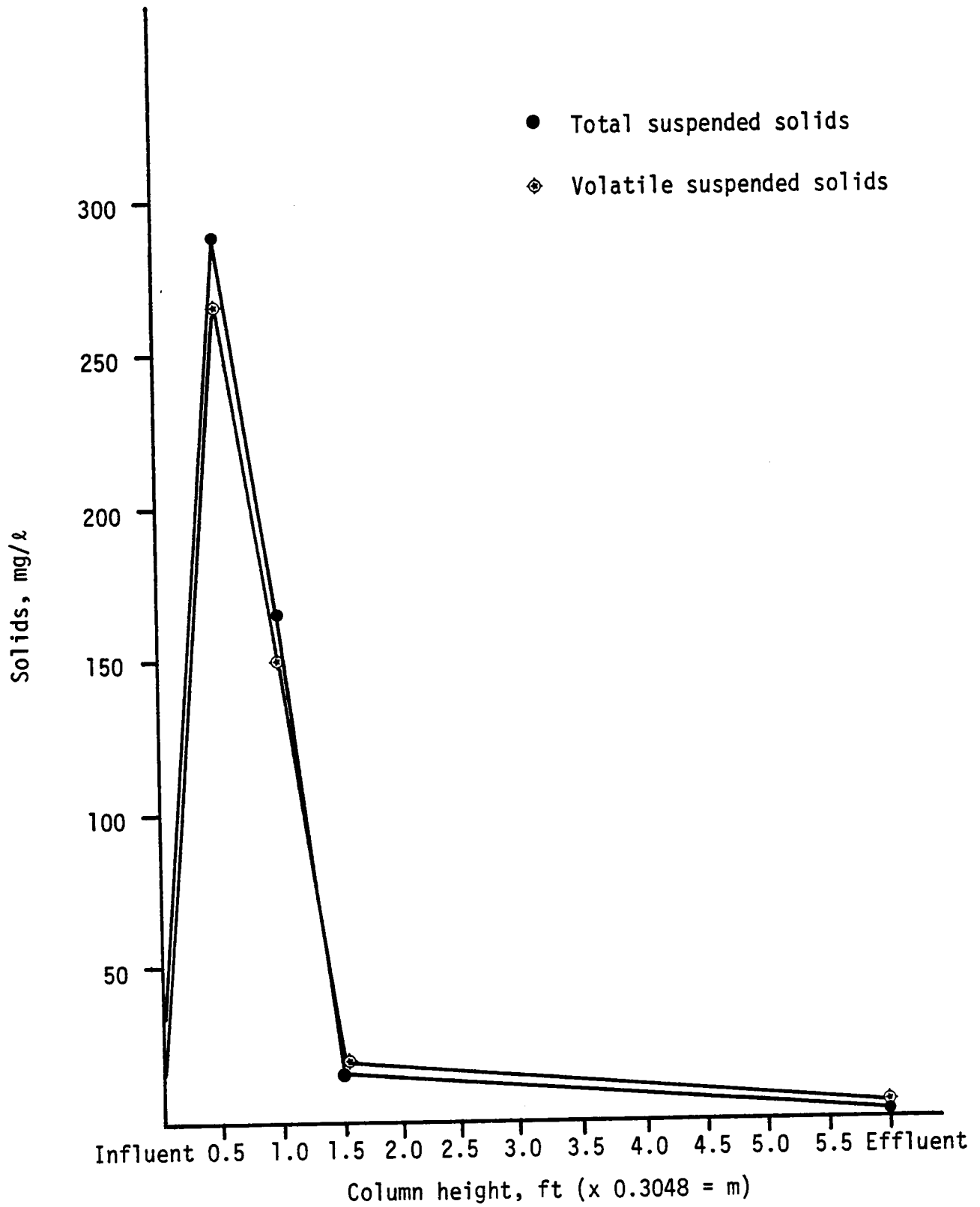


FIGURE 11. Solids profile from phase no. 4.

During all phases, samples were collected in clear plastic bottles, and the solids could be observed. The samples always appeared the same during each phase. At 0.5 and 1.0 feet, the samples were very turbid and contained dark particles; at 1.5 feet, the samples were less turbid; at 2.0 and 2.5 feet, there always were large flat particles, but the solution was clear, not turbid. The remainder of the samples from the higher regions of the bioreactor appeared clear. All of the solids would centrifuge into a pellet (preparation of SCOD and volatile acids samples) leaving a clear solution.

Ammonia production. Results of average effluent ammonia concentrations and the ranges found are given in Table 3, page 28. These concentrations appeared to be a function of flow rate: for a given influent SCOD concentration, effluent ammonia concentration decreased as flow rate (HLR) was doubled. This effect is illustrated in Figure 12. With a shorter HLR, there appears to be less time for ammonia accumulation within the ANFLOW bioreactor. A greater difference in effluent ammonia concentration was noted at the higher influent SCOD concentrations. During phases 5 and 6, the same range of effluent ammonia concentration was found, but at the higher HLR, the average concentration was slightly less.

The effect of OLR on effluent ammonia concentration is illustrated in Figure 13. By comparing the slopes of these lines, it was found that at the higher HLR, the rate of increase of effluent ammonia concentration was less. If the ammonia concentrations are converted into loadings, that is, grams per day of ammonia formed, production was found to be less at the lower HLR, illustrated in Figure 14. However, if the parameters are normalized, that is, each parameter contains the flow

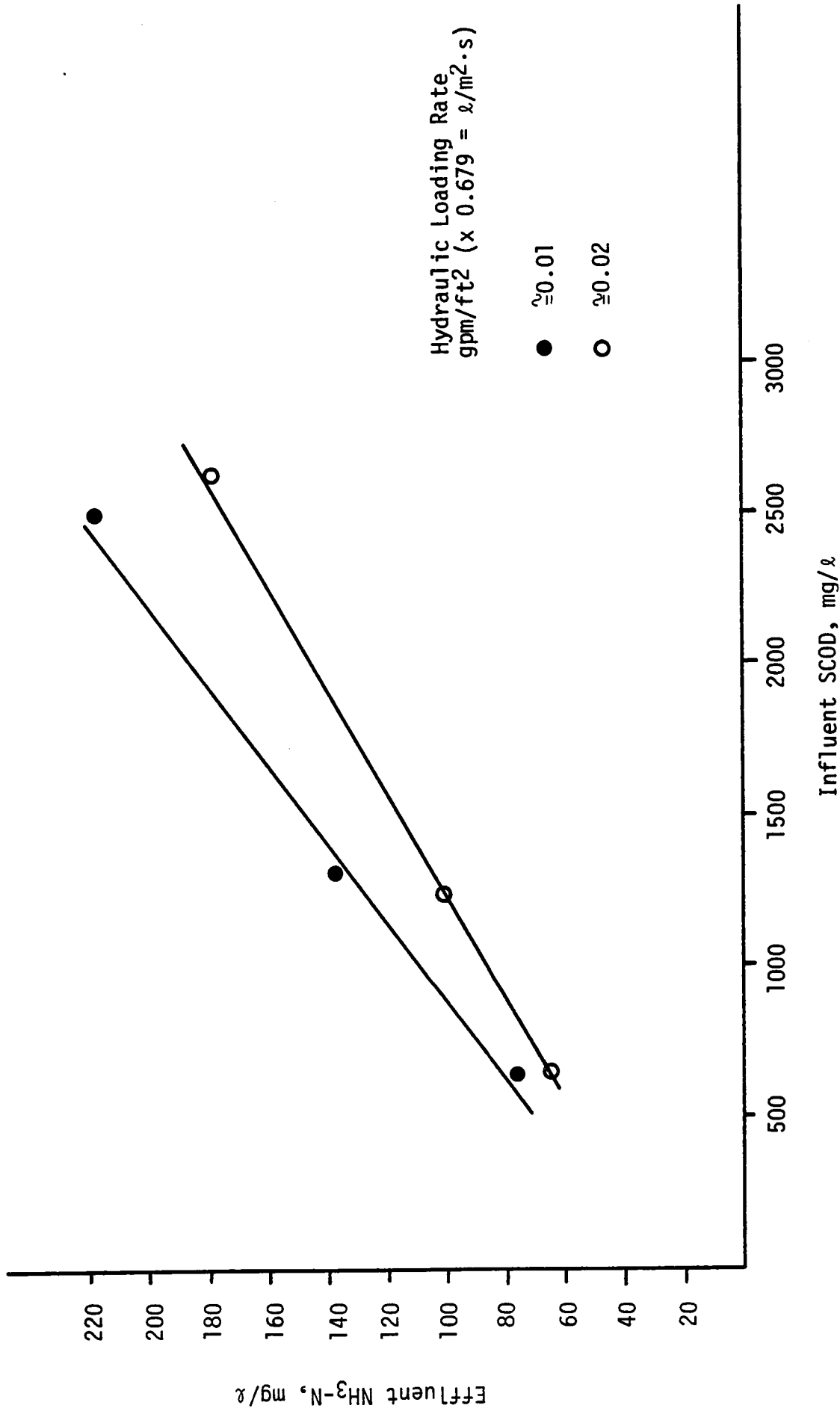


FIGURE 12. Effect of influent soluble chemical oxygen demand (SCOD) on effluent ammonia-nitrogen concentration.

