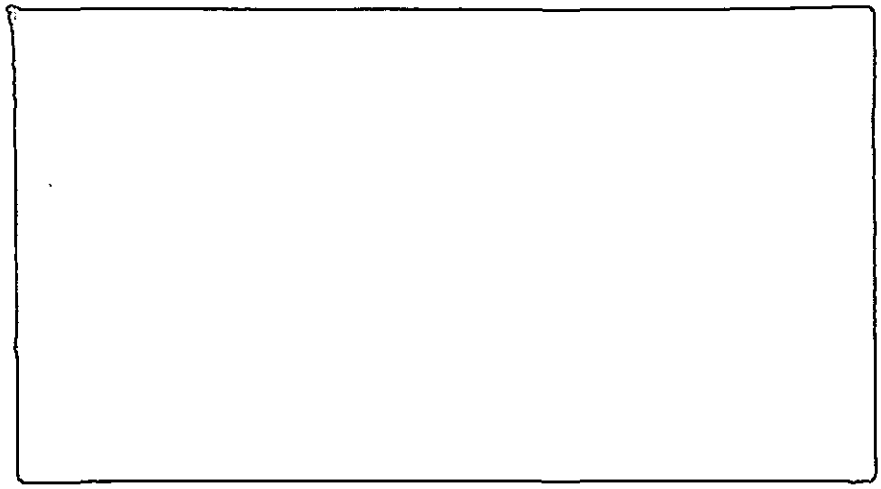
Environmental Engineering



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THE EFFECTS OF TEMPERATURE AND NUTRIENT-LIMITATION ON AN ANAEROBIC FILM EXPANDED BED REACTOR TREATING A HIGH STRENGTH WASTE

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ABSTRACT

The influence of temperature and trace nutrients on the anaerobic film expanded bed process were studied in this investigation. A reactor was constructed and operated in the mesophilic temperature range. Whey, a dairy industry waste product, was supplied to the reactor at an influent concentration of 10 g/l and at a constant HRT. Experimental results show that temperature had relatively little impact on reactor performance as defined by COD removal. Activation energies determined from Arrhenius temperature dependence plots were found to be on the order of 2000-3000 cal/mole. Q_{10} values were found to be on the order of 1.2 which suggests the overall reaction is diffusion limited. Based on the above criteria, the anaerobic film expanded bed process was compared to the anaerobic slurry and activated sludge processes and found to be far less temperature dependent.

Trace nutrients were found to significantly influence reactor performance. Whey powder supplemented with nitrogen and phosphorus, was found to be nutrient-limited by either Ni, Fe, or Co, or some combination of those elements. After the addition of the above elements to the reactor feed, COD removal efficiencies increased and volatile organic acids decreased. Previous anaerobic studies with the same batch of whey but with different dilution water were very successful and it was assumed that most cheese wheys contained these essential elements. However, results from this study demonstrate that careful attention to nutrient requirements must be made for successful anaerobic industrial waste treatment.

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CHAPTER I

INTRODUCTION

Anaerobic processes have been utilized in wastewater treatment for at least 100 years (53). However broad scale application of anaerobic treatment has been limited to stabilization of municipal and industrial sludges and agricultural residues (53, 83). Recently new anaerobic process configurations have been engineered and are finding use in many facets of biological water and wastewater treatment. One such process is the anaerobic expanded bed (AFEB) reactor.

Since 1974, the AFEB process has seen development from bench scale tests through pilot scale operation and several full scale designs. The process has proven successful for the treatment of many industrial wastes at both high and low concentrations on lab scale reactors. Currently, the AFEB process is being investigated for the treatment of municipal wastewater at the pilot scale level (80). Switzenbaum and Danskin (81) found the AFEB process to be a viable alternative for the disposal of raw whey produced in the cheese manufacturing process. They were able to achieve a soluble chemical oxygen demand (COD) removal efficiency of approximately 87 percent at a hydraulic retention time of 12 hours and an influent whey concentration of 10,000 mg/l. Hickey and Owens (31) found the AFEB process to be a successful treatment method for wastewater from four different industries involved in dairy and chemical products, and from food processing and

soft drink bottling. They were able to achieve more than a 90 percent BOD_5 removal efficiency with the AFEB process in all cases. They were also able to achieve the same results with heat treatment liquor produced from the thermal conditioning of primary and waste-activated sludge. Likewise, Sutton and Li (78) were able to attain significant COD reductions with the AFEB process in treating several industrial effluents including wastewater from both the cheese and soy processing industires. In addition to the above treatability studies, earlier work by Switzenbaum and Jewell (83) demonstrated that the AFEB process was capable of successfully treating a low strength waste over a wide variety of organic volumetric loading rates and at reduced temperatures.

Common to the above studies is the evaluation of process performance as a function of influent concentration and hydraulic retention time. Except for the study by Switzenbaum and Jewell (83), the evaluation of process performance as a function of temperature has been limited.

The purpose of the study is to more accurately define the effect of temperature on the AFEB process treating a high strength waste and to compare the results obtained to others in the literature. Specific objectives include:

1) The evaluation of the effect of temperature on an AFEB reactor operating at a constant hydraulic retention time and influent substrate concentration.

2. The comparison of the effect of a nutrient limited substrate and a nutrient supplied substrate on the performance of the AFEB reactor.
3. The determination of activation enzymes with the Arrhenius expression and comparison of the values obtained to other biological systems.
4. The calculation and comparison of Q_{10} values.
5. The monitoring of the AFEB reactor at different temperatures to compare gas production rates, biomass concentration, suspended solids, and volatile acids concentration.

CHAPTER II

Background

2.1. Microbiology of anaerobic digestion. Anaerobic digestion involves the conversion of organic matter to methane and carbon dioxide by bacterial action. The process is complex, includes several types of bacterial populations, and contains many, as yet, ill-defined ecological niches. The bacteria associated with the process represent at least three groups - the fermentative bacteria, the H_2-producing acetogenic bacteria, and the methanogenic bacteria (15). A diagram of the fermentative process is shown in Figure 1.

In the anaerobic digestion process, biodegradable organic compounds are first hydrolyzed and degraded to yield a multitude of simplier compounds such as organic acids, carbon dioxide, and hydrogen gas. These compounds are subsequently converted to CH_4 and more CO_2 (27). The bacteria responsible for the initial breakdown of the more complex compounds are collectively referred to as nonmethanogenic bacteria. The nonmethanogenic population consists of two groups of bacteria, the fermentative or acid forming bacteria and the hydrogen-producing acetogenic bacteria. The third group of bacteria, the methanogens, carry out the production of methane gas.

The process of anaerobic digestion proceeds first with the hydroloysis of macromolecular compounds. The hydrolyzed products are used as substrates by the acid forming organisms. Metabolic end-products from the acid forming organisms include hydrogen, acetate, carbon dioxide, propionate, butyrate, and valerate. The latter organic

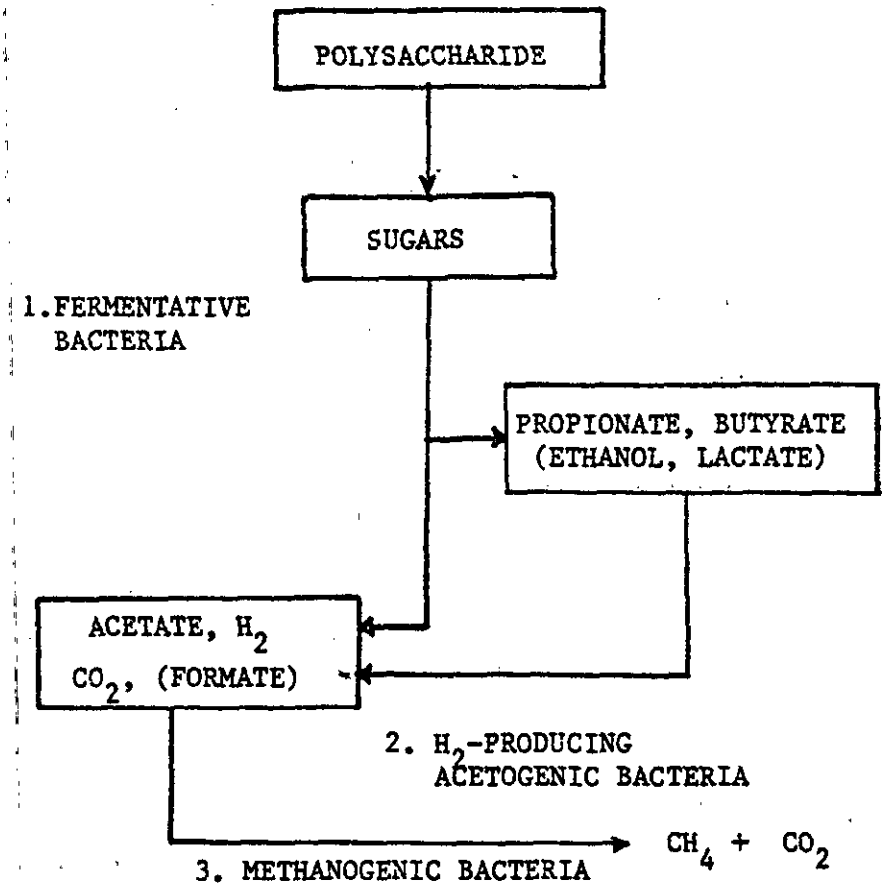

Figure 1. A scheme showing the three general metabolic groups of bacteria in methane fermentation (after Bryant (15)).

acids, along with ethanol and lactate, are converted to acetate, hydrogen, and carbon dioxide by the hydrogen producing acetogenic bacteria (Bryant, 15).

The H_2-producing acetogenic bacteria play an important role in the anaerobic digestion process. As stated above, their metabolic end-products include acetate and hydrogen. Acetate is an immediate precursor to methane production. Hydrogen is also required by methanogenic bacteria but high levels of hydrogen inhibit methanogenesis. McCarty (51) has reported that although only a few methanogens can utilize acetate as a substrate, it is the immediate precursor of 72 percent of the methane produced through the reduction of a complex waste (Figure 2). Kirch and Sykes (39) have reported that the accumulation of H_2 may have an inhibitory effect on the conversion of acetate to methane and the oxidation of propionate. Bryant (15) has suggested that H_2 is a major regulator of the anaerobic process. If the partial pressure of H_2 increases above a certain level due to stresses on the methanogens, the catabolism of pyruvate to acetate, CO_2, and H_2 decreases resulting in a buildup of propionate, butyrate, and valerate. Since the methanogens are stressed, the organic acids will accumulate resulting in the breakdown of the fermentative process.

The third group of organisms, the methanogenic bacteria, are very substrate specific and live in commensal interaction with the nonmethanogenic organisms; they depend on the first group of organisms for their substrate. At one time methanogenic bacteria, were classified according to substrate, as presented in Table 1. Methanogens obtain energy for growth from electrons generated in their oxidation

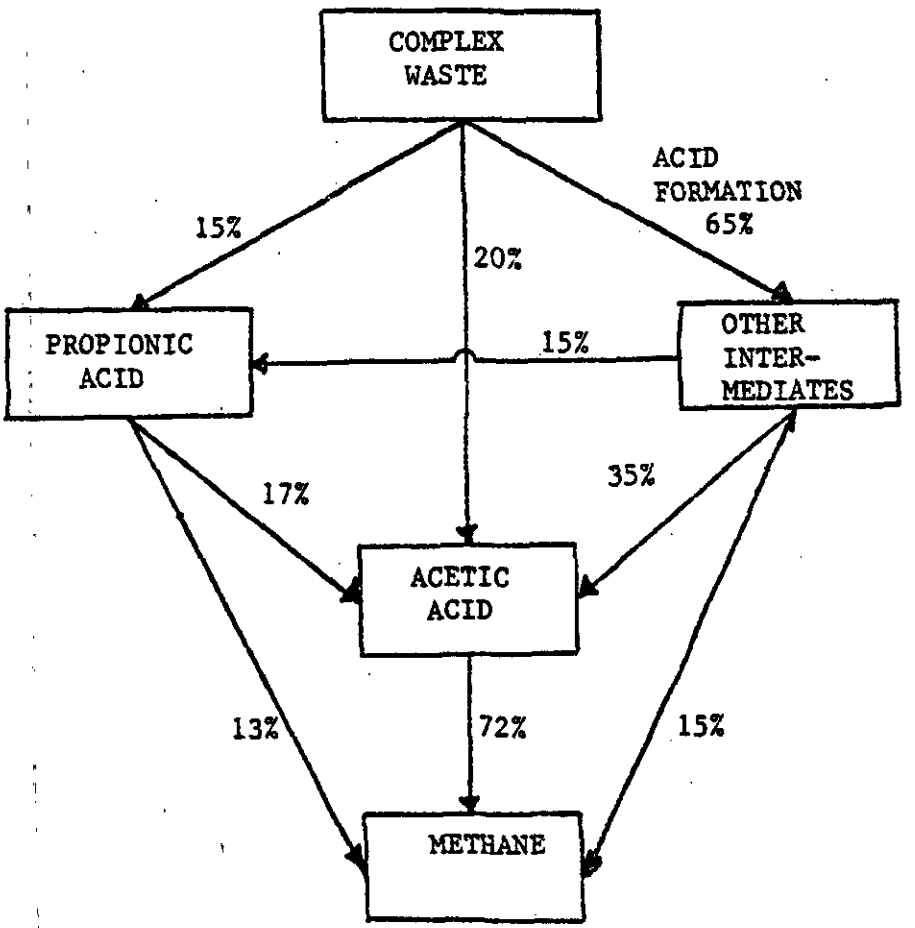

Figure 2. Pathways in methane fermentation of complex wastes (after McCarty (51)).