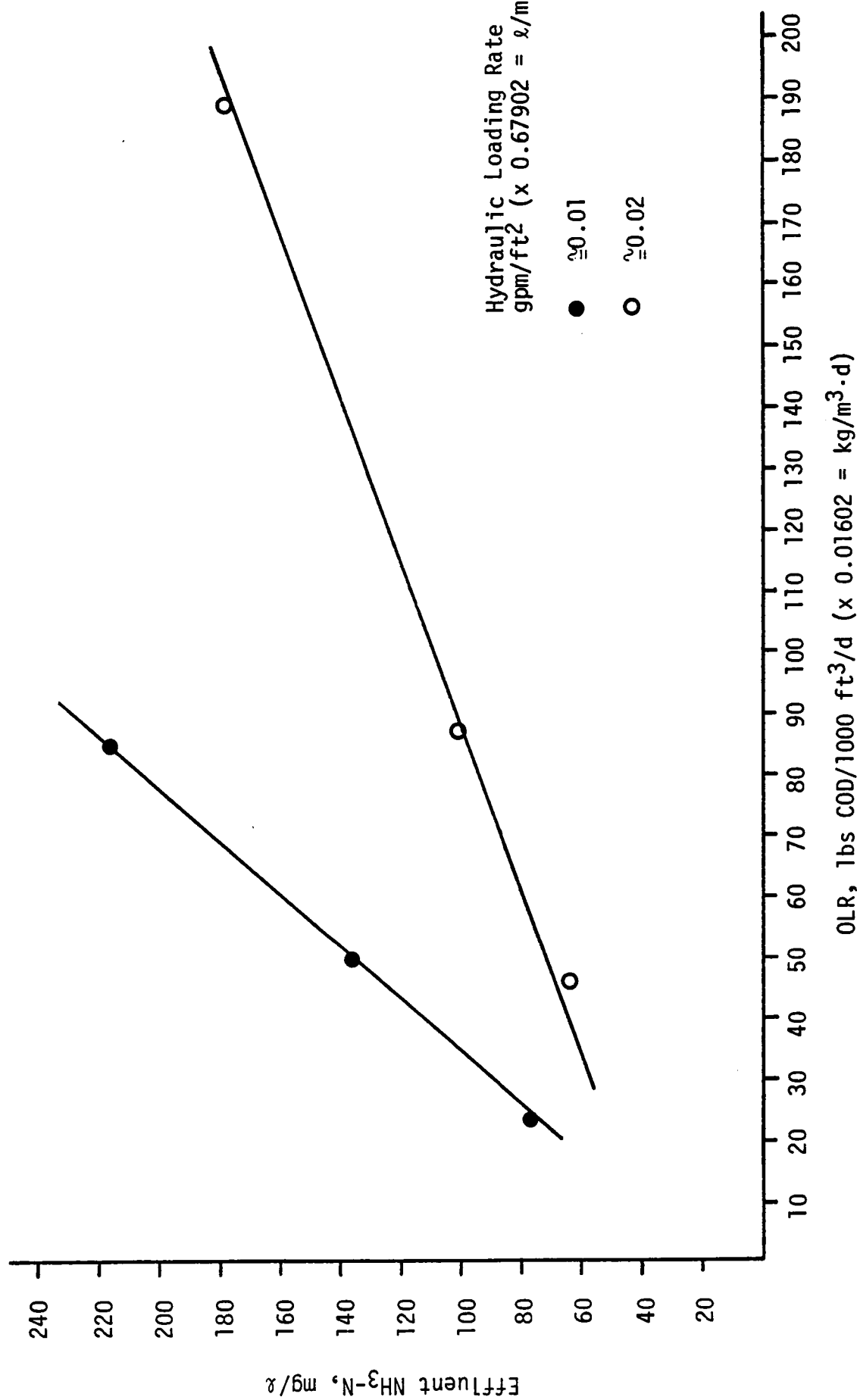


FIGURE 13. Effect of organic loading rate (OLR) on effluent ammonia-nitrogen concentration.

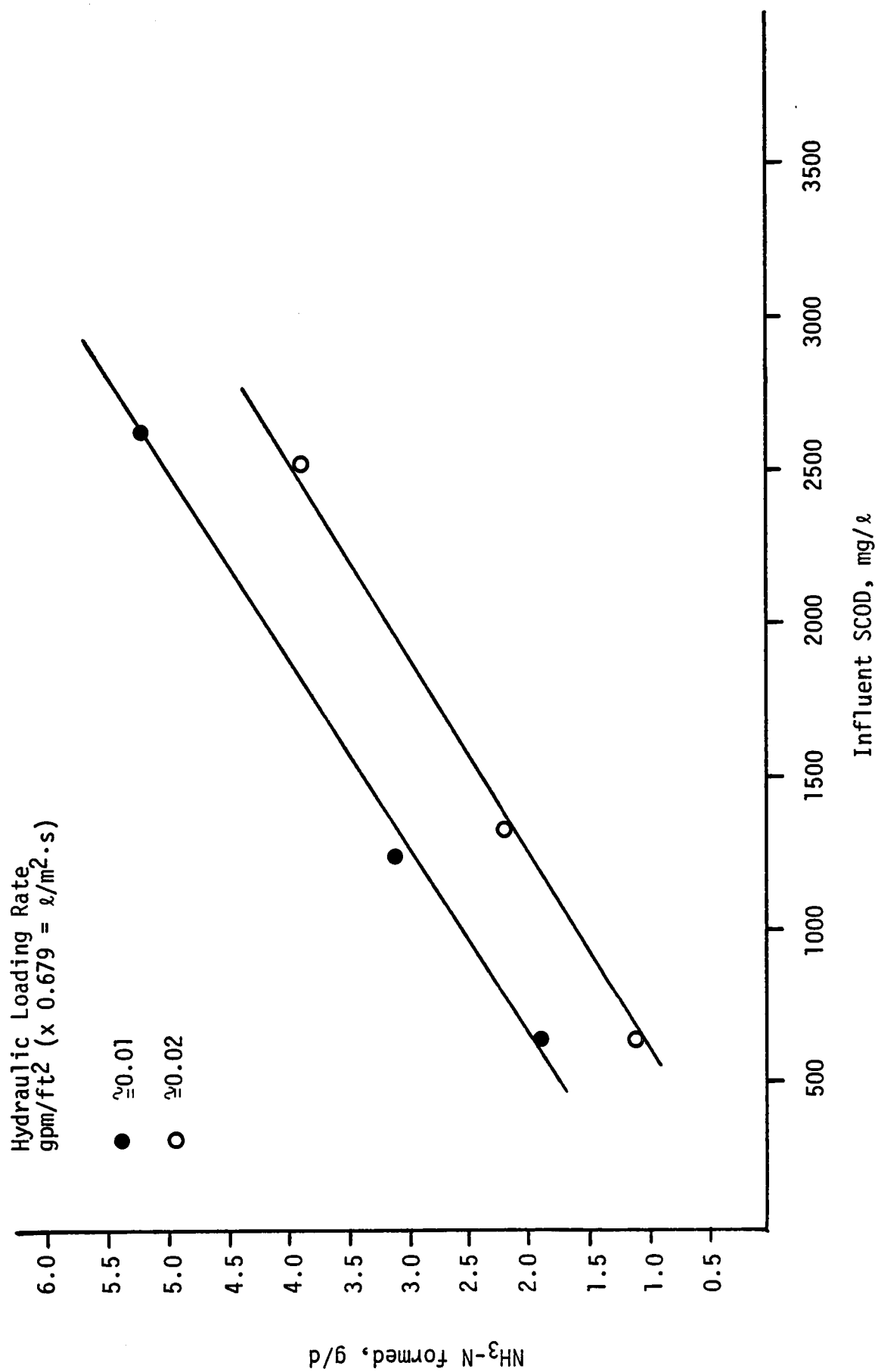


FIGURE 14. Effect of influent soluble chemical oxygen demand (SCOD) on ammonia-nitrogen formed.

rate, then the grams per day of ammonia formed was linear as a function of OLR as illustrated in Figure 15.

The profile of ammonia concentration along the length of the ANFLOW bioreactor is shown in Figure 16. Actual values are given in Tables B-3, C-3, D-3, E-4, and G-3 in the Appendix. Figure 16 illustrated the difference and variations of ammonia observed at the higher influent SCOD concentrations (phases 1 and 2). At the lowest influent SCOD concentrations, less variation was observed in the profile.

The profile showed a gradual increase in ammonia concentration from 0.5 ft to the effluent. Decreases were noted in the profile which suggest formation and uptake of ammonia within the bioreactor were not consistent. Ammonia uptake for cell synthesis occurred at a lower rate than ammonia formation.

Usually only duplicate samples of influent, effluent and the aerobic bioreactor effluent were analyzed for TKN. However, once during phase 1, a profile of TKN was analyzed. This profile is illustrated in Figure 17, which showed that organic-nitrogen was completely converted to ammonia-nitrogen be 1.5 feet within the ANFLOW bioreactor. In this profile, there was apparently no further ammonia accumulation after 1.5 feet.