Table 1. Methane Bacteria Classified According to Substrate (after Barker (9))

I. Rod-shaped cells

Non-sporulating: Methanobacterium

1. Mbact. formicium: formate, CO, H_2
2. Mbact. propionicum: propionate
3. Mbact. sohngenii: acetate, butyrate

II. Spherical cells

Non-sarcina arrangement: Methanococcus

1. Mc. mazei: acetate, butyrate
2. Mc. vannielii: formate, H_2

Sarcina arrangement: Methanosarcina

1. Ms. barkerii: methanol, acetate, Co, H_2
2. Ms. methanica: acetate, butyrate (?)

of H_2 (83). The common factor relating methanogens is methane production.

Methanogens isolated from different environments tend to be unrelated morphologically and range from minute cocci and larger sarcina to individual and chain forming bacilli (39). Mutualism is a common and interesting interaction within the methanogenic group. For example, it was thought for many years that Methanobacterium omelianskii existed in pure culture. It was later discovered that M. omeliamskii exists as a mutalistic interaction between two rod-shaped bacteria. The methane producing organism oxidizes gaseous hydrogen with the subsequent reduction of CO_2 to CH_4. Its counterpart oxidizes methanol to acetic acid and hydrogen gas. The counterpart is, however, inhibited by hydrogen gas and depends on the methane producing organism to keep the concentration low. The methane producing organism, in turn, depends upon its associate for a supply of hydrogen (27).

Recently it has become evident that the methanogens are clearly a unique group of protists. Balch et al. (8) have shown that the methanogens are phylogenetically distinct from typical procaryotes, and it has been proposed by Woese and Fox (90) that the methanogens be classified as members of the archaebacteria, a discrete biological grouping.

In the past, methanogens have been considered very sensitive obligate anaerobes. However, new evidence is emerging which indicate they are hardier than previously believed. Taylor (84) has reported

that the methanogens may not deserve their label as the most sensitive microorganisms in the microbial consortium.

The delicate balance among the mutualistic groups of bacteria is the biggest source of trouble in the process of anaerobic digestion and often leads to difficulty in the operation of digesters. Even though anaerobic digestion is a complex process, it has been said, that with few exceptions, most wastes susceptible to aerobic treatment can also be treated anaerobically (51).

2.2. Microbial films. Atkinson and Davies (3) have suggested that any surface in contact with a nutrient medium containing microorganisms will eventually become biologically active due to the adhesion of microorganisms from the bulk solution. The formation of attached microbial film in a fixed film reactor is a pre-requisite for successful waste treatment.

A microbial film consists of a gelatinous mass of microorganisms stuck together by extracellular secretions of tangled polysaccharide fibers. The polysaccharide fibers extend from the surface of the microorganism and form a felt-like "glycocayx" surrounding an individual cell or a colony of cells and enables the microorganisms to adhere to solid surfaces (83). Costerton et al. (20) have suggested that the glycocalyx is essential to the biological success of most bacteria in most of the natural environments in which they are observed. Costerton et al. (20) have also reported a "consortium" effect relating the adherence of a particular bacterial species to a favorable niche close to the source of a necessary nutrient.

Microbial films have been shown to form in conditions of both low and high substrate concentrations (83, 94). Nordin et al. (62) showed the importance of ionic strength to microbial adhesion on solid surfaces. In a later study, Atkinson and Fowler (5) emphasized the importance of pH in determining the formation of microbial films. They concluded that microbial film formation involved complex biological and physiochemical factors and that there is no single explanation for the phenomenon of adhesion of microorganisms to surfaces.

2.3. The AFEB process. A generalized schematic of the anaerobic film expanded bed (AFEB) process is shown in Figure 3. The process consists of a column of inert sand sized particles (approximately 500 μm) which expand as a result of the upward direction of the recycle flow. The inert particles provide a support surface for the growth of microorganisms. Since the support particles are small, the system has a large surface area to volume ratio and can maintain a large population of bacterial mass. The AFEB process is also a completely mixed system and provides excellent contact between biomass and substrate. Since the microorganisms are attached, the system enables long solid retention times with concomitant short hydraulic retention times. Switzenbaum (79) and Meunier and Wilson (57) have described the expanded bed process as an optimal biological reactor in terms of efficiency.

The AFEB process is similar to the fluidized bed process in chemical engineering. The theory and application of fluidized beds

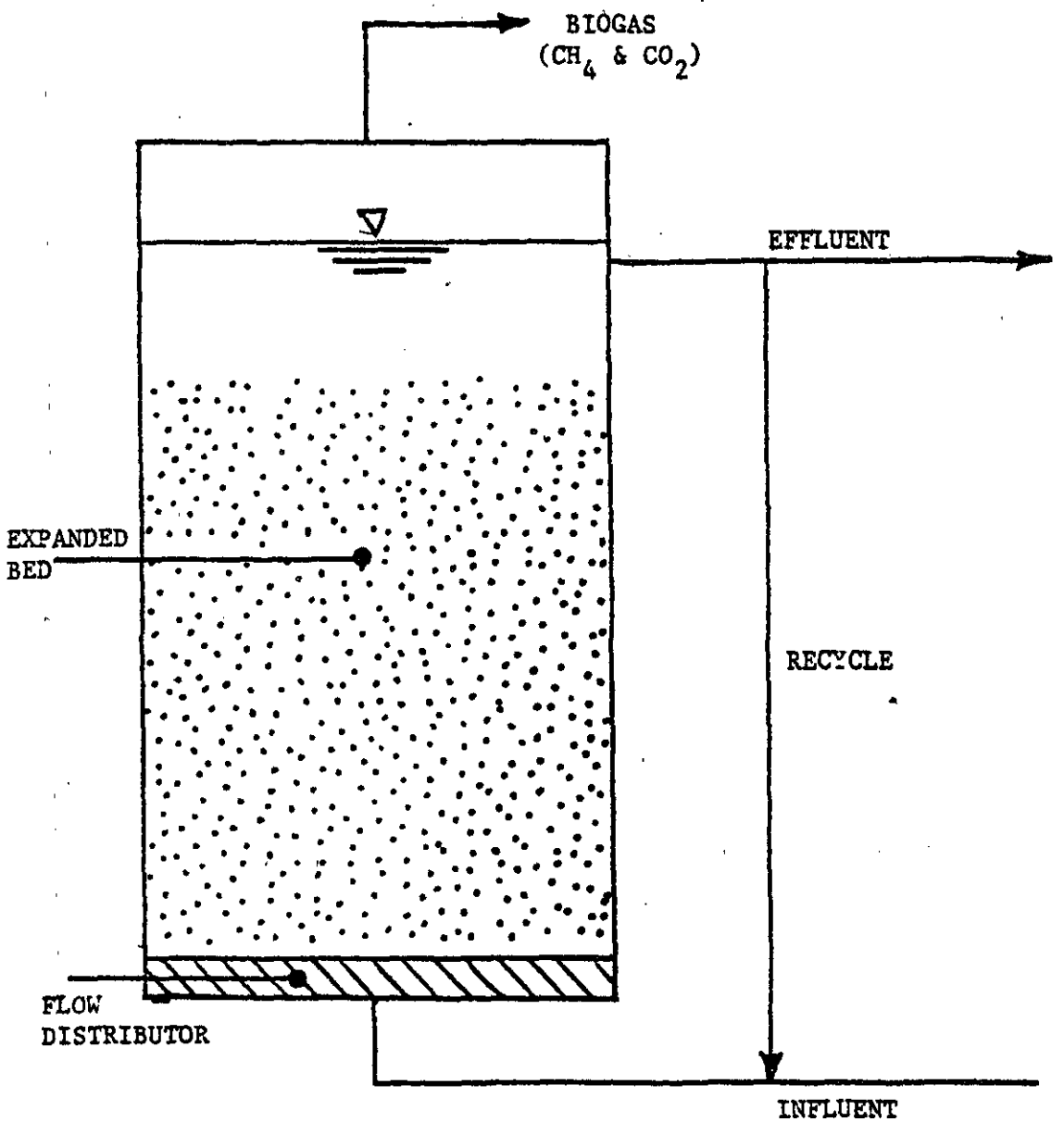

Figure 3. Generalized schematic of the anaerobic film expanded bed process.

have been presented in several text and reference books (Zenz and Othmer (92), McCabe and Smith (50), Perry and Clinton (66), Bennet and Myer (12)). Generally, the fluidized bed process refers to gas-solids contacting where fluidization is accomplished by the movement of a gaseous stream through a bed of solid particles. The process is commonly employed in combustion systems.

In most cases fluidization refers to a more than doubling of the static bed volume (79). Reactors that have a smaller degree of expansion have been termed "expanded beds" (79, 81, 83). The term "expanded bed" assumes an additional meaning in biological systems. Since biomass grows on the support media, the particles become less dense, and at a given recycle flow rate, the bed volume expands to a greater degree. Thus aerobic systems, with higher biomass yields, would cause a greater bed expansion than lesser yielding anaerobic systems (79). In this study the terms expanded and fluidized are synonomous and refer to the general process shown in Figure 3.

2.4. Biofilm in fixed-film reactors. Biofilm thickness is an important parameter in the operation of fixed-film reactors. Thick films tend to slough off as organisms nearest the support surface are starved of nutrients (83). The starvation results in unwanted endogenous respiration or even the production of toxic end products which cause the film to detach from the support surface (4).

Thin films have been regarded as more efficient for waste conversion. Hawkes (29) and McKinney (55) have observed that maximum efficiency in trickling filters occurs with thin films. The

qualitative statements of the two authors have been supported by other investigators (32, 41, 58, 68, 86) as reported estimates of effective film depth ranges from 0.7 μm to 120 μm (83).

An advantage of the AFEB process is the maintenance of a thin film due to particle abrasion. Atkinson and Davies (3) report that in the fluidized bed process frequent particle to particle contacts occur which causes the film to maintain a dynamic steady state between growth and attrition of microbial mass. Thus thick biofilms do not develop and whenever other parameters (e.g. environmental conditions and substrate flux) are maintained at steady state, the support particles contain a near constant biomass.

2.5. Development of the AFEB process. The origins of the anaerobic film expanded bed process extend to bioengineering technology. Barker et al. (10) are cited as using a fluidized bed reactor for starch hydrolysis. Other investigators have reported that the fluidized bed process is applicable to immobilized enzyme technology and growth associated systems (Cheryon et al. (18); Lieberman and Ollis (47); O'Neill et al. (64); Atkinson and Davies (3)).

Cooper and Wheeldon (19) have reviewed the development of the expanded bed process for wastewater treatment. They report that the process derived from independent work on denitrification by Jeris et al. (33) and by Baily and Thomas (7).

The application of the expanded bed process for anaerobic treatment is attributed to work conducted in the laboratory of Dr. William J. Jewell at Cornell University (80). Comparing film and

slurry reactors, Jewell and Mackenzie (35) were able to achieve twice the organic removal capacity in the film system under similar conditions. Subsequently, work by Leuschner (45) demonstrated that the expanded bed process would work under anaerobic conditions. Later, Jewell and coworkers demonstrated the AFEB process was capable of treating dilute synthetic organic wastewater (83), domestic sewage (36), and dairy cow manure (34). Switzenbaum and Danskin (81), Hickey and Owens (31), and Sutton and Li (78) have contributed to the development of the AFEB process by demonstrating that it is capable of treating various industrial wastes at different operating conditions. Very recent work by Schraa and Jewell (71) has shown that the AFEB process is capable of converting soluble organic substrate to methane and carbon dioxide under thermophilic conditions.

2.6. Temperature effects on the anaerobic digestion process. Temperature is an important factor determining the rate of biological activity and is therefore an important environmental requirement for biological treatment processes. Brock (14) states that temperature can affect living organisms in two opposing ways. As temperature rises, the rate of chemical and enzymatic activity in the cell increases and growth becomes faster. On the other hand, many cellular components such as proteins and nucleic acids are sensitive to high temperatures and may be irreversibly inactivated.

Temperature effects in anaerobic digestion processes are particularly important due to interacting bacterial populations. Since different species of bacteria have different optimal temperatures, they

respond to temperature change in qualitatively similar but quantitatively dissimilar ways. Data in the literature indicate that the methanogenic bacteria are relatively temperature sensitive. Lawrence and McCarty (44) found the maximum specific growth rate for methanogens in an acetate reactor to decrease from 8.1 mg/mg-day at 35°C to 4.8 mg/mg-day at 30°C.

Little quantitative information exists on the effect of temperature upon the nonmethanogenic bacteria. O'Rourke (65) found lipid degradation to be reduced in a sewage sludge digester operating at 15°C. However, he found significant removal at 25°C and 20°C. This suggests that the lipid-degrading bacteria are also sensitive to low temperature.

The sensitivity of anaerobic suspended growth systems to temperature is well documented and often cited as a major disadvantage (27, 51, 56, 93). Speece and Kem (73) reported that a drop in temperature from 35°C to 27°C reduced the methane production rate in an anerobic slurry system by 80 percent. Further, an anaerobic slurry system that has been developed at one temperature is likely to have a different balance of microorganisms than a reactor developed at another temperature. Changes of only a few degrees may cause a major imbalance in the microbial population which can lead to process failure (27).

In contrast, data pertaining to the AFEB process indicate the system is capable of functioning at reduced temperatures. Switzenbaum and Jewell (83) found the process to attain high organic removal

efficiencies down to 10°C. Though temperature was found to be an important variable affecting process efficiency, the process was shown to compensate well for changes in temperature. Even at reduced temperatures, they found the process to respond well to shock loadings of two to three times the normal influent concentration (36).

Likewise, Hickey and Owens (31), in whey treatability studies, found the COD removal efficiency to decrease by only eight percent in an AFEB reactor when the temperature was reduced from 35°C to 24°C.

2.7. Nutrient requirements of the anaerobic digestion process. Early work by Sawyer (69) documented nutrient requirements concerning carbon, nitrogen, and phosphorus in aerobic biological treatment. Since Sawyer's work, considerable information has been gained by others concerning the role of nutrients in aerobic waste treatment. However, it has only been in the recent past that the role of nutrients in the anaerobic process has been closely examined. The lack of information has been attributed to the difficulty of growing pure cultures of methanogens in pure and relatively simple substrates.

Speece and McCarty (74) cite work from a number of investigators that describe the need of a growth promoting substance for methane bacteria. Huekelekian and Heineman (30) concluded from experiments involving the seeding of digesters that the chief value of digested sludge was in the production of favorable environmental conditions and not in the number of bacteria added. Stander (68) studied the treatability of winery waste by anaerobic digestion and found that periodic additions of raw sewage sludge was necessary to operate the

process at high loading rates. This indicated that some growth promoting substances were present in the sludge but not in pure winery waste. In studies which involved the digestion of acetic acid, McCarty and Vath (54) found it was necessary to add supernatant liquor solids from a domestic sewage sludge digester.

An indepth study by Speece and McCarty (74) which concerned the accumulation of biological solids in anaerobic digestion, found the addition of inorganic salts alone to pure organic substrates enabled satisfactory digestion. In a preliminary investigation to their study, the authors performed experiments to determine if some selected pure compounds would promote satisfactory acetate digestion in a reactor purged of the original seed sludge. Of the compounds studied, they found thiamine, proline, gylcine, benzimidazole, cobalt chloride, and ferric chloride to yield positive stimulation and increase acetate utilization rates. Glycine, proline, benzimidazole and cobalt indicated vitamin B_{12} as a stimulatory compound but additions of vitamin B_{12} failed to stimulate acetate digestion.

For their biological solids accumulation experiment, a series of anaerobic reactors were supplied a substrate that was either a carbohydrate, a protein, or a fatty acid. In addition to one of the above carbon sources, the following inorganic salts were added: $NaHCO_3$, $(NH_4)\ HPO_4$, $MgSO_4$, NH_4Cl, KCl, $MgCl_2$. Then stimulants from the preliminary acetate utilization study were added and reactor performance monitored. Of the list of stimulants, it was found necessary only to add $FeCl_3$ along with the above inorganic salts to obtain satisfactory digestion.

Other investigators have recently begun to examine the role of various trace elements required by methanogenic bacteria. Jones and Stadman (38) studied the effects of selenium and tungsten on Methanococcus vaniellii and found both elements to have a stimulatory effect on growth and on levels of formate dehydrogenase activity of the cells. Taylor and Pritt (85) found iron and nitrogen sources to be growth limiting in a culture of Methanobacterium thermoautotrophicum. Schonheit et al. (70) studied the growth requirement of Methanobacterium autotrophicum for nickel, cobalt, and molybdenum. Murray and van den Berg (61) studied the effect of nickel, cobalt, and mobybdenum on the performance of a methanogenic fixed film reactor. They found that single additions of nickel and cobalt, but not molybdenum, stimulated the conversion of acetic acid to methane and carbon dioxide. In combination tests, they found nickel and cobalt especially stimulated performance, and the addition of molybdenum to slightly stimulate reactor performance. Work by Diekert et al. (22, 23, 24, 25) has proved to be particularly important in determining nickel dependence in a coenzyme possibly unique to methanogens. Diekert et al. (23) found that nickel is an essential element in factor F_{430} and that iron, cobalt and molybdenum are not involved. Of specific interest is the work by Speece et al. (75) involving nickel stimulation in anaerobic digestion. They found that nickel, in combination with other supplements significantly increased the acetate utilization rate of their anaerobic digester to 51 g/l-day (as compared to 3.3 g/l-day for conventional high-rate digestion). In the absence of nickel, the maximum acetate utilization rate was 15 g/l-day.

C H A P T E R I I I

Experimental Procedure

3.1. Scope of study. The purpose of this research was to examine the effects of temperature and nutrient limitation on an anaerobic film expanded bed (AFEB) reactor treating a highly concentrated influent substrate. The results of the study would be useful for comparing the AFEB process to other wastewater treatment processes and for comparison to a previous study which involved the effects of temperature, and organic and hydraulic loading rates on the AFEB process treating dilute organic wastes (83).

Reactor performance was evaluated at seven different temperatures (54, 49, 44, 35, 30, 25, and 20°C) for a nutrient-limited substrate. Reactor performance was evaluated at three different temperatures (35, 30, and 25°C) for a nutrient-supplied substrate.

This study was conducted in the laboratory at the bench scale level.

3.2. Experiment Design. Figure 4 is a schematic of the experiment. To maintain temperature control, the AFEB reactor was housed in an incubator (Fisher Low Temperature Incubator Model 300). Influent substrate was continuously pumped to the reactor at an average rate of 614 cm^3 per day with a Cole-Parmer Model 7565 Masterflex pump. The influent substrate was constantly stirred with a magnetic stirrer and stored in a refrigerator. Gases and liquid effluent left the reactor in separate lines and were collected outside of the incubator.

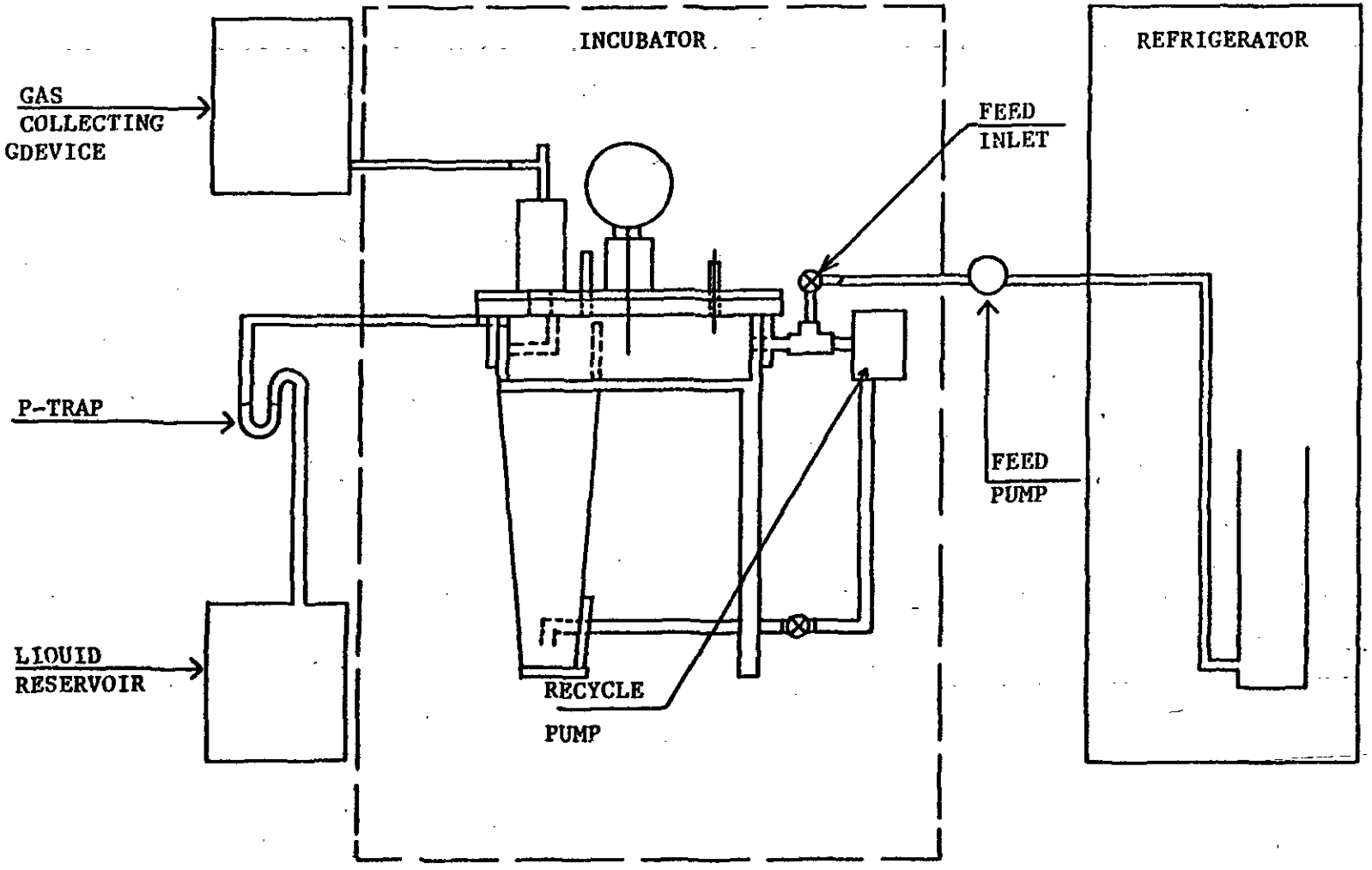

Figure 4. Experiment design.

Gas produced by the reactor was collected and measured daily in a gas collection device specially constructed for the experiment. To assure the maintenance of anaerobic conditions in the reactor, the liquid effluent passed through a P-trap before being collected in the reservoir.

3.2.1. Reactor design. A schematic of the AFEB reactor is shown in Figure 5 and reactor dimensions are listed in Table 2. The reactor was constructed from 3/8-inch thick cast acrylic (ANSI Z97.1-1972) manufactured by the Polycast Technology Corporation, Stamford, Connecticut. Total reactor volume was 2729 cm^3. The tapered portion of the reactor resembled an inverted pyramid, contained a volume of 1012 cm^3, and housed 368 cm^3 of aluminum oxide particles which served as the support media for the biofilm.