The nitrogen mass balance for all six experimental phases is given in Table 4. This table includes mainly data from the TKN analyses. Since the TKN analyses were performed only twice per phase and thus at different time periods, the effluent ammonia concentrations were not equal to those average values given in Table 3, page 28. It is significant to note that at the higher HLR (and thus the shorter HRT), the effluent samples always contained organic-nitrogen. This suggests that

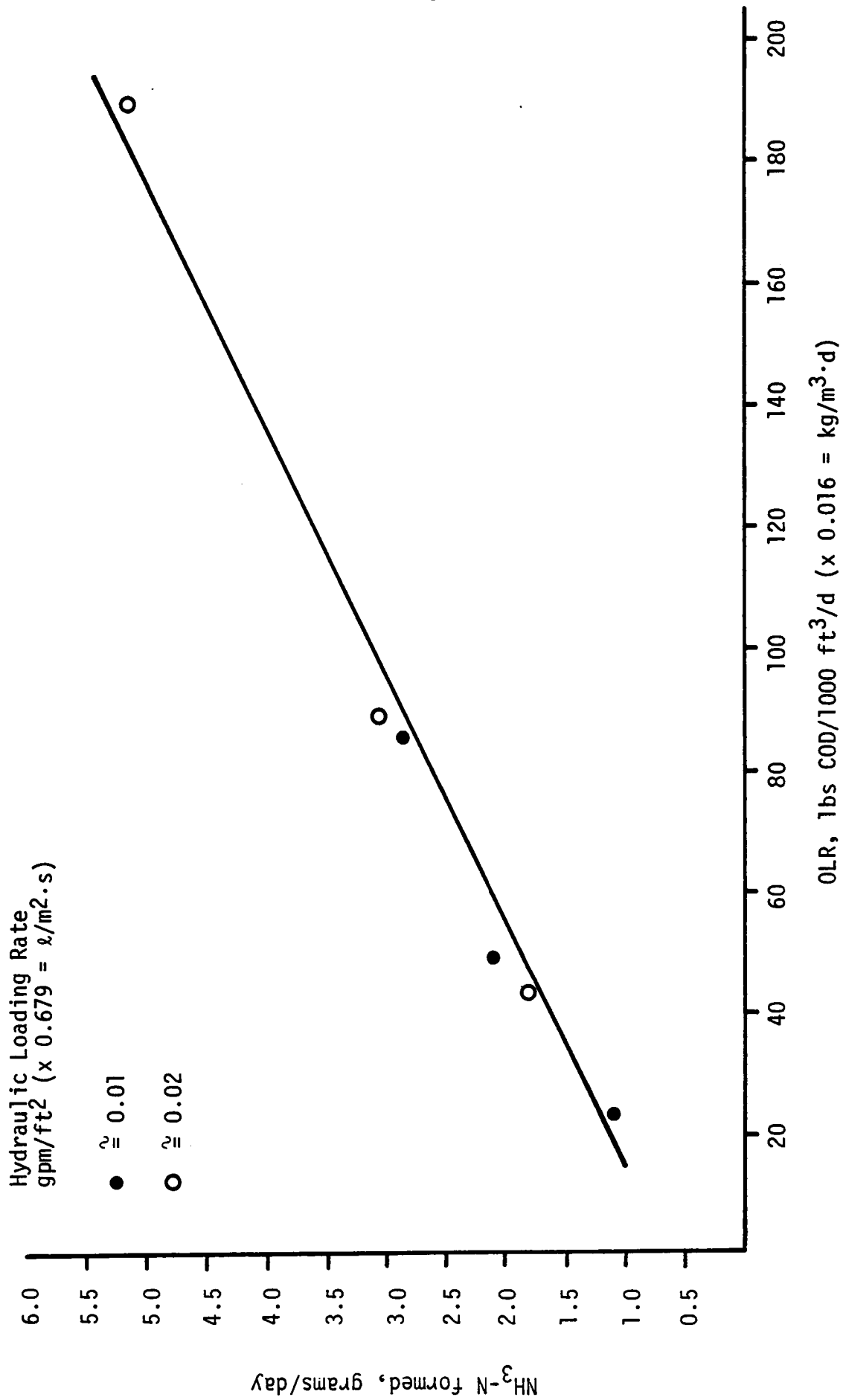


FIGURE 15. Effect of organic loading rate (OLR) on ammonia-nitrogen formed.

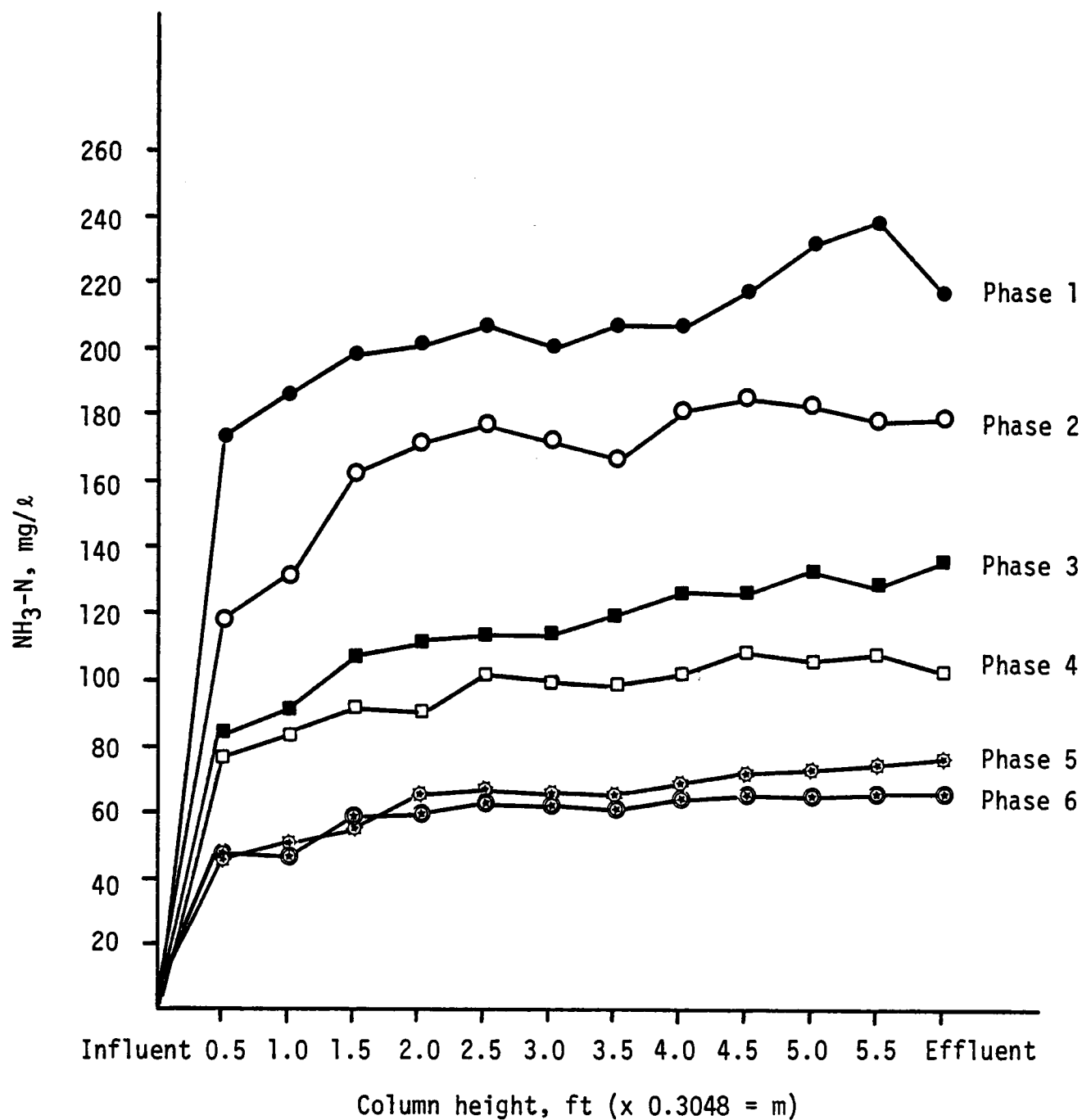


FIGURE 16. Ammonia-nitrogen concentration profile.