Attached above the tapered portion of the AFEB reactor was a sealed tank which served as a reservoir for the recycle pump. The recycle reservoir tank was also equipped with:

(a) an effluent weir which protected the effluent discharge line from clogging, stabilized effluent flow, and directed recycle flow towards the recycle pump inlet;

(b) a separating weir which extended the hydraulic column above the tapered portion of the reactor and prevented smaller aluminum oxide particles from being entrained in the recycle flow;

Table 2. Reactor Dimensions

Total Overall Dimensions:	Length 32 cm
	Height 55 cm
	Width 15 cm
Recirculation Tank:	Length 25.2 cm
Dimensions(inside)	Width 8.0 cm
	Depth 8.7 cm
Recirculation Tank Total	
Liquid Volume:	1717 cm^3
Tappered Tank:	Height 27.8 cm
	Base 3.3 x 3.3 cm
	Top 8.4 x 8.4 cm
Tappered Tank	
Total Liquid Volume:	1012 cm^3
Height of Expanded Bed:	21 cm
Volume of Expanded Bed:	605 cm^3

NOTE: Expanded bed volume was calculated from

$$\text{volume} = \frac{1}{3} H \left(A_B + A_T + \sqrt{A_B \cdot A_T}\right)$$

where: H = height

A_B = area of the base

A_T = area of the top

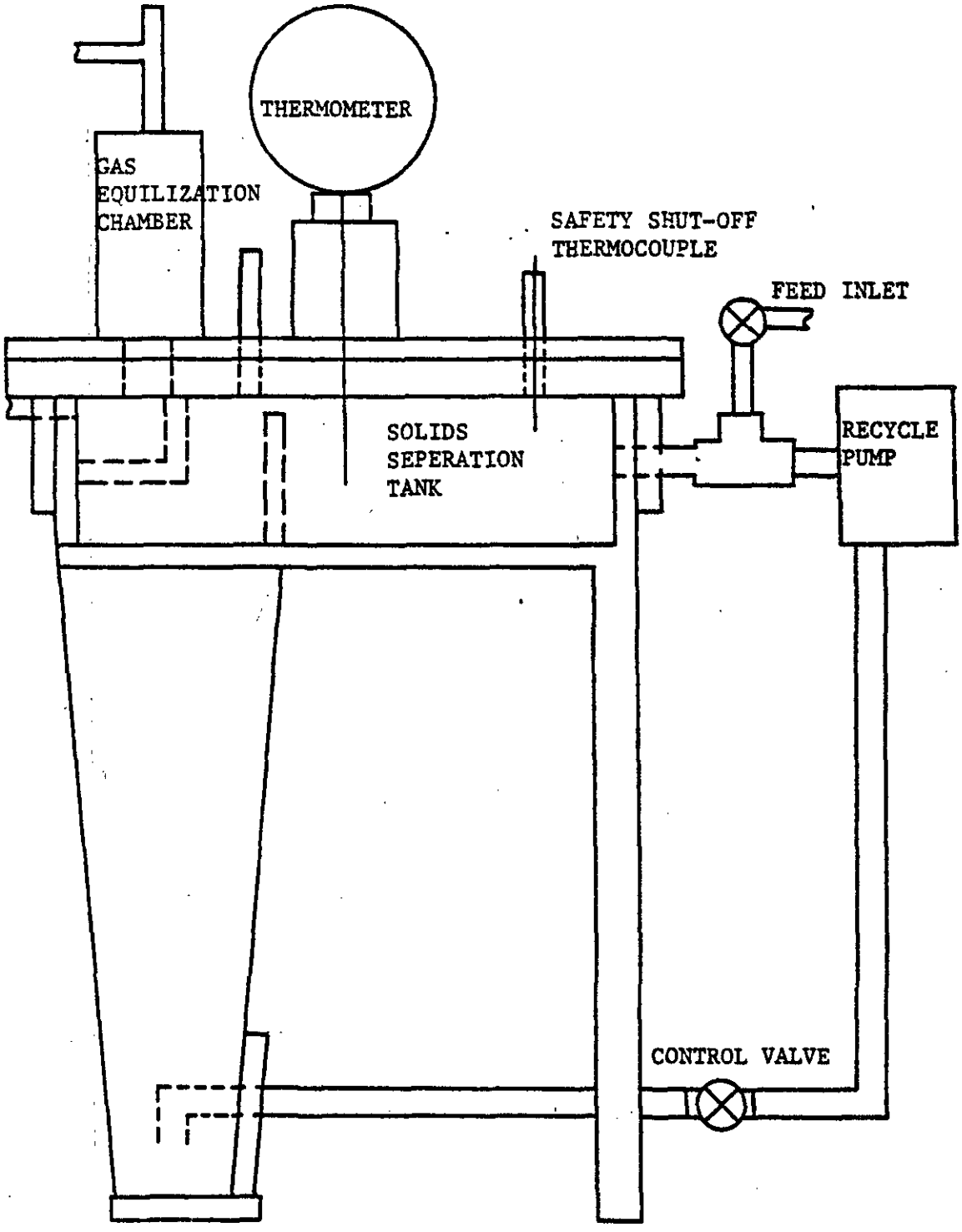

Figure 5. Reactor design.

(c) a liquid sampling portal which was sealed with a septum to enable sampling with a syringe;

(d) a temperature portal for a bimetalic Precision Instrument Thermometer;

(e) a thermocouple portal for a high temperature safety shut-off relay;

(f) a gas vent; and

(g) a recyle discharge outlet.

Affixed above the gas vent was a small gas stabilization chamber which prevented moisture and biofilm from entering the collection line to the gas collector. The gas stabilization chamber also provided a small reservoir for gas sampling which enabled a more accurate gas sample. Coupled to the top of the gas stabilization chamber and the gas collection line was a gas sampling portal. The gas sampling portal was sealed with a septum for sampling with a syringe.

The recycle discharge outlet was on the end of the recycle reservoir tank opposite the effluent discharge line. Attached to the recycle discharge outlet was the influent substrate line. Positioning the influent substrate line at the recycle discharge outlet provided immediate mixing as the recycle discharge outlet was on the suction side of the recycle pump.

Reactor recycle was provided by a Little Giant Model QE38N centrifugal force pump which also served to expand the support media. Recycle flow was controlled with a metering ball value (Cole-Parmer C-1360-40) calibrated in five degree intervals. The recycle flow

entered the bottom of the tapered portion of the reactor and was directed downward to provide a more thorough mixing of the support media and to discourage line clogging during shutdown periods.

3.2.2. AFEB reactor operation. The operation of the AFEB reactor required first adding the biofilm support media in the tapered portion of the reactor. Next the reactor was filled with water and the top of the recycle reservoir tank was bolted into place. The recycle pump was then primed with a 50 ml syringe through a valve installed on the pump for priming purposes. The metering control valve was adjusted to control the expansion of the support media after the recycle flow had achieved enough velocity to expand the bed and expel trapped air. Through the course of the experiment, the recycle flow rate was adjusted to maintain an expanded bed height of 21 cm which corresponds to an expanded bed volume of 605 cm^3. After initial startup, the reactor proved to be highly reliable and provided uninterupted performance except for scheduled shut-down for sampling purposes.

3.2.3. Gas collection and measurement. Gas produced by the reactor was collected and measured daily with a gas collection device specifically constructed for the experiment (Figure 6). Dimensions of the gas collection device are listed in Table 3. The device consisted of a plexiglass gas collecting cylinder, sealed on the top and suspended over a standpipe in a cylinder containing water. The standpipe was connected to the gas stabilization chamber on the reactor. The gas collecting cylinder was counter-balanced with weights so that gas produced in the reactor caused the cylinder to rise. The vertical distance the gas collecting cylinder rose was measured

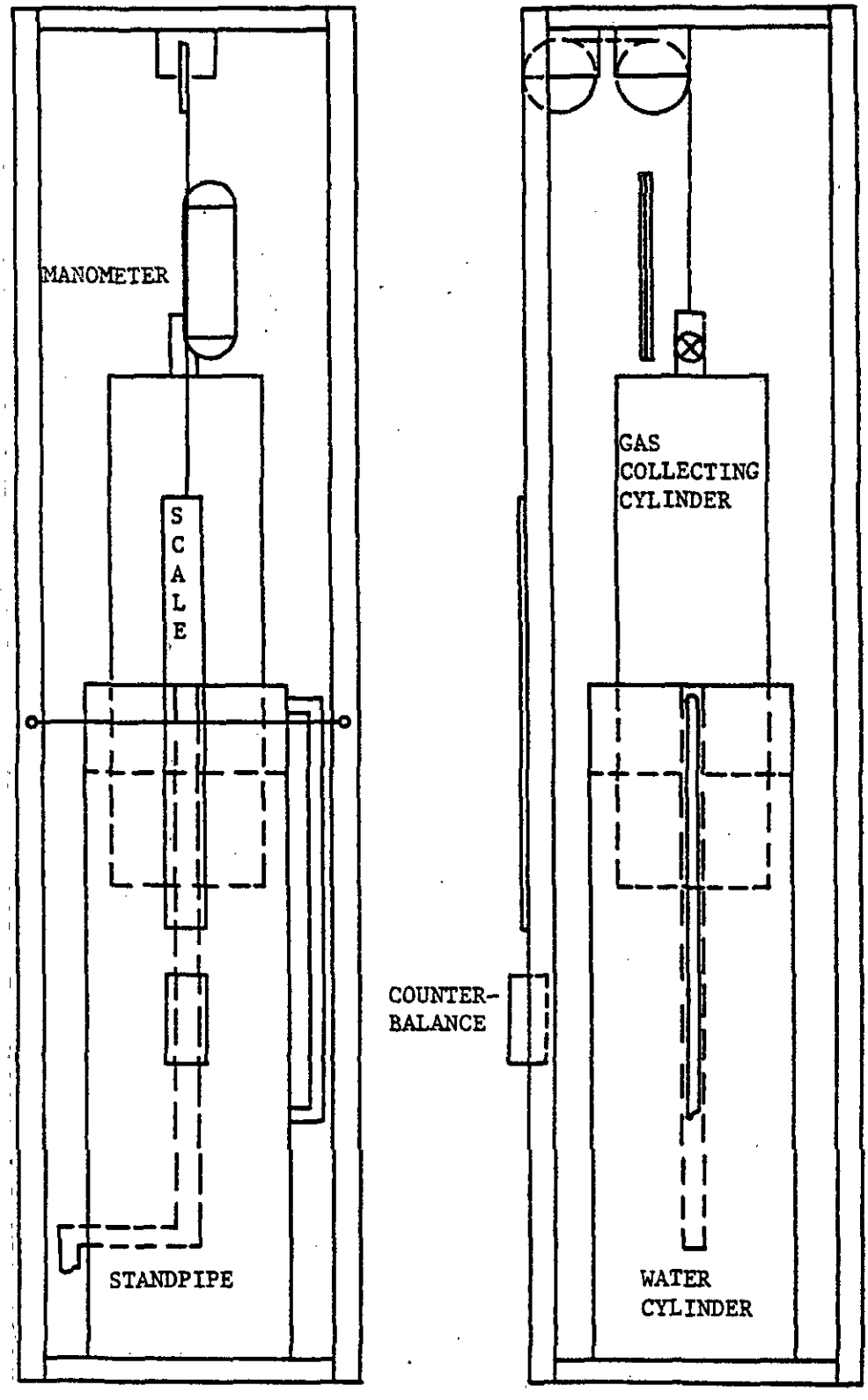

Figure 6. Gas collection device.

Table 3. Gas Collecting Device Dimensions

Total Overall Dimensions:	Height	103.5 cm
	Width (front)	25.7
	Width (side)	25.4 cm
Gas Collecting Cylinder:	Height (outside)	39.1 cm
	Height (inside)	38.1 cm
	Diameter (outside)	11.4 cm
	Diameter (inside)	10.033 cm
Outer (Water Containing) Cylinder:	Height	51.4 cm
	Diameter (outside)	15.7 cm
	Diameter (inside)	15.2 cm
Standpipe:	Length	40.6 cm
	Diameter (inside)	0.64 cm

with the scale that had the counterweight attached to it. The level of the water that had been displaced by the gas collecting cylinder was measured with a sight glass attached to the outer cylinder. A small manometer was connected to the top of the gas collecting cylinder so that the system could be equalized to atmospheric pressure for measurement purposes.

A calibration check of the gas collecting device was accomplished by injecting known volumes of air into the gas collection line with a 50 cm^3 syringe. Figure 7 shows the graph produced from the calibration check. Daily gas production rates were calculated at room temperature and were determined from the total changes in height including the water level change of the gas collecting cylinder. The calculated gas volume was then converted to standard temperature (0°C). Pressure changes were not accounted for as the resultant volume change was deemed insignificant.

The gas collection device worked very well over the entire course of the experiment.

3.2.4. Influent substrate reservoir. The influent substrate reservoir consisted of a 1000 cm^3 graduated cylinder installed in refrigerator (Figure 4). The graduated cylinder was connected to the feed pump with a one-fourth inch PVC pipe fitting located near the bottom of the cylinder. The substrate was constantly stirred with a magnetic stirrer and the refrigerator was maintained at 8 to 10°C.

3.3. Influent Substrate. Cheese whey was chosen as the influent substrate as it has proven to be easily fermentable. Fermenting

DIAMETER OF GAS COLLECTING CYLINDER (INSIDE) = 10.033 cm

Δ VOLUME = $\Pi R^2 \Delta H$

Δ VOLUME = 7.9059 ΔH

Where ΔH = Change in height in mm

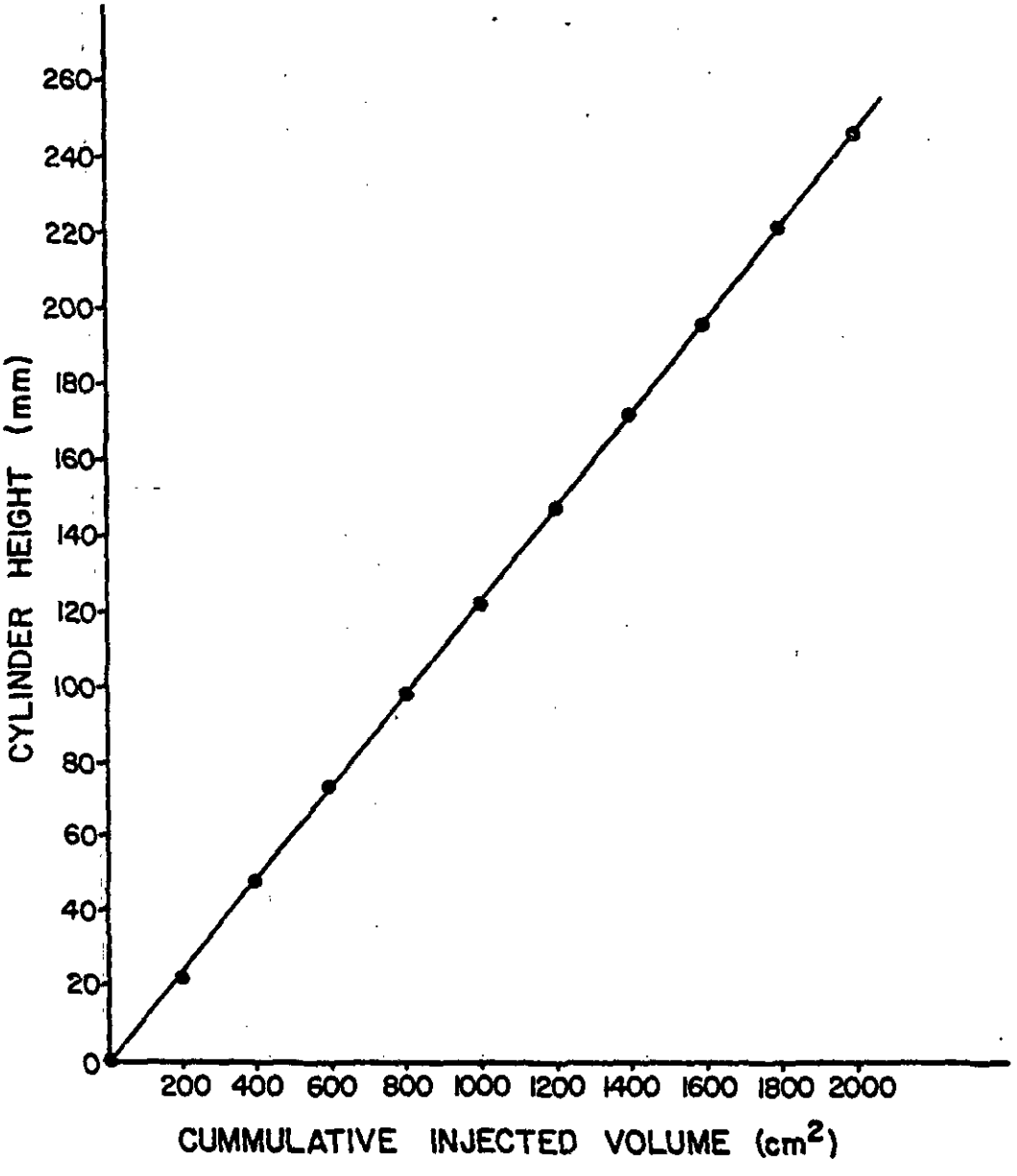

Figure 7. Gas collection calibration graph.

whey to methane gas was initially investigated by Buswell et al. (16). Switzenbaum and Danskin (81) and Hickey and Owens (31) have shown the AFEB process to be an extremely efficient system for treating whey. Switzenbaum and Danskin (81) were able to attain a 93.1 percent COD removal efficiency with an AFEB reactor treating sweet whey at 10,000 mg COD/l, at 28°C, and an organic volumetric loading rate of 8.9 Kg COD/m^3/day. Hickey and Owens (31) were able to attain an 89.5 percent COD removal efficiency with the AFEB reactor at 35°C treating acid whey at 9,025 mg COD/l and an organic load of 4.5 Kg COD/m^3/day.

Sweet whey powder was used as the substrate in this study to avoid storage problems of whole whey. Tables 4 and 5 list some nutritional properties, and Table 6 list some chemical properties of whey powder.

A Fisher Model 300 electrobalance accurate to 0.01 g was used to weigh all ingredients for both the nutrient-limited and nutrient-supplied substrates (including the nutrient salt reagents). In both the nutrient-limited and nutrient-supplied experiments, the substrate was prepared in 3.5 liter volumes, stored at 4°C, and added daily to the constantly stirred substrate reservoir.

Substrate Compositions and Flow Rate

3.3.1. Nutrient-limited substrate composition. Initially, this study involved only the examination of the effect of temperature on the AFEB process and did not include the effects of nutrient limitation. The substrate formula chosen for the study (Table 7) had a C/N/P ratio of 10.75:2.33:1, respectively, and was the same

Table 4. Amino Acid Composition of Whey Powder (13)

Amino Acid	mg/mg Whey Powder
Lysine	8.13
Histidine	1.31
Ammonia	0.76
Argenine	2.78
Aspartic	9.55
Threonine	5.18
Serine	4.98
Glutomic	20.70
Proline	7.83
Glycine	1.67
Alanine	5.03
Cystine	N.C.
Valine	5.68
Methionine	1.25
Isoleucine	5.45
Leucine	10.30
Tyrosine	3.17
Phenylalanine	3.35
Tryptophan	6.32

Table 5. Composition of Whey (87).

Vitamin	Fluid Whey (a)	Dried Whey (b)
Vitamin A	11	50
Thiamine	0.4	3.7
Riboflavin	1.2	23.4
Nicotinic Acid	0.85	9.6
Pantothenic acid	3.4	47.3
Vitamin B_6	0.42	4.0
Biotin	0.014	0.37
Folic Acid	-	0.89
Vitamin B_{12}	0.002	0.021
Vitamin C	13	-
Vitamin E	-	-
Choline	-	1356

(a) mg/l except Vitamin A (IU/100 ml).

(b) mg/Kg except Vitamin A (IU/100 g).

Table 6. Analysis of Sweet Whey Powder*
(1 g/l solution)

Parameter	Value	Method of Analysis
Total COD	997.5 mg/l	Dichromate Refleux
Soluble COD	826.3 mg/l	Dichromate Refleux
pH	6.5	Glass electrode
Total Kjeldahl Nitrogen	19.2 mg/l	Indophenol
Ammonia nitrogen	0.46 mg/l	Indophenol
Total phosphorus	5.0 mg/l	Ascorbic Acid
Soluble Orthophosphorus	2.9 mg/l	Ascorbic Acid
Total protein	323 mg/l	Buiret
Suspended Solids	62.3 mg/l	Glass fiber filter
Volatile Suspended Solids	59.7 mg/l	Glass fiber filter
BOD_L	928.1 mg/l	Thomas method
K (base e)	0.142 day^{-1}	Thomas method
Potassium	16.7 mg/l	Atomic Absorption Spectrophotometry
Sodium	6.6 mg/l	Atomic Absorption Spectrophotometry
Calcium	5.9 mg/l	Atomic Absorption Spectrophotometry
Magnesium	1.08 mg/l	Atomic Absorption Spectrophotometry
Alkalinity	13.45 mg/l as $CaCO_3$ to pH 4.8	Potentiometric titration
Carbon**	0.3868 g/g whey powder	Combustion
Hydrogen**	0.0564 g/g whey powder	Combustion
Nitrogen**	0.0198 g/g/whey powder	Combustion

* values obtained from reference (81).
** values determined in this study.

Table 7. Nutrient-Limited Substrate Formula

Per liter of Substrate:

10 g Powdered whey

5 g $NaHCO_3$*

10 ml of 1 M$(NH_4)_2HPO_4$**

* Arm and Hammer Baking Soda
** Analytical grade ragent

formula used by Switzenbaum and Danskin (81). It was assumed, as in the Switzenbaum and Danskin study, that trace nutrient requirements would be met by the tap water used for dilution.

In their study, Switzenbaum and Danskin (81) were able to attain a 93.1 percent COD removal efficiency treating an influent substrate of 10,000 mg COD/1 at an organic volumetric loading rate of 8.9 Kg COD/m^3/day. and at a reactor temperature of 28°C. Using the same substrate formula and concentration but at a reactor temperature of 35°C and an organic loading of 10 Kg COD/m^3/day, the highest COD removal efficiency during this phase of this study was 60.3 percent. It was assumed that the poor removal efficiency was due to a nutritional limitation and some necessary nutrient was absent in the tap water that was used for dilution but was present in the tap water used by Switzenbaum and Danskin. It must be stressed that this is the only detail that differed from the earlier study (aside from reactor configuration). In order to facilitate data collection and to possibly determine temperature effects on a nutritionally limited system, it was decided to continue with the nutrient-limited substrate and add nutrient salts at a later time.

3.3.2. Nutrient-supplied substrate composition. The nutrient supplied substrate composition is given in Table 8. The C/N/P ratio of the nutrient-supplied substrate was 12.32/3.69/1, respectively. The formula for the nutrient-supplied substrate stemmed from original work by Speece and McCarty (74) concerning nutrient requirements in anaerobic digestion and from semi-continuous batch reactor studies

Table 8. Nutrient-Supplied Substrate Formula

Per liter of substrate:

10g whey powder

5g $NaHCO_3$[1]

99 ml of Salt I

99 ml of Salt II

1.29 ml of 1000 ppm Nickel Standard[2]

Salt I, per liter of solution[3]:

11.40g $(NH_4)\ HPO_4$

2.01g $MgCl_2 \cdot 6H_2O$

Salt II, per liter of solution[3]:

14.00g NH_4Cl

2.00g KCl

7.30g $FeCl_3 \cdot 6H_2O$

0.30g $CoCl_2 \cdot 6H_2O$

5.00g $MgCl_2 \cdot 6H_2O$

1. Arm and Hammer Baking Soda.
2. Fisher Brand Atomic Absorption Spectrophotometry Standard
3. Analytical grade reagents.

conducted by Switzenbaum and Danskin (81). The addition of nickel originated from investigations concerning the importance of nickel to methanogenic bacteria by Whitman and Wolf (88) and by Diekert et al. (25).

3.3.3. Influent flow rate. The influent flow rate to the AFEB reactor was measured by simultaneously valving off the flow from the substrate reservoir and valving on the flow from a 50 ml buret containing influent feed. The system incorporated two three-way valves which aided in removing small amounts of air entrapped in the feed line and in removing precipitates formed from the substrate. The results of 46 flow measurements taken over the course of the experiment are given in Table 9. The average influent flow during the study was 614 cm^4/day and the standard deviation was 109 cm^3/day.

3.4. Biofilm support material. The biofilm support material used in this study was porous aluminum oxide particles manufactured by Corning Glass Works. The support particles had been used in previous studies (81, 83) and were sieved for uniformity. The physical characteristics of the material included: a particle density of 2.79 g/cm^3, a loose bulk density of 0.6 g/cm^3, an apparent diameter of 500 micrometers, and a calculated surface area of 45,216 cm^2/1000 cm^3. This material was chosen because it had an already existing attached anaerobic biofilm which reduced start-up time. Other considerations for its selection included its uniformity and its ability to be ashed to measure biofilm volatile organic matter.

Table 9. Influent Flow Rate

	Volume (cm^3)	Elapsed Time(sec)	Feed Rate cm^3/day		Volume (cm^3)	Elapsed Time(sec)	Feed Rate cm^3/day
1	50.0	6516.2	663	24	36.0	4741.0	656
2	50.0	7230.6	597	25	28.5	3851.4	639
3	50.0	5865.5	736.5	26	36.1	4372.9	713
4	51.0	6164.8	715	27	31.05	3719.7	721
5	52.0	6144.5	731	28	26.3	2732.3	832
6	42.5	5466.3	672	29	35.15	4065.4	747
7	50.0	5858.6	737	30	30.0	4621.4	561
8	34.0	3692.7	792	31	21.0	3999.3	454
9	40.0	6662.7	519	32	21.3	3673.1	501
10	33.5	5326.7	487	33	32.5	6173.6	455
11	20.6	3600.0	487	34	24.7	4337.2	492
12	39.6	5313.1	644	35	29.1	5086.4	494
13	36.8	4906.0	648	36	26.0	3505.7	641
14	41.6	6061.9	593	37	32.5	4547.2	618
15	34.6	5083.3	588	38	30.9	3886.9	687
16	22.9	3902.5	507	39	35.8	4310.3	718
17	29.2	4500.	562	40	38.0	4911.5	668
18	37.85	5848.2	562	41	44.8	5702.3	679
19	29.9	6326.7	408	42	39.1	5013.5	674
20	37.1	3994.3	802	43	31.0	4045.2	662
21	17.7	3700.4	413	44	33.4	4250.3	679
22	27.8	4973.1	483	45	28.2	4070.0	599
23	20.8	3955.2	454	46	25.7	3980.3	558

n = 46

Average = 614 cm^3/day

Standard deviation = 109 cm^3/day

Analytical Methods

3.5.1. Gas composition. A GOW-MAC 550 thermal conductivity gas chromatograph coupled to a Fisher Recordall-Series 5000 strip chart recorder was used to determine gas composition. The separating column was stainless steel, six feet long by one-fourth inch in diameter, and packed with 80/100 mesh Porapak Q packing. Gas samples were collected from the gas sample part on the reactor (Figure 4-2). and injected into the gas chromatograph with disposable 1 cm^3 tuberculin syringes. Instrument conditions are given in Table 10.