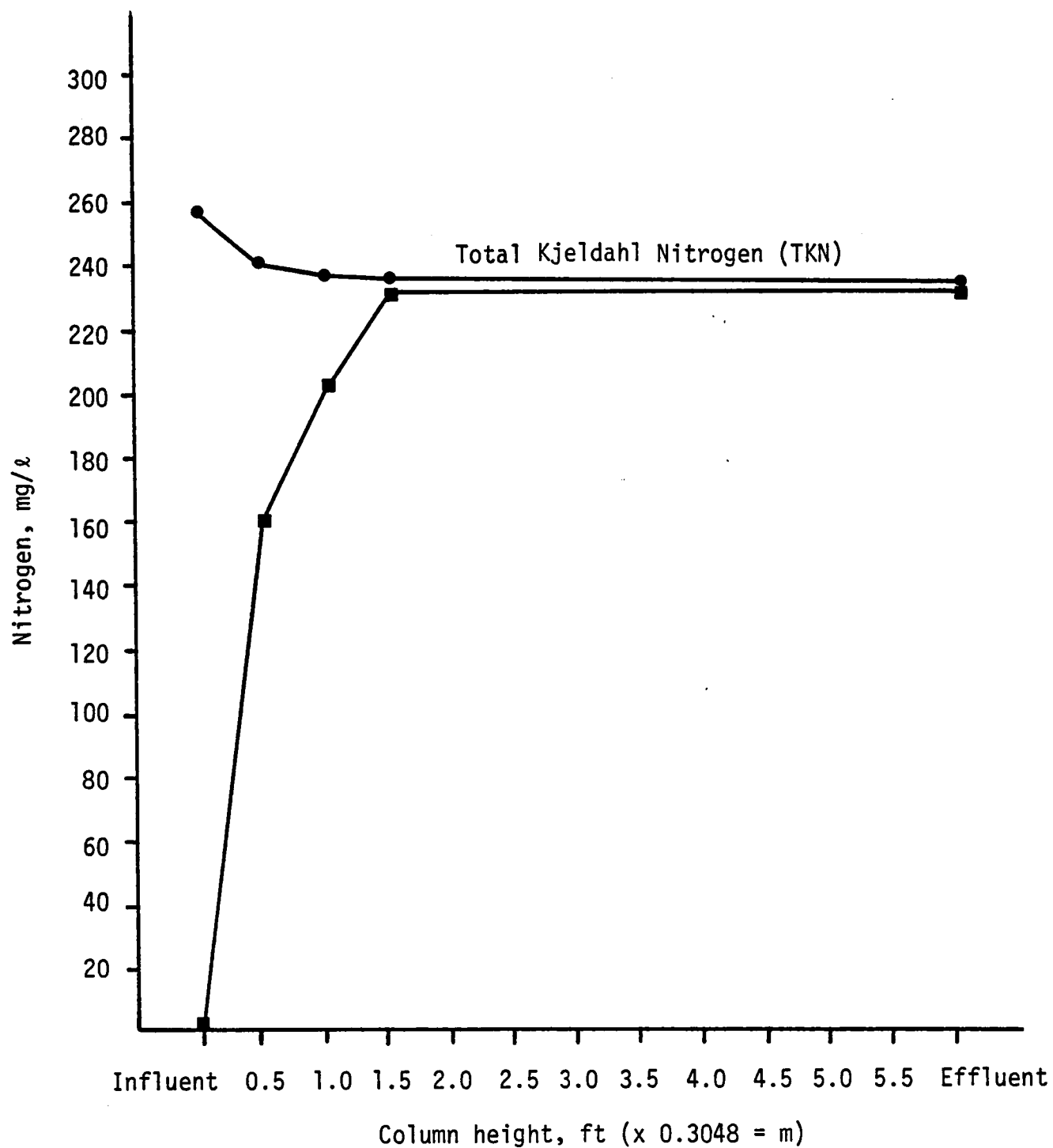


FIGURE 17. Conversion of organic-nitrogen to ammonia-nitrogen in ANFLOW bioreactor during phase no. 1.

Table 4. Nitrogen Mass Balance*

Phase No.	1	2	3	4	5	6
Influent Total Kjeldahl-Nitrogen (TKN), mg/liter	258	240	165	192	68	82
Influent NH ₃ -N, mg/liter	≈0	≈0	≈0	≈0	≈0	≈0
Influent Organic-N, mg/liter	258	240	165	192	68	82
Effluent TKN, mg/liter	235	196	130	141	58	78
Effluent NH ₃ -N, mg/liter	235	182	130	121	58	63
Effluent Organic-N, mg/liter	0	14	0	20	0	15
Average Hydraulic Residence Time (HRT), hours	≈41	≈20	≈41	≈20	≈41	≈20
Rate of Nitrogen Assimilation** mg/liter/hr	0.56	2.2	0.85	2.6	0.24	0.20
Average NO ₃ +NO ₂ -N, mg/liter	<1	<1	<1	<1	<1	<1
Average Aerobic HRT, hours	≈3	≈1.5	≈3	≈1.5	≈3	≈1.5
Aerobic TKN, mg/liter	--	40	5	22	3	<1
Percent Reduction of TKN (ANFLOW to Aerobic)	--	80	96	82	95	99

Table 4. Continued

Phase No.	1	2	3	4	5	6
Aerobic NH ₃ -N, mg/liter	--	38	3	22	3	<1
Average Aerobic NO ₃ +NO ₂ -N, mg/liter	5	15	35	25	40	35
Influent Organic-N Loading Rate grams/day	3.45	6.91	2.47	5.94	1.01	2.36
NH ₃ -N Formed, grams/day	2.90	5.20	2.20	3.10	1.10	1.90

*Data collected were non-routine and at different time periods than values in Table 3, page 28
(Summary of Average Results).

**Difference between influent and effluent TKN per residence time.

there was not enough residence time for complete organic-nitrogen conversion to ammonia-nitrogen or that mass transfer of organic into regions of active biomass had been decreased. The rates of nitrogen assimilation were calculated and also given in Table 4. At the shorter HRT, the assimilation rates were higher except during phases 5 and 6 (the lowest influent SCOD concentrations). These results suggested that convective mass transfer of nitrogen to biomass were enhanced by the higher flow rates at higher influent SCOD concentrations (or higher influent organic-nitrogen). During phases 1, 2, 3, and 4, and 50% increase in flow rate resulted in a 74% and a 67% increase, respectively, in the rate of nitrogen assimilation.

To understand the effect of influent TKN (in this study influent TKN was also influent organic-nitrogen) on effluent ammonia-nitrogen concentration, the influent was converted into a loading rate in grams per day; the effect of organic-nitrogen loading rate on effluent ammonia-nitrogen concentration is illustrated in Figure 18. It was observed that at the lower flow rates, the ammonia concentration in the effluent was less. The three points for each flow rate in Figure 18 appeared to be more of an exponential curve rather than a straight line, but this was not conclusive without additional data points. However, if the parameters are normalized, that is, a flow rate is included in both parameters, then it was observed the ammonia formed in grams per day was linear as a function of influent organic-nitrogen loading rate (in grams per day) as illustrated in Figure 19. Although there were scattered points on this straight line, the effect did appear to be linear; these analyses were not routine and thus there were not enough data points for sufficient statistical analyses.

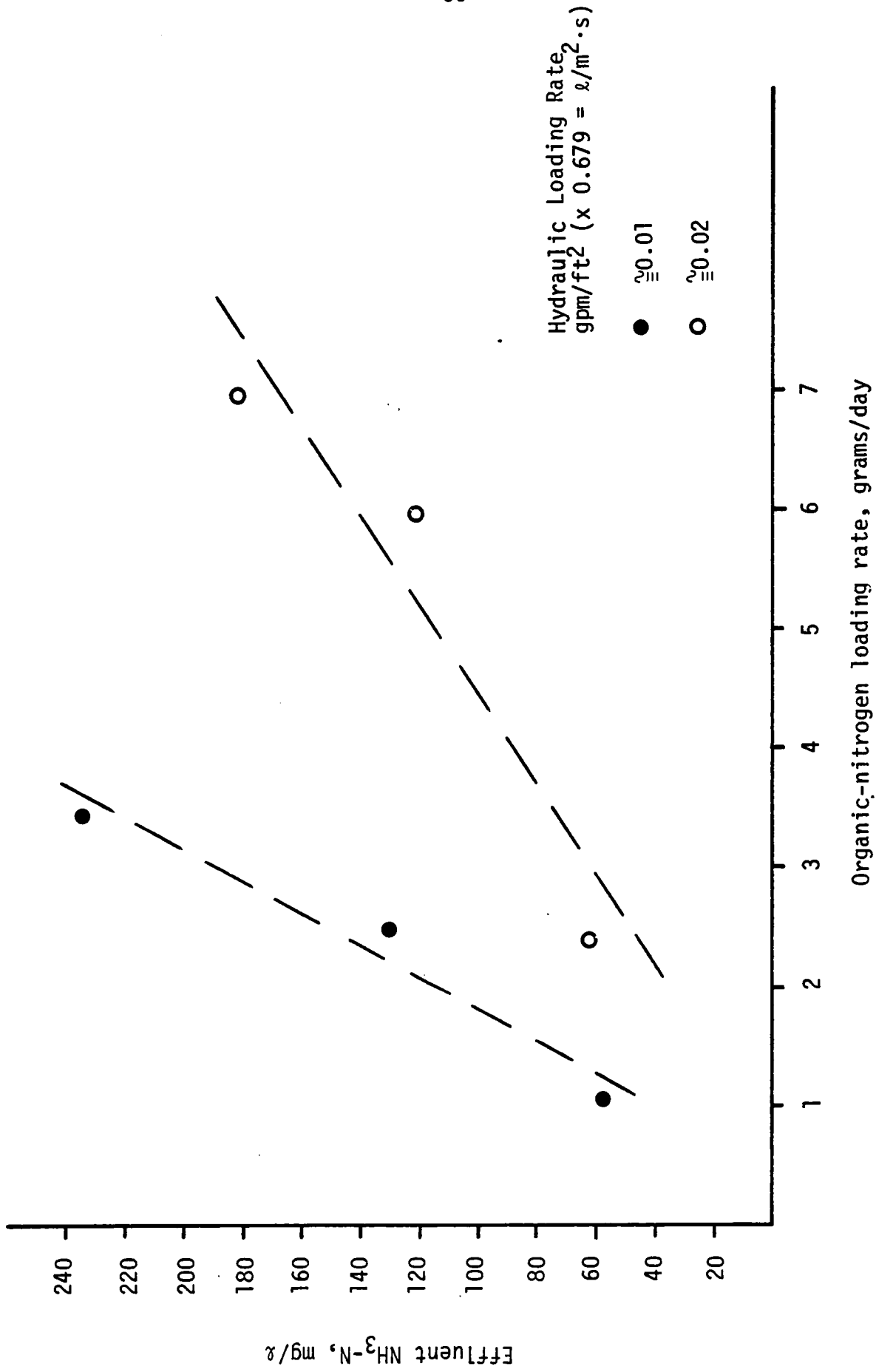


FIGURE 18. Effect of organic-nitrogen loading rate on effluent ammonia-nitrogen concentration.