3.5.2. pH. A Fisher Accumet pH Meter Model 600 equipped with a combination electrode was used to determine pH values. The sensitivity of the pH meter was 0.1 pH units.

3.5.3. Chemical oxygen demand. Chemical oxygen demand (COD) measurements were determined by using a modification of the Jirka and Carter method (40). A Bausch and Lomb Spectronic 20 was used for the spectrophotometric measurements. A 10,000 mg/l standard stock COD solution was prepared by dissolving 8.500 g of potassium acid pthalate in distilled water and diluting to one liter.

The digestion solution was prepared by adding 167 ml of concentrated sulfuric acid to 500 ml of distilled water. Subsequently, 17.00 g of mercuric sulfate and 10.216 g of potassium dichromate were added into the solution which was then cooled and diluted to one liter.

Table 10. Gas Chromatograph Conditions

Carrier Gas:	Helium
Flow Rate:	30 ml/min
Injection Port Temperature:	110°C
Column Temperature:	80°C
Detector Temperature:	70°C
Bridge Current:	6 ma
Attenuator Setting:	16
Recorder Setting:	10 mv full scale
Recorder speed:	0.5 in/min

The catalyst solution was prepared by adding 22.00g of silver sulfate to a 4 Kg bottle of concentrated sulfuric acid.

Kimax culture tubes (25 x 150 mm) with teflon lined screw caps were used as both digestion tubes and cuvettes for the spectrophotometric analysis. An appropriate sample volume (usually 2 ml) was introduced into the culture tube, then an appropriate amount of distilled water was added to bring the diluted volume to 10 ml. Next 6 ml of digestion solution and 14 ml of catalyst solution were added. The tubes were capped and inverted at least three times to mix contents. At least two blanks and a set of standards from 100 to 1000 mg COD/l were prepared for each set of samples.

After the addition of the digestion and catalyst solutions, samples and standards were heated in a forced air oven at 150°C for two hours. Then the tubes were cooled, rinsed with distilled water, wiped dry, and measured at 600 nm. A calibration curve was prepared from the standards and the COD of each sample calculated.

3.5.4. Suspended and volatile suspended solids. Suspended solids were determined according to the procedure outlined on page 94 of Standard Methods (76). Whatman GF/A (4.25 cm) glass microfiber filters (Whatman Ltd., England) were used. Filters were prewashed with three 20 ml washings of distilled water, dried at 103°C for at least one hour, and dessicated for at least one hour before use. The filtering apparatus used was a pyrex glass Millipore Filter Holder (Millipore Corporation, Bedford, Massachusetts).

Volatile suspended solids were determined by ashing the dried filter from the suspended solids determination at 550°C according to the procedure outlined on page 95 of Standard Methods (76).

3.5.5. Biofilm volatile organic matter. The volatile organic matter of the biofilm was determined by collecting approximately 0.5g of aluminum oxide support material in a preweighed Whatman GFA glass microfibre filter that had been folded in the shape of a cone. A small vacuum pressure (about 1 lb gauge) was applied to the filter to remove excess moisture. The filter and media were then dried at 103°C for one hour, dessicated for one hour, and weighed. The filter and sample were then ashed at 550°C for 15 minutes, cooled in a dessicator for at least one hour, and again weighed. A blank which consisted of a folded filter containing unused aluminum oxide media was carried through each analysis. Blank values were subtracted from sample values to correct for error due to weight loss of the aluminum oxide support media.

3.5.6. Volatile organic acids. Volatile organic acids were measured by the chromatographic separation method as described on page 467 of Standard Methods (76). The sodium hydroxide titrant was potentiometrically standardized with 0.0500 N potassium hydrogen phthalate solution to pH 8.7. The standardization procedure is given on page 251 of Standard Methods (76).

3.5.7. Carbon,hyrogen, and nitrogen analysis. Carbon, hydrogen, and nitrogen (CHN) values were determined by the University of Massachusetts

Microanalysis Laboratory with a Perkin-Elmer 240 Elemental Analyzer. The Microanalysis Laboratory is located in the Graduate Research Center - Tower B.

3.5.8. Scanning electron photomicrographs. Scanning electron photomicrographs were made by Professor Stanley Holt and his assistant, Ms. Erika Musante, in the Microbiology Department at the University of Massachusetts/Amherst. A JOEL Model JSM 25 S scanning electron microscope and Polaroid Type 665 film were used. A complete list of analytical equipment used in this study is provided in Table 11.

Table 11. Analytical Equipment

pH meter:	Fisher Accumet pH Meter Model 600
Spectrophotometer:	Bausch and Lomb Spectronic 20
Gas Chromatograph:	GOW:MAC 550 Model 69-570
Chart Recorder:	Fisher Recordall Series 5000 Model B5117-51
Analytical Balance:	Mettler H31AR
Electrobalance:	Fisher Model 300
Forced Air Oven:	Blue M Model SW17TA
Muffle Furnance:	Thermolyne Model CPSA8720
Carbon, Nitrogen, Hydrogen, Analysis:	Perkin-Elmer Model 240 Elemental Analyzer
Electron photomicrographs:	JOEL Model JSM 25S Scanning Electron Microscope**

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* Microanalysis Laboratory, Graduate Research Center, University of Massachusetts/Amherst.

** Biology Department, Morrill Science Center, University of Massachusetts/Amherst.

C H A P T E R I V

Experimental Results

This experiment was originally started in October 1980 at Clarkson College of Technology, Potsdam, New York. The experiment was relocated to the University of Massachusetts/Amherst during July 1981. Extensive equipment redesign was conducted from August 1981 to May 1982. Data collection was performed from May 1982 through mid-September 1982.

A summary of the results of the AFEB study is presented in this chapter followed by a discussion of the results in Chapter V. Data collected from the AFEB reactor during a thermophilic stage of operation is presented in Appendix A.

4.1. Summary data. Summary data for each of the temperatures evaluated in both the nutrient-limited study and the nutrient-supplied study are presented in Tables 12 and 13. The AFEB reactor was operated at each of the temperatures listed in both of the studies for nine days (8.9 detention times based on expanded bed volume) to attain pseudo-steady-state conditions. Following the nine day interium, data were collected for three consecutive days except for the 30°C run in the nutrient-limited study which was only two consecutive days. All the data presented in Tables 12 and 13 represent average values except suspended solids, volatile suspended solids, and biofilm volatile matter which were determined from grab samples taken at the end of each run. Influent COD was determined on the specific batch of substrate used during the respective temperature evaluation. Methane

Table 12. Summary Data: Nutrient-Limited Experiment

Temperature, °C	35	30	25	20
Avg. daily gas production (cm^3/day @ 0°C and atmospheric pressure)	967	939	734	622
% CH_4	65.7	67.0	56.0	53.5
% CO_2	34.3	33.0	44.0	46.5
CH_4 Production (cm^3/day)	635	629	411	333
CO_2 Production (cm^3/day)	332	310	323	289
Influent COD (mg/l)	9580	9231	9720	9719
Effluent Soluble COD (SCOD) (mg/l)	3800	4385	4927	5040
Effluent Total COD (TCOD) (mg/l)	3855	4848	6097	6400
SCOD removal (mg/l)	5780	4846	4793	4679
TCOD removal (mg/l)	5725	4383	3623	3319
SCOD removal efficiency (%)	60.3	52.5	49.3	48.0
TCOD removal efficiency (%)	60.0	47.5	37.2	34.0
Suspended solids (mg/l)	252	452	608	632
Volatile suspended solids (mg/l)	252	309	569	604
Biofilm volatile organic matter (mg/g)	37	50	52	57
Volatile organic acids (mg/l as CH_3COOH)	1911	3317	3487	3680
pH	6.9	6.9	6.9*	6.7*

*25 percent NaOH solution added to maintain pH.

Table 13. Summary Data: Nutrient-Supplied Experiment

Temperature, °C	35	30	25
Average Daily gas production (cm^3/day @ 0°C and atmospheric pressure)	1445	1016	1778
% CH_4	56.9	54.0	54.2
% CO_2	43.1	46.0	45.8
CH_4 Production (cm^3/day)	822	549	964
CO_2 Production (cm^3/day)	623	467	814
Influent COD (mg/l)	9780	9820	10160
Effluent soluble COD (SCOD) (mg/l)	1995	2410	3590
Effluent total COD (TCOD) (mg/l)	2968	2795	4667
SCOD removed (mg/l)	7785	7410	6570
TCOD removed (mg/l)	6812	7025	5493
SCOD removal efficiency (%)	79.6	75.5	64.7
Suspended solids (mg/l)	121	133	303
Volatile suspended solids (mg/l)	99.7	130	255
Biofilm volatile organic matter (mg/g)	39	41	51
Volatile organic acids (mg/l as CH_3COOH)	627	687	823
pH	7.0	6.8	6.8*

*25 percent NaOH added to matinain pH.

and carbon dioxide concentration of the biogas were normalized to 100 percent. Samples which required storage were preserved by lowering the pH to 2.0 and freezing.

4.1.1. Influence of temperature on gas production. The influence of temperature on gas production is shown in Figures 8, 9, and 10. The average daily gas production showed a decreasing trend with decreasing temperature in the nutrient-limited experiment but no trend could be related to temperature in the nutrient-supplied experiment.

Methane production was determined by multiplying the percent methane composition times the corresponding average daily gas production rate. Methane production versus temperature is plotted in Figure 9. Like the average daily gas production rate versus temperature plot, the methane production rate versus temperature plot shows a decreasing trend with decreasing temperature for the nutrient-limited experiment and no particular trend for the nutrient-supplied experiment.

Percent methane composition versus temperature is shown in Figure 10. Methane composition increased slightly from 35°C to 30°C then decreased steadily at 25°C and 20°C for the nutrient-limited experiment. The nutrient-supplied experiment showed a slight decrease in methane composition with decreasing temperature. One interesting point to note is that the methane composition was higher for the nutrient-limited experiment than for the nutrient-supplied experiment.

4.1.2. Influence of temperature on COD removal rates. Figures 11, 12, 13 and 14 show the relationship of effluent chemical oxygen demand to reactor temperature. Figures 11 and 12, respectively show the total

chemical oxygen demand (TCOD) and the soluble chemical oxygen demand (SCOD) of the effluent for both the nutrient-limited and the nutrient-supplied experiments. The TCOD was determined on the sample as taken from the reactor. The SCOD was determined on the filtrate obtained by filtering the sample through a Whatman GF/A (4.25 cm) glass microfiber filter. Figures 13 and 14 show the TCOD and the SCOD removal efficiencies as a function of reactor temperature for both the nutrient-limited and the nutrient-supplied experiments.

The highest COD removal efficiencies were attained at 35°C for both of the experiments. Decreasing COD removal efficiency was also observed with decreasing temperature for both of the experiments. An important result is that the COD removal efficiency for the nutrient-supplied experiment is considerably higher than for the nutrient-limited experiment. At 35°C, the SCOD removal efficiency for the nutrient-supplied experiment was more than 1.3 times the COD removal efficiency for the nutrient-limited experiment. And at 25°C, the COD removal efficiency for the nutrient-supplied experiment was more that 1.4 times the COD removal efficiency of the nutrient-limited experiment.