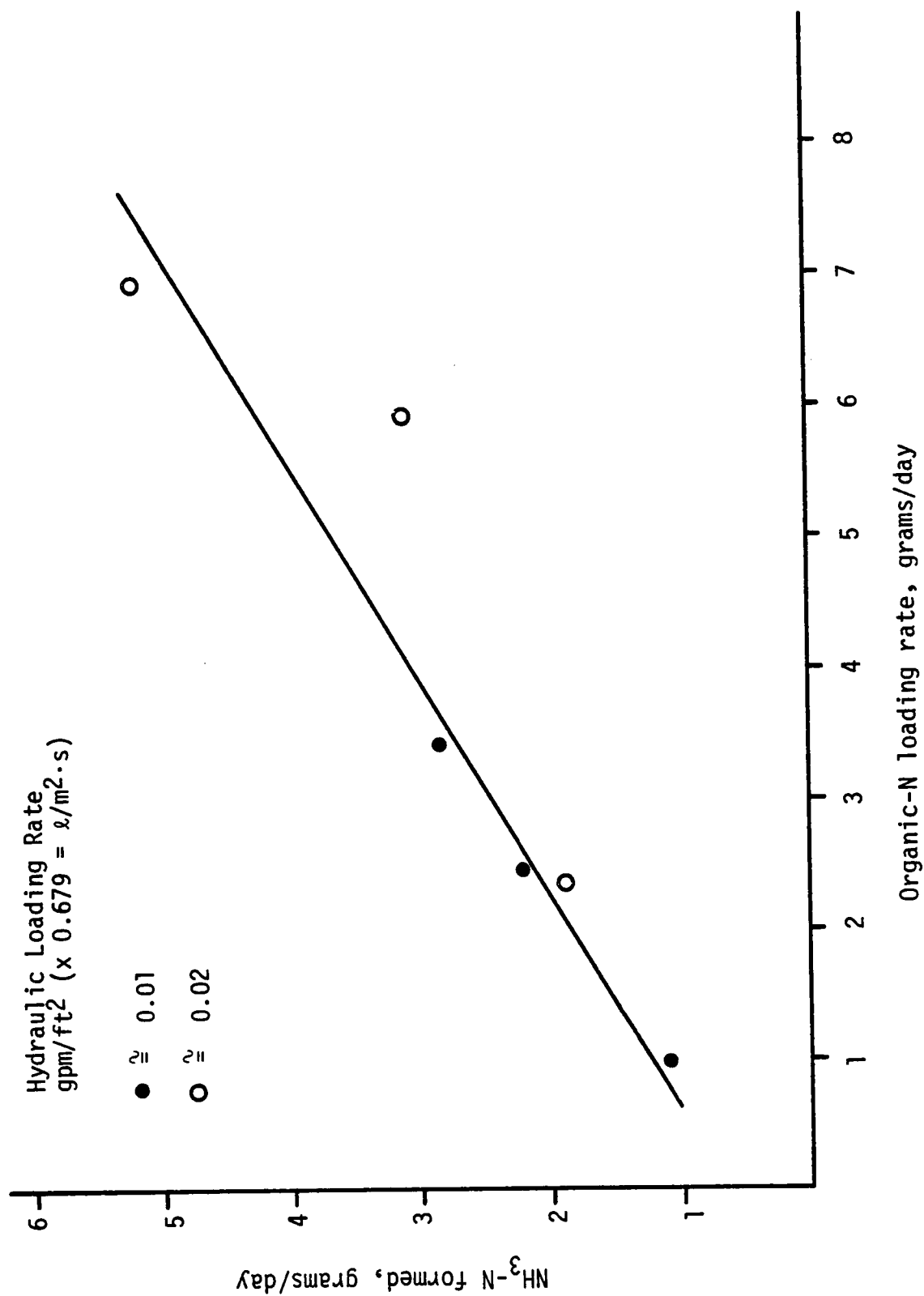


FIGURE 19. Effect of organic-nitrogen loading rate on ammonia-nitrogen formation.

Aerobic bioreactor nitrification. The results of nitrification are given in Table 3, page 28. Ammonia-nitrogen removal occurred to some extent in all phases. Best removal rates were seen at the lower OLRs and the lower HLR (where HRT was longer). During phase 5, ANFLOW effluent ammonia-nitrogen was reduced to an average of 3 mg/liter in the aerobic bioreactor; during this phase, the lowest influent SCOD and the lower flow rate were used. During all phases except phase 1, ammonia reduction exceeded 80% even at the HRT of one to five hours.

Aerobic bioreactor samples were analyzed for TKN except during phase 1. These results are given in Table 4. Over 80% removal of TKN occurred in the aerobic bioreactor. During phase 6, 99% removal of TKN was observed.

The aerobic bioreactor effluent was also analyzed for nitrate+nitrite-nitrogen, as given in Table 4. During the experimental phases of best ammonia-nitrogen reduction, nitrate+nitrite-nitrogen ranged between 25-40 mg/liter.

During phase 2, the average aerobic ammonia-nitrogen concentration was 35 mg/liter. At this short HRT, there was not enough time for nitrification to completely occur. On the last day of this experimental phase, the effluent from the ANFLOW bioreactor was discontinued from feeding into the aerobic bioreactor and then aerobic bioreactor effluent samples were collected periodically and analyzed for ammonia-nitrogen. These results are given in Table 5; it was observed that longer HRTs will enhance efficiency of nitrification.

Table 5. Nitrification with Increased Residence Time During Phase 2

Hydraulic Residence Time hours	Aerobic NH ₃ -N mg/liter
1.5	40
3.0	2
5.0	1

Disruptions in steady state. Failures in the pumping system or other maintenance problems caused a few disruptions of continuous feeding or loss of liquid volume. Occasionally breaks in the pump lines developed between the pump and feed tank. Thus air was pumped into the ANFLOW bioreactor, often as long as overnight. The gas compositions analyzed on the following day showed the same concentrations of CO₂, but the other gases increased to around 50% and methane decreased to around 30%. Normally the other gases were less than or equal to 10% of the total gas composition. Gradually the other gases decreased and within three days, gas composition returned to normal. Recovery did not appear to be related to HRT. The SCOD removal did not fluctuate during these disruptions.

Other breaks in the feed lines occurred between the pump and the ANFLOW bioreactor; thus the liquid volume drained out. Once a clamp on one of the lower sample ports broke releasing the liquid volume. After the repairs and feed was continuously pumped to restore the volume, effluent quality did not show significant changes in SCOD or ammonia-nitrogen. Thus, ANFLOW bioreactors showed capabilities of withstanding disruptions in flow and tolerating exposure to air.

V. CONCLUSIONS

The ANFLOW bioreactor was demonstrated as an efficient approach to soluble chemical oxygen demand (SCOD) removal for a range of SCOD concentrations and two hydraulic loading rates (HLRs). Profiles of SCOD, volatile acids, and solids concentrations showed distinct regions of complex organics breakdown and waste stabilization within the ANFLOW bioreactor. As influent SCOD concentrations and flow rates changed, profiles of SCOD and volatile acids concentrations did not show major changes or shifts within the bioreactor. The most efficient SCOD removal was seen at high organic loading rates (OLRs). The SCOD removal was higher at the doubled flow rate for a given influent SCOD; effluent concentrations of SCOD were less at the higher flow rate for a given OLR. However, in terms of overall bioreactor efficiency, the SCOD removal rate was linear as a function of increasing OLR.

Gas production was doubled at the higher flow rate for a given influent SCOD, but overall production was linear as a function of increasing OLR. The best quality of off-gas in terms of percentage methane was observed when influent SCOD was less than 1500 mg/liter. As would be expected, the largest volume of gas was produced during the highest OLR; however, percentage of methane was lowest. These results become important when considering utilizing the off-gas as an energy source: large volumes of off-gas are desired but higher percentage of methane would reduce the amount of scrubbing to purify the off-gas.

Effluent quality of the ANFLOW bioreactor did not change when perturbations of steady-state conditions occurred. Exposure to air,

although short, did not appear to effect the performance of the ANFLOW bioreactor.

The effluent ammonia concentration was less at the higher flow rate for a given influent SCOD, OLR, or organic-nitrogen loading rate. At lower influent SCOD concentrations, the effect of flow rate was not as significant as during use of high influent SCOD concentrations. The figures presented could be used for predicting effluent quality in terms of ammonia-nitrogen concentrations for a given SCOD concentrations, OLR, or organic-nitrogen loading rate at two HLRs. When the variables were normalized, ammonia formation in terms of grams per day produced was linear as a function of increasing OLR.

It was demonstrated that the ammonia can be nitrified with an aerobic upflow bioreactor with only 5% of the volume of the ANFLOW bioreactor. This aerobic filter was working under stress conditions due to the short HRTs. It is, therefore, expected that a larger volume aerobic filter could nitrify the effluent ammonia-nitrogen of the ANFLOW bioreactor to meet compliance regulations.

Effluent from the ANFLOW bioreactor could be disposed by land treatment methods as an alternative to unit processes. The effluent from the ANFLOW bioreactor may also be valuable as a fertilizer or a supplement to fertilizers containing ammonium compounds. Another alternative use of the effluent could be as a feed source in algae and/or fish culturing and harvesting.

A soluble wastewater appears to be best suited for ANFLOW bioreactor treatment although it is feasible that the off-gas could be used to generate power to run grinder pumps for wastewaters with high solids concentrations. Domestic wastewater would be preferred over industrial

wastewater since industrial wastes may contain toxic substances, for example, high concentrations of heavy metals. However, on-site treatment of a specific industrial waste of a known composition, for example, dairy wastes or meatpacking wastes rather than electroplating wastes or a complex of various types of wastes, should be considered for ANFLOW bioreactor wastewater treatment. High loading rates would be necessary for consideration of utilizing the methane produced. Agricultural uses of the ANFLOW bioreactor seem to be the most suitable: animal wastes could be treated with subsequent use of the methane produced to run generators for grinder pumps; the effluent from the bioreactor could then be used as a fertilizer or fertilizer supplement.

At lower loading rates, the ANFLOW bioreactor was still capable of efficient COD removal with lower amounts of ammonia-nitrogen produced. However, the low amounts of gas produced would not be sufficient for recovery as an energy source. The ANFLOW bioreactor can withstand disruptions in loading rates if it is necessary to change influent conditions in order to predict a specified effluent quality.

LIST OF REFERENCES

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1. Genung, R. K., W. W. Pitt, Jr., G. M. Davis, and J. H. Koon, "Energy Conservation and Scale-Up Studies for a Wastewater Treatment System Based on a Fixed-Film, Anaerobic Bioreactor," paper presented at the Second Symposium on Biotechnology in Energy Production and Conservation, Gatlinburg, TN, October 2-5, 1979.
2. Koon, John H., Gary M. Davis, Richard K. Genung, and W. Wilson Pitt, "The Feasibility of an Anerobic Upflow Fixed-Film Process for Treating Small Sewage Flows," presented at Energy Optimization of Water and Wastewater Management for Municipal and Industrial Applications Conference, New Orleans, LA, December 1979.
3. Genung, R. K., et al., "Pilot Plant Demonstration of an Anaerobic Fixed-Film Bioreactor for Wastewater Treatment," *Biotechnol. and Bioeng.* 74, 117 (1978).
4. Metcalf and Eddy, Inc., Wastewater Engineering: Treatment, Disposal, Reuse, Second Edition, McGraw-Hill, New York, 1979.
5. Andrews, J. F., R. D. Cole, E. A. Pearson, "Kinetics and Characteristics of Multistage Methane Fermentations," Sanitary Engineering Research Laboratory (SERL-Berkeley, California), No. 64-11, December 1964.
6. Chynoweth, David P., Sambhunath Ghosh, Martha L. Packer, "Anaerobic Processes--Literature Review," *JWPCF* 51(6), 1200 (1979).
7. Kroeker, E. J., D. D. Schulte, A. B. Sparling, H. M. Lapp, "Anaerobic Treatment Process Stability," *JWPCF* 51(4), 718 (1979).
8. Ghosh, S., D. P. Chkynoweth, M. L. Packer, F. S. Sedzielarz, A. C. Roess, and E. M. Van Ryzin, "Anaerobic Processes--Literature Review," *JWPCF* 50(6), 2388 (1978).
9. McCarty, Perry L., "Anaerobic Waste Treatment Fundamentals," *Public Works*, Parts 1-4, September, 107-112, October, 123-126, November 91--94, December 95-99 (1964).
10. Fannin, Kerley F., David P. Chynoweth, Sambhunath Ghosh, and Vipul J. Srivastave, "Anaerobic Processes," *JWPCF* 52(6), 1182 (1980).
11. Young, James C. and Perry L. McCarty, "The Anerobic Filter for Waste Treatment," *JWPCF* 41(5), Part 2, R160 (1969).
12. McFarlane, Paul N., and Henryk Melcer, "The Occurrence of Purple Sulfur Bacteria in Anaerobic Lagoons--Theory and Application," Proc. of the 32nd Industrial Waste Conference, Purdue University, 497 (1977).

13. Mountfort, D. O. and R. A. Asher, "Changes in Proportions of Acetate and Carbon Dioxide Used as Methane Precursors During the Anaerobic Digestion of Bovine Waste," *Applied and Environmental Microbiology* 35(4), 648 (1978).
14. Ghosh, Sambhurath, John R. Conrad, and Donald L. Klass, "Anaerobic Acidogenesis of Wastewater Sludge," *JWPCF* 47(1), 30 (1975).
15. Jagnow, G., K. Haider, and P-Chr. Ellwardt, "Anaerobic Dechlorination and Degradation of Hexachlorocyclohexane Isomers by Anaerobic and Facultative Anaerobic Bacteria," *Arch. Microbiol.* 115, 285 (1977).
16. Jensen, S., R. Gothe, and M.-O. Kindshdt, "Formation of bio(p-chlorophenyl)-acetonitrile (p,p'-DDCN) from P,P'-DDT in Anaerobic Sewage Sludge," *Nature* 240, 420 (1972).
17. Gossett, James M., Perry L. McCarty, Jerry C. Wilson, and Don S. Evans, "Anaerobic Digestion of Sludge from Chemical Treatment," *JWPCF* 50(3), 533 (1978).
18. McCarty, Perry L. and Rosa E. McKinney, "Salt Toxicity in Anaerobic Digestion," *JWPCF* 33(4), 399 (1961).
19. Van Velson, A.F.M., "Adaptation of Methanogenic Sludge to High Ammonia-Nitrogen Concentrations," *Water Res. (G.B.)* 13, 995 (1979).
20. Albertson, Orris E., "Ammonia Nitrogen and the Anaerobic Environment," *JWPCF* 33(9), 978 (1961).
21. Switzenbaum, M. S., and W. J. Jewell, "Anaerobic Attached Film Expanded Reactor Treatment of Dilute Organics," paper presented at the 51st Annual Conference, WPCF, Anaheim, CA, October 1978.
22. Norrman, Jonas, and Bjorn Forstell, "Anaerobic Wastewater Treatment in a Two-Stage Reactor of a New Design," Proceedings of 32nd Industrial Waste Conference, Purdue University, 387 (1977).
23. DeWalle, F. B., and E. S. K. Chian, "Kinetics of Substrate Removal in a Completely Mixed Anaerobic Filter," *Biotechnology and Bio-engineering* 18, 1275 (1976).
24. Young, James C., "Chemical Methods for Nitrification Control," *JWPCF* 45(4), 637 (1973).
25. Trulsson, Sven-Gunnar, "Ammonia Recovery from Wastewaters in Packed Columns," *JWPCF* 51(10), 2513 (1979).
26. Haug, Roger T., and Perry L. McCarty, "Nitrification with Submerged Filters," *JWPCF* 44(11), 2086 (1972).

27. Tamblyn, Thomas A., and Bryan R. Sword, "The Anaerobic Filter for the Denitrification of Agricultural Subsurface Drainage," Proceedings of the 24th Industrial Waste Conference, Part 2, Purdue University, 1135 (1969).
28. Young, James C., E. Robert Baumann, and Danny J. Wall, "Packed-Bed Reactors for Secondary Effluent BOD and Ammonia Removal," JWPCF 47(1), 48 (1975).
29. Prakasam, T. B. S., C. Lue-Hing, E. Bogvsch, D. R. Zenz, "Pilot-Scale Studies of Single-Stage Nitrification," JWPCF 51(7), 1904 (1979).
30. Jeris, John S., Roger W. Owens, Robert Hickey, and Frank Flood, "Biological Fluidized-Bed Treatment for BOD and Nitrogen Removal," JWPCF 49(5), 816 (1977).
31. Argaman, Yerahmiel, and Eliahu Miller, "Modeling Recycled Systems for Biological Nitrification and Denitrification," JWPCF 51(4), 749 (1979).
32. Klapwijk, A., C. Jol, and H. J. G. W. Donker, "The Application of an Upflow Reactor in the Denitrification Step of Biological Sewage Purification," Water Research (G.B.) 13, 1009 (1979).
33. Jeris, John S., and Roger W. Owens, Pilot-Scale, High-Rate Biological Denitrification," JWPCF 47(8), 2043 (1975).
34. Andreoli, Aldo, Nichloas Bartiluicci, Robert Forgione, and Royal Reynolds, "Nitrogen Removal in a Subsurface Disposal System," JWPCF 51(4), 841 (1979).
35. Davies, T. R., "Isolation of Bacteria Capable of Utilizing Methane as a Hydrogen Donor in the Process of Denitrification," Water Research 7, 575 (1973).
36. Sollo, F. W., H. F. Mueller, and T. E. Larson, "Denitrification of Wastewater Effluents with Methane," JWPCF 48(7), 1840 (1976).
37. Wilkinson, T. G., and D. E. F. Harrison, "The Affinity for Methane and Methanol of Mixed Cultures Grown on Methane in Continuous Culture," J. Appl. Bact. 36, 309 (1973).
38. Mason, Jan, "Communication--Methane as a Carbon Source in Biological Denitrification," JWPCF 49(5), 855 (1977).
39. Narkis, N., M. Rebhun, and Ch. Sheindorf, "Denitrification at Various Carbon to Nitrogen Ratios," Water Research 13, 93 (1979).
40. Hancher, C. W., et al., "Operation of a Fluidized-Bed Bioreactor for Denitrification," Biotechnol. Bioeng., 8, 361 (1978).

41. McCarty, Perry L., Louis Beck, and Percy St. Ament, "Biological Denitrification of Wastewaters by Addition of Organic Materials," Proceedings of the 24th Industrial Waste Conference, Part 2, Purdue University, 1271 (1969).
42. Buchanan, R. E., and N. E. Gibbons, editors, Bergey's Manual of Determinative Bacteriology, Eighth Edition, Williams and Wilkins Co., Baltimore, 1974.
43. Standard Methods for the Examination of Water and Wastewater, 14th Edition, American Public Health Association, 1976.
44. Manual of Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, 1974.