4.1.3. Influence of temperature on volatile organic acids production. Figure 15 shows the relationship of volatile organic acids (VOA) concentration and reactor temperature. Volatile organic acids increased with decreasing reactor temperature for both the nutrient-limited and the nutrient-supplied experiments. But, the increase was much greater for the nutrient-limited experiment. Equally significant is the difference between the VOA concentration in each of

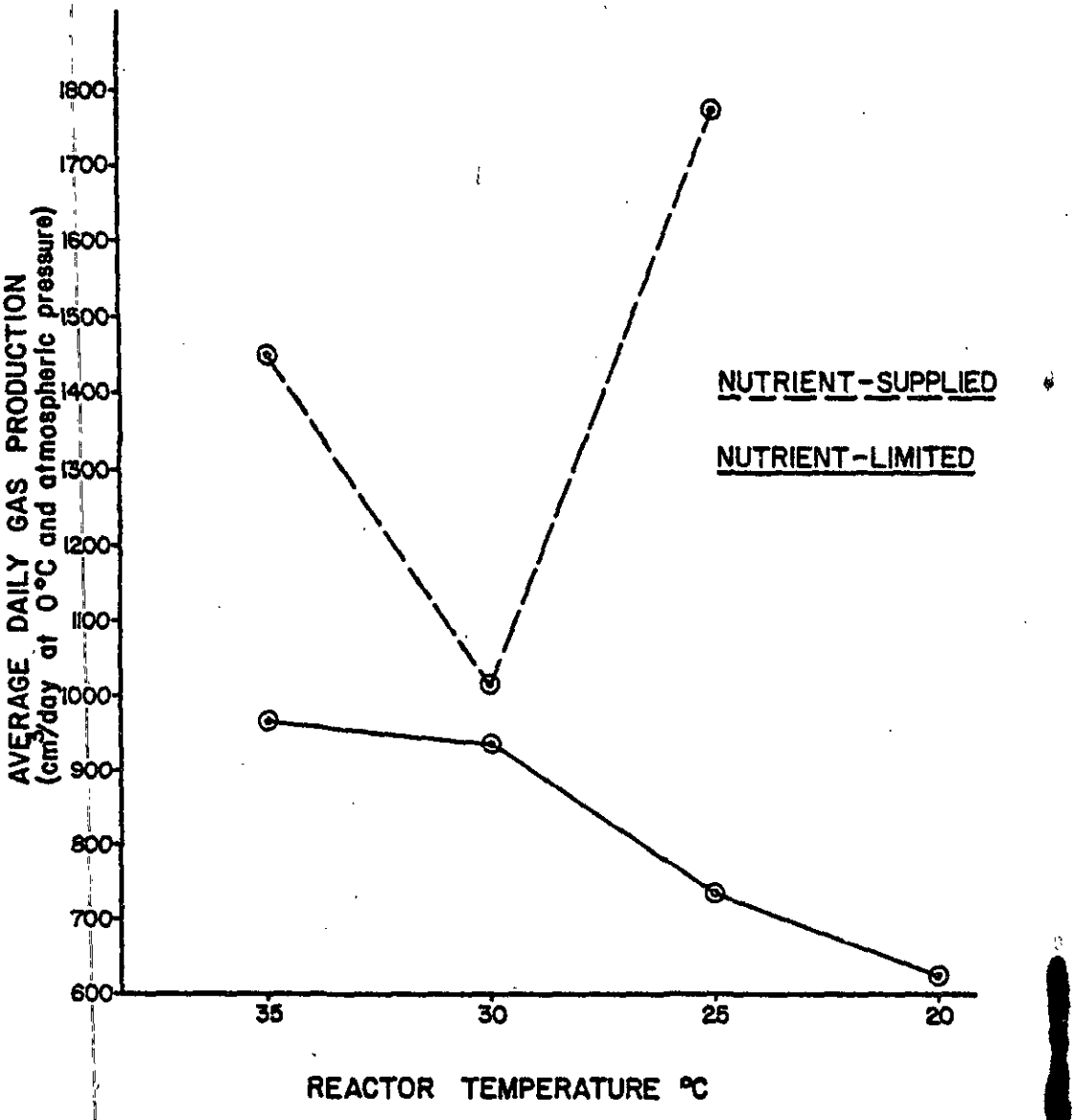

Figure 8. Influence of temperature on average daily gas production rate.

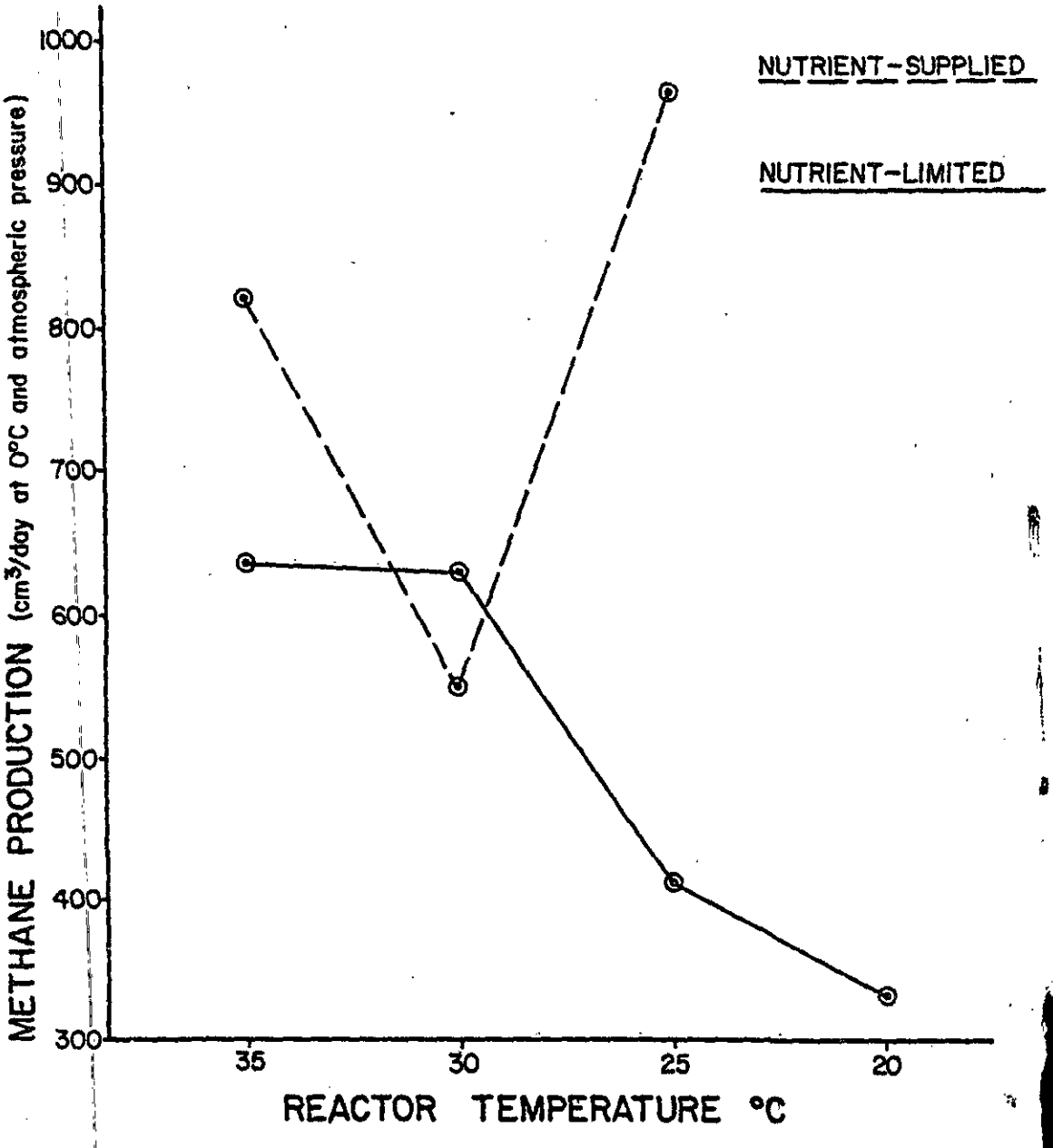

Figure 9. Influence of temperature on methane production rate.

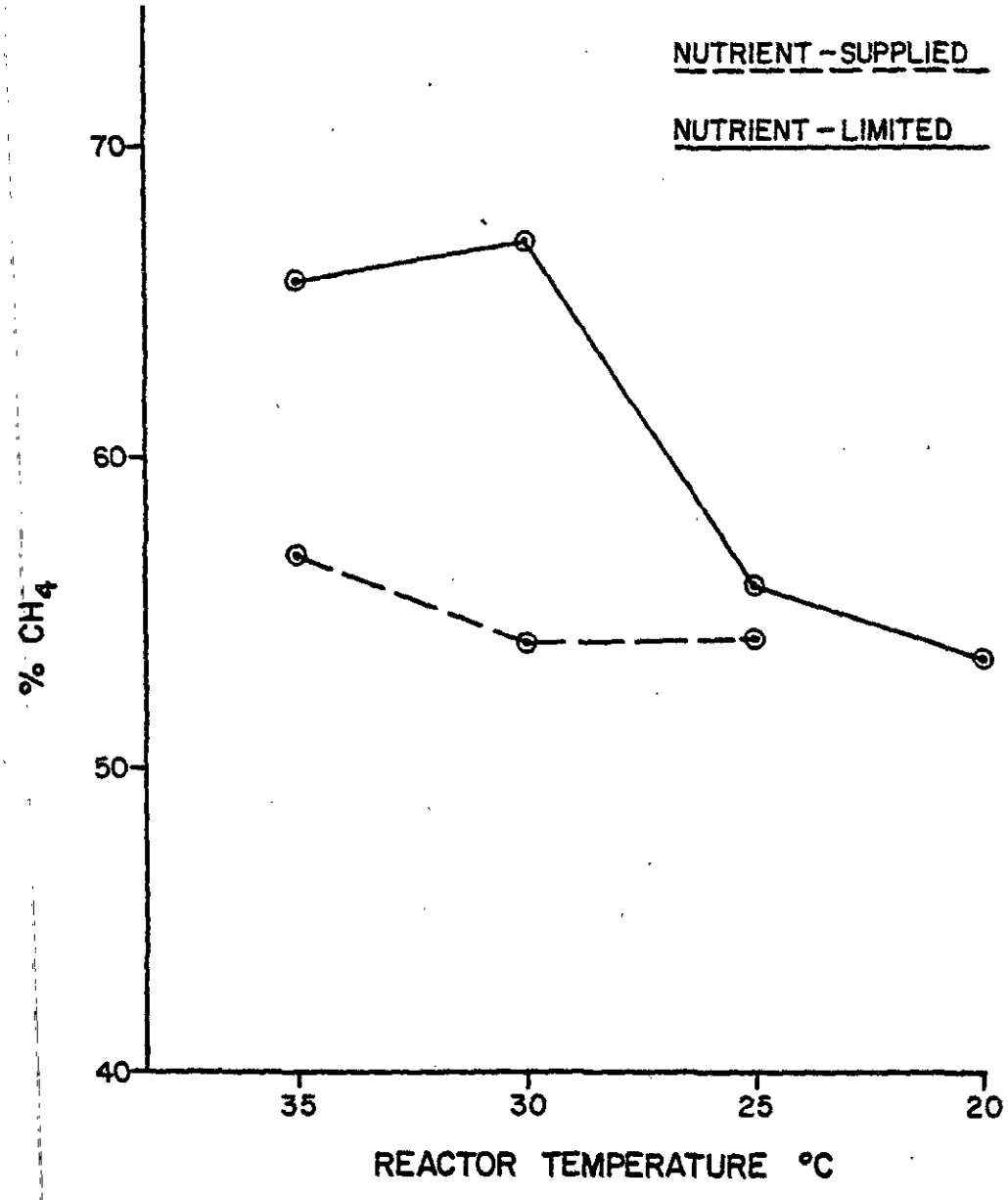

Figure 10. Influence of temperature on methane composition.

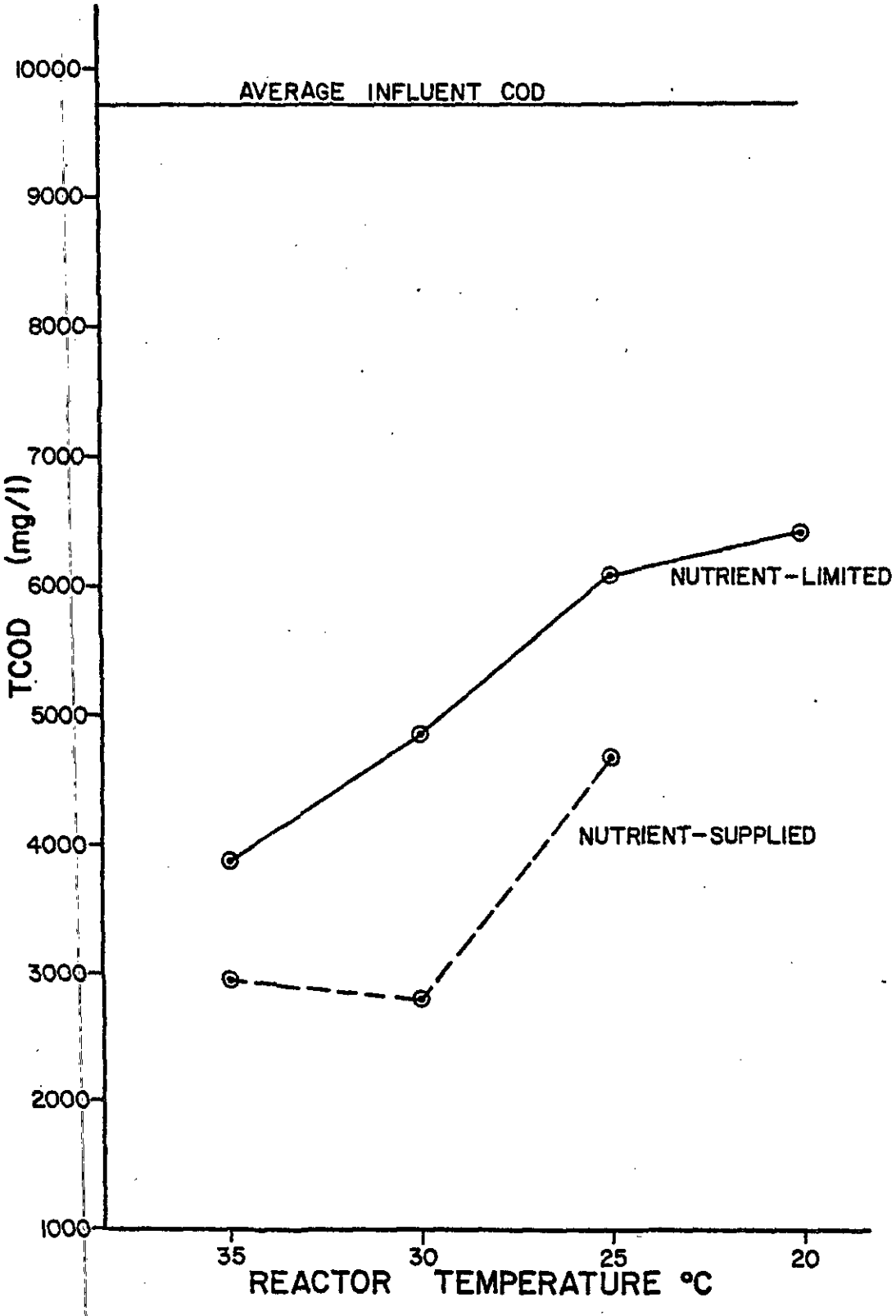

Figure 11. Influence of temperature on effluent concentration as TCOD.