APPENDIXES

APPENDIX A. SUMMARY OF CHEMICAL OXYGEN DEMAND ANALYSES

Table A-1. Results of Chemical Oxygen Demand Profile Studies (mg/liter)

Phase No.	1	2	3	4	5	6
Column Height, ft						
Influent	2500	2570	1350	1250	640	665
0.5	960	1572	715	730	243	318
1.0	600	994	545	500	183	230
1.5	195	333	160	235	92	107
2.0	185	306	140	160	81	103
2.5	181	210	136	128	76	90
3.0	179	179	134	118	75	92
3.5	(177)	(177)	(132)	(116)	(75)	(88)
4.0	(175)	(175)	(130)	(114)	(75)	(86)
4.5	(173)	(174)	(128)	(112)	(74)	(84)
5.0	(171)	(173)	(126)	(110)	(74)	(82)
5.5	(169)	(172)	(124)	(108)	(74)	(80)
Effluent	167	171	122	106	74	78

NOTE: Numbers in parentheses are extrapolated.

APPENDIX B. SUMMARY OF PHASE NO. 1

Table B-1. Operating Conditions in Phase No. 1*

Parameter	Value
Organic Loading Rate (OLR) lbs COD/1000 ft ³ /d (kg/m ³ ·d)	84.5 (1.35)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² ·s)	0.007 (0.005)
Aerobic HLR gpm/ft ² (liter/m ² ·s)	0.147 (0.100)
Hydraulic Residence Time (HRT), hours	≈41
Aerobic HRT, hours	≈3

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table B-2. Volatile Acids Profile in Phase No. 1*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				
	Acetic	Propionic	Isolutyric	Butyric	Total
Influent	200	125	5	10	335
0.5	400	400	10	20	830
1.0	210	195	5	5	420
1.5	50	20	-	-	70
2.0	20	10	-	-	30
2.5	5	-	-	-	5
3.0	-	-	-	-	-

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table B-3. Data for Performance Analyses in Phase No. 1*

Parameter	Average	Standard Deviation	Range
Gas Production liter/hr	0.83	0.06	0.06-1.00
Percent Methane	71.7	2.07	68.6-79.3
Percent Carbon Dioxide	25.7	2.45	18.3-28.3
Percent Other Gases	2.6	-	-
Flow Rate, ml/min	9.3	2.3	8.5-11.5
pH--Influent	6.5	0.08	6.4-6.6
Effluent	7.2	.099	7.1-7.5
Aerobic pH	8.0	0.46	7.6-8.2
Chemical Oxygen Demand (COD), mg/liter			
Influent	2500	121	2280-2675
Effluent	167	19	150-207
Alkalinity, mg/liter as CaCO ₃			
Influent	1850	150	1500-2000
Effluent	3200	300	2800-3350
NH ₃ N, mg/liter			
Influent	14	7	4-29
0.5 ft	173	19	146-202
1.0 ft	186	26	142-219
1.5 ft	198	26	137-234
2.0 ft	201	29	178-244
2.5 ft	207	30	137-252
3.0 ft	200	34	141-255
3.5 ft	207	31	161-247
4.0 ft	206	29	148-256
4.5 ft	217	33	157-300
5.0 ft	232	41	168-311
5.5 ft	238	44	175-328
Effluent	217	42	160-341

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX C. SUMMARY OF PHASE NO. 2

Table C-1. Operating Conditions in Phase No. 2*

Parameter	Value
Organic Loading Rate (OLR) lbs COD/1000 ft ² ·d (kg/m ³ ·d)	189 (3.03)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² ·s)	0.015 (0.010)
Aerobic HLR gpm/ft ² (liter/m ² ·s)	0.316 (0.215)
Hydraulic Residence Time (HRT), hours	≈20
Aerobic HRT, hours	≈1.5

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table C-2. Volatile Acids Profile in Phase No. 2*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				
	Acetic	Propionic	Isobutyric	Butyric	Total
Influent	155	50	-	-	205
0.5	468	225	50	50	793
1.0	320	134	25	25	504
1.5	141	40	10	10	201
2.0	140	42	5	5	192
2.5	109	18	trace	trace	127
3.0	75	7			82
3.5	73	5			78
4.0	70	3			73
4.5	68	-			68
5.0	60				60
5.5	55				55
Effluent	50				50

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table C-3. Data for Performance Analyses in Phase No. 2*

Parameter	Average	Standard Deviation	Range
Flow Rate, ml/min	20.0	1.66	17.5-22.5
Gas Production, liter/hr	1.8	0.16	1.6-2.0
Percent Methane	66.2	1.27	65.6-68.0
Percent Carbon Dioxide	31.9	1.6	29.6-35.0
Percent Other Gases	1.9	-	-
Chemical Oxygen Demand, mg/liter			
Influent	2619	241	2237-2850
Effluent	156	19	132-183
Aerobic	167	-	-
pH--Influent	6.7	0.55	6.3-7.9
Effluent	7.3	0.08	7.2-7.4
Aerobic pH	7.6	0.12	7.5-7.8
Alkalinity, mg/liter as CaCO ₃			
Influent	1865	179	1690-2180
Effluent	2930	152	2730-3100
Aerobic	2135	207	2010-2500
NH ₃ -N, mg/liter			
Influent	2	2	1-6
0.5 ft	118	28	69-169
1.0 ft	132	26	90-166
1.5 ft	162	33	126-221
2.0 ft	172	33	125-218
2.5 ft	177	30	138-214
3.0 ft	172	35	134-223
3.5 ft	166	33	136-232
4.0 ft	181	41	144-266
4.5 ft	185	37	152-236
5.0 ft	182	45	147-272
5.5 ft	178	47	150-279
Effluent	179	32	141-250
Aerobic	35	3	33-40

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX D. SUMMARY OF PHASE NO. 3

Table D-1. Operating Conditions in Phase No. 3*

Parameter	Value
Organic Loading Rate (OLR) 1bs COD/1000 ft ² /d (kg/m ³ /d)	49.2 (0.78)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² .s)	0.008 (0.005)
Aerobic HLR gpm/ft ² (liter/m ² .s)	0.164 (0.112)
Hydraulic Residence Time (HRT), hours	≈41
Aerobic HLR, hours	≈3

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table D-2. Volatile Acids Profile in Phase No. 3*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				Total
	Acetic	Propionic	Isobutyric	Butyric	
Influent	140	10			150
0.5	225	125			350
1.0	170	100			270
1.5	50	5			55
2.0	5	5			10
2.5	-	-			
3.0	-	-			

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table D-3. Data for Performance Analyses in Phase No. 3*

Parameter	Average	Standard Deviation	Range
Gas Production, liter/hr	0.40	0.04	0.32-0.48
Percent Methane	78.5	1.44	76.2-81.0
Percent Carbon Dioxide	17.4	1.59	15.4-19.8
Percent Other Gases	4.1	-	-
Flow Rate	10.4	0.57	4.5-11.5
Chemical Oxygen Demand, mg/liter			
Influent	1310	270	936-1880
Effluent	121	16	96-155
Aerobic	166	23	143-196
Influent pH	7.3	0.48	6.6-8.2
Effluent pH	7.2	0.20	6.9-7.6
Aerobic pH	7.2	0.28	6.8-7.6
Alkalinity, mg/liter as CaCO ₃			
Influent	2000	300	1700-2200
Effluent	3000	350	2600-3300
Aerobic	1800	200	1650-2000
NH ₃ -N, mg/liter			
Influent	10	5	1-30
0.5 ft	83	19	66-102
1.0 ft	91	21	69-125
1.5 ft	107	20	73-140
2.0 ft	111	22	67-139
2.5 ft	113	24	71-152
3.0 ft	113	24	66-152
3.5 ft	119	26	67-167
4.0 ft	126	28	62-163
4.5 ft	126	28	64-171
5.0 ft	133	28	61-176
5.5 ft	129	30	61-178
Effluent	136	33	104-183
Aerobic	11	8.6	2-25

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX E. SUMMARY OF PHASE NO. 4

Table E-1. Operating Conditions in Phase No. 4*

Parameter	Value
Organic Loading Rate lbs COD/1000 ft ³ /d (kg/m ³ ·d)	86.9 (1.39)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² ·s)	0.016 (0.011)
Aerobic HLR gpm/ft ² (liter/m ² ·s)	0.340 (0.231)
Hydraulic Residence Time (HRT), hours	≈20
Aerobic HRT, hours	≈1.5

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table E-2. Volatile Acids Profile in Phase No. 4*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				Total
	Acetic	Propionic	Isobutyric	Butyric	
Influent	250	150	10		410
0.5	350	280	5	5	640
1.0	210	150	5	10	375
1.5	50	25	-	-	75
2.0	25	30			55
2.5	20	5			25
3.0	20	5			25
3.5	10	-			25
4.0	-				10

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table E-3. Solids Profiles in Phase No. 4*

Height Above Feed Point (ft)	Solids (mg/liter)	
	Total Suspended	Volatile Suspended
Influent	38	35
0.5	285	265
1.0	163	150
1.5	13	15
Effluent	2	5

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table E-4. Data for Performance Analyses in Phase No. 4*

Parameter	Average	Standard Deviation	Range
Flow Rate, ml/hr	21.5	2.1	17.5-22.5
Gas Production, liter/hr	0.66	0.14	0.47-0.86
Percent Methane	76.7	1.93	72.8-79.3
Percent Carbon Dioxide	17.2	2.5	13.7-22.2
Percent Other Gases	6.1	-	-
Chemical Oxygen Demand, mg/liter			
Influent	1236	361	832-1800
Effluent	106	22	71-150
Aerobic	128	-	-
pH--Influent	7.0	0.49	6.4-7.9
Effluent	7.1	0.16	6.9-7.4
Aerobic	7.2	0.19	6.9-7.5
Alkalinity, mg/liter as CaCO ₃			
Influent	1350	412	1000-1800
Effluent	1750	200	1400-2000
Aerobic	1000	300	500-1300
NH ₃ -N, mg/liter			
Influent	13	11	3-37
0.5 ft	76	13	73-93
1.0 ft	83	25	64-118
1.5 ft	91	23	61-116
2.0 ft	90	19	58-106
2.5 ft	101	22	66-122
3.0 ft	99	23	67-123
3.5 ft	98	21	64-115
4.0 ft	101	19	69-117
4.5 ft	107	20	72-134
5.0 ft	105	22	71-127
5.5 ft	107	21	72-130
Effluent	101	18	63-122
Aerobic	18	11	6-44

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX F. SUMMARY OF PHASE NO. 5

Table F-1. Operating Conditions in Phase No. 5*

Parameter	Value
Organic Loading Rate ₃ 1bs COD/1000 ft ³ /d (kg/m ³ ·d)	23.5 (0.38)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² ·s)	0.015 (0.010)
Aerobic HLR gpm/ft ² (liter/m ² ·s)	0.163 (0.111)
Hydraulic Residence Time (HRT), hours	≈41
Aerobic HRT	≈3

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table F-2. Volatile Acids Profile in Phase No. 5*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				Total
	Acetic	Propionic	Isobutyric	Butyric	
Influent	110	0			110
0.5	80	30			110
1.0	85	20			105
1.5	40	5			45
2.0	35	0			35
2.5	30				30
3.0	25				25
3.5	10				10
4.0	-				-

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table F-3. Data for Performance Analyses in Phase No. 5*

Parameter	Average	Standard Deviation	Range
Flow rate, ml/min	10.3	0.84	9.0-11.0
Gas Production, liter/hr	0.18	0.04	0.11-0.24
Percent CH ₄	78.0	0.79	76.8-78.9
Percent CO ₂	14.8	0.98	13.4-16.6
Percent Other Gases	7.2	1.18	5.7-9.4
Chemical Oxygen Demand, mg/liter			
Influent	631	85	570-760
Effluent	70	7	61-81
Aerobic	64	8	52-77
pH--Influent	7.5	0.33	7.1-8.0
Effluent	7.5	0.74	7.4-7.6
Aerobic	7.6	0.12	7.4-7.8
Alkalinity, mg/liter as CaCO ₃			
Influent	1961	137	1750-2150
Effluent	2368	88	2230-2470
Aerobic	1926	61	1820-2010
NH ₃ -N, mg/liter			
Influent	3	3	1-11
0.5 ft	46	6	40-56
1.0 ft	50	6	42-62
1.5 ft	56	6	48-65
2.0 ft	65	5	58-70
2.5 ft	67	6	58-80
3.0 ft	64	5	58-74
3.5 ft	66	5	58-72
4.0 ft	69	4	63-75
4.5 ft	72	5	64-79
5.0 ft	73	5	64-78
5.5 ft.	74	6	63-81
Effluent	76	12	56-90
Aerobic	3	0.99	2-5

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX G. SUMMARY OF PHASE NO. 6

Table G-1. Operating Conditions in Phase No. 6*

Parameter	Value
Organic Loading Rate lbs COD/1000 ft ³ /d (kg/m ³ .d)	45.7 (0.732)
Hydraulic Loading Rate (HLR) gpm/ft ² (liter/m ² .s)	0.015 (0.010)
Aerobic HLR gpm/ft ² (liter/m ² .s)	0.317 (0.215)
Hydraulic Residence Time (HRT), hours	≈20
Aerobic HRT, hours	≈1.5

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table G-2. Volatile Acids Profile in Phase No. 6*

Height Above Feed Point (ft)	Volatile Acids (mg/liter)				Total
	Acetic	Propionic	Isobutyric	Butyric	
Influent	100	75	5		180
0.5	150	50	-		200
1.0	75	20			95
1.5	25	15			40
2.0	15	-			15
2.5	-				-
3.0					

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

Table G-3. Data for Performance Analyses in Phase No. 6*

Parameter	Average	Standard Deviation	Range
Flow Rate, ml/min	20.0	1-19	18.4-21.6
Gas Production, liter/hr	0.35	0.58	0.25-0.39
Percent CH ₄	78.6	2.3	75.9-83.4
Percent CO ₂	14.1	2.4	9.9-18.8
Percent Other Gases	7.3	-	-
Chemical Oxygen Demand, mg/liter			
Influent	632	101	438-766
Effluent	79	7	68-86
Aerobic	58	2	56-60
pH--Influent	7.5	0.39	6.7-8.1
Effluent	7.4	0.17	7.2-7.6
Aerobic	7.8	0.37	7.3-8.2
Alkalinity, mg/liter as CaCO ₃			
Influent	2400	318	2170-2650
Effluent	2500	300	2320-2750
Aerobic	2210	-	-
NH ₃ -N, mg/liter			
Influent	10	5	1-29
0.5 ft	46	2	42-48
1.0 ft	49	5	43-54
1.5 ft	58	6	50-67
2.0 ft	60	5	55-69
2.5 ft	63	7	56-75
3.0 ft	64	6	58-72
3.5 ft	62	9	53-78
4.0 ft	65	9	54-81
4.5 ft	65	9	54-83
5.0 ft	65	9	58-82
5.5 ft	66	9	59-85
Effluent	65	10	54-90
Aerobic	9	7	1-28

*All values pertain to the ANFLOW bioreactor except where aerobic (bioreactor) is mentioned. All aerobic data are for effluent only.

APPENDIX H. CALCULATIONS

- Organic Loading Rate (OLR)

$$\begin{array}{c} \text{Average influent} \\ \text{chemical oxygen demand} \\ \text{(COD), g/liter} \end{array} \times \begin{array}{c} \text{Average flow} \\ \text{rate, ml/min} \end{array} \times \frac{1 \text{ lb}}{454 \text{ g}} \times \frac{60 \text{ min}}{\text{hr}} \times \frac{24 \text{ hr}}{\text{d}}$$

$$\text{OLR} = \text{lbs COD/d/ft}^3 \times 1000$$

$$= \text{lbs COD/d/1000 ft}^3$$

$$\text{Bioreactor volume} = 6 \text{ ft by } 8 \text{ in. diameter}$$

$$= 2.09 \text{ ft}^3$$

$$\text{Hydraulic Residence Time (HRT) from dye test found to be 41 hours}$$

$$\text{Bioreactor volume} = (\pi r^2 L) (\text{void fraction})$$

$$\theta = \frac{V(\text{v.f.})}{Q}$$

$$\text{where } \theta = \text{detention time, hours}$$

$$V = \text{volume, liters}$$

$$Q = \text{flow, liters/hr}$$

$$\text{v.f.} = \text{void fraction}$$

$$41 \text{ hours} = \frac{(2.09 \text{ ft}^3)(\text{v.f.}) (28.32 \text{ liters/ft}^3)}{\text{liters/d}}$$

$$\text{using } 0.010 \text{ liters/min} = 0.6 \text{ liters/hr}$$

$$\text{v.f.} = 0.42$$

$$2.09 \text{ ft}^3 \times 0.42 = 0.8778 \text{ ft}^3 \text{ bioreactor volume}$$

$$1 \text{ bs COD/1000 ft}^3 \div 0.8778 = 1 \text{ bs COD/1000 ft}^3/\text{d for ANFLOW bioreactor}$$

$$\text{OLR} \times 0.01602 = \text{kg/m}^3 \cdot \text{d}$$

- Hydraulic Loading Rate (HLR)

$$\text{Average flow rate} \div \text{bioreactor cross section}$$

$$= \text{gpm/ft}^2$$

$$\times 0.67902 = \text{liters/m}^2 \cdot \text{s}$$

- $\text{ft}^3 \text{CH}_4/\text{lb COD removed}$

$$(\text{liters/d gas} \times 0.0353 \times \% \text{CH}_4) \div$$

$$(\text{g/liter average influent COD} \times \% \text{ removal} \times 1 \text{ lb}/454 \text{ g} \\ \times \text{average flow rate liter/min} \times 60 \text{ min/hr} \times 24 \text{ hr/d})$$

- Organic-Nitrogen Loading Rate

$$\begin{array}{l} \text{influent} \\ \text{organic-N, g/liter} \end{array} \times \begin{array}{l} \text{average flow} \\ \text{rate, ml/min} \end{array} \times \frac{1 \text{ liter}}{1000 \text{ ml}} \times 60 \text{ min/hr} \times 24 \text{ hrs/d}$$

$$= \text{grams/d}$$

VITA

Debby Hines was born April 15, 1952, in Knoxville, Tennessee. She later lived in Indiana where she attended elementary and high schools. She graduated from high school in May 1970. She enrolled at Indiana University in August 1970 and graduated in August 1974 receiving a Bachelor of Arts degree in Biological Sciences.

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