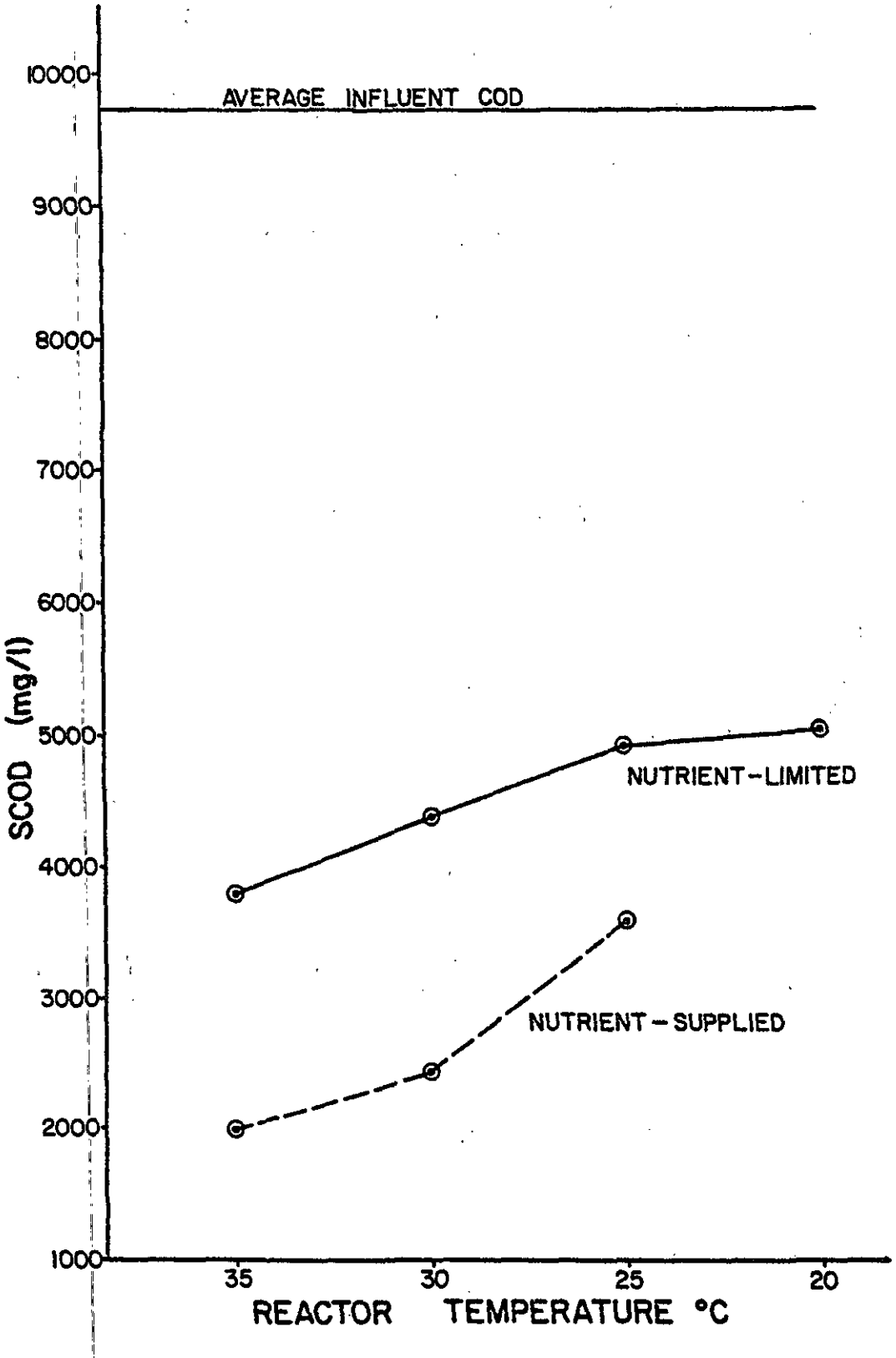

Figure 12. Influence of temperature on effluent concentration as SCOD.

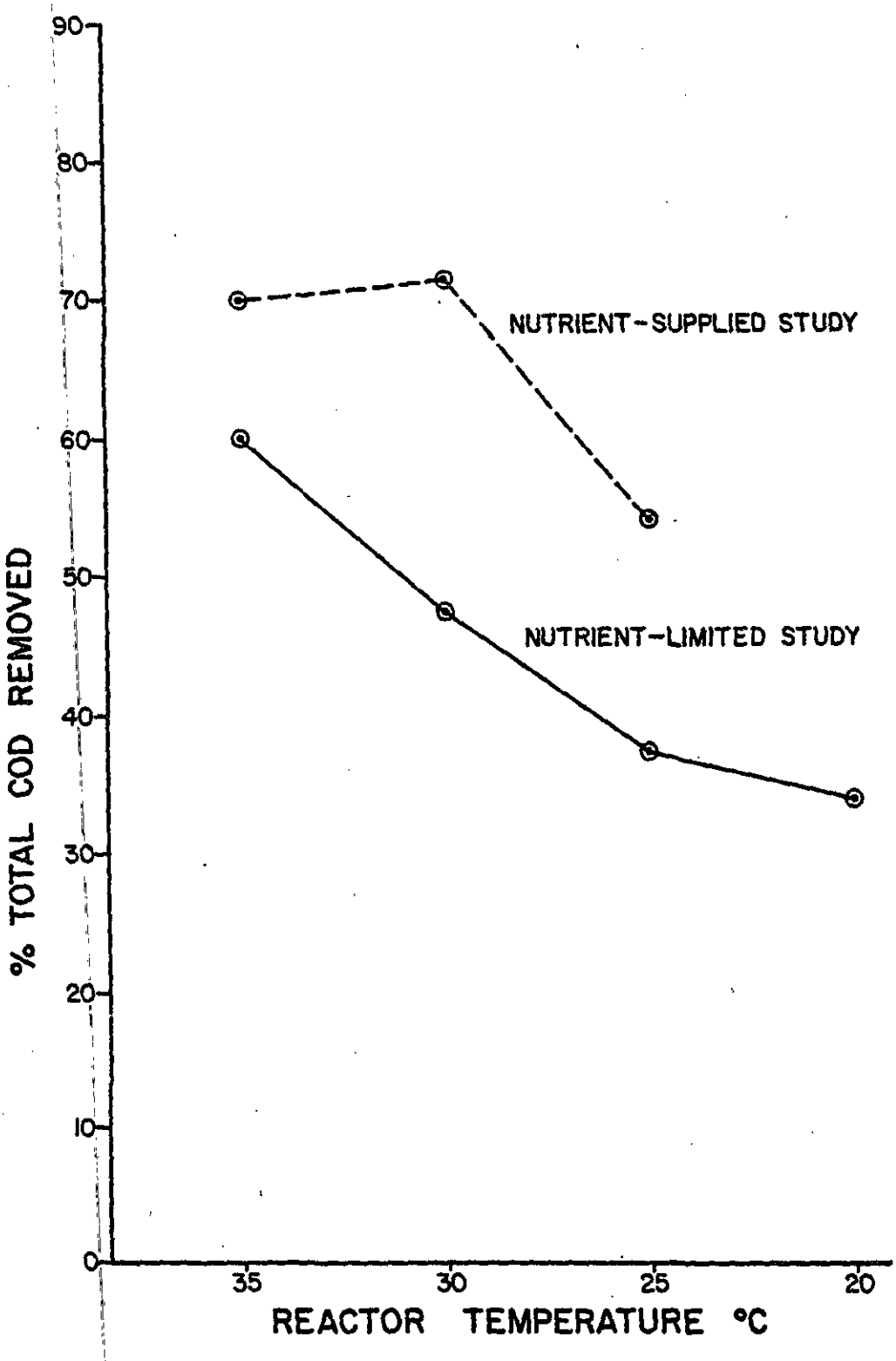

Figure 13. Influence of temperature on TCOD removal efficiency.

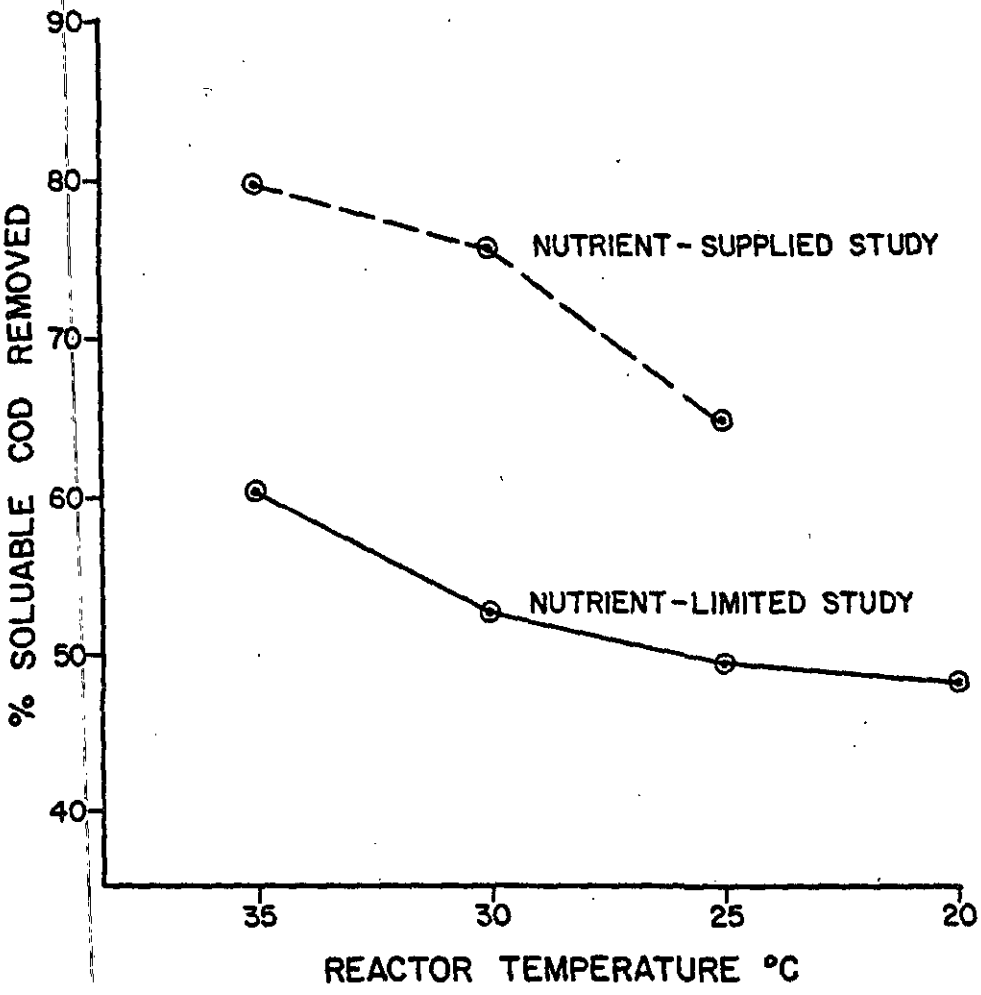

Figure 14. Influence of temperature on SCOD removal efficiency.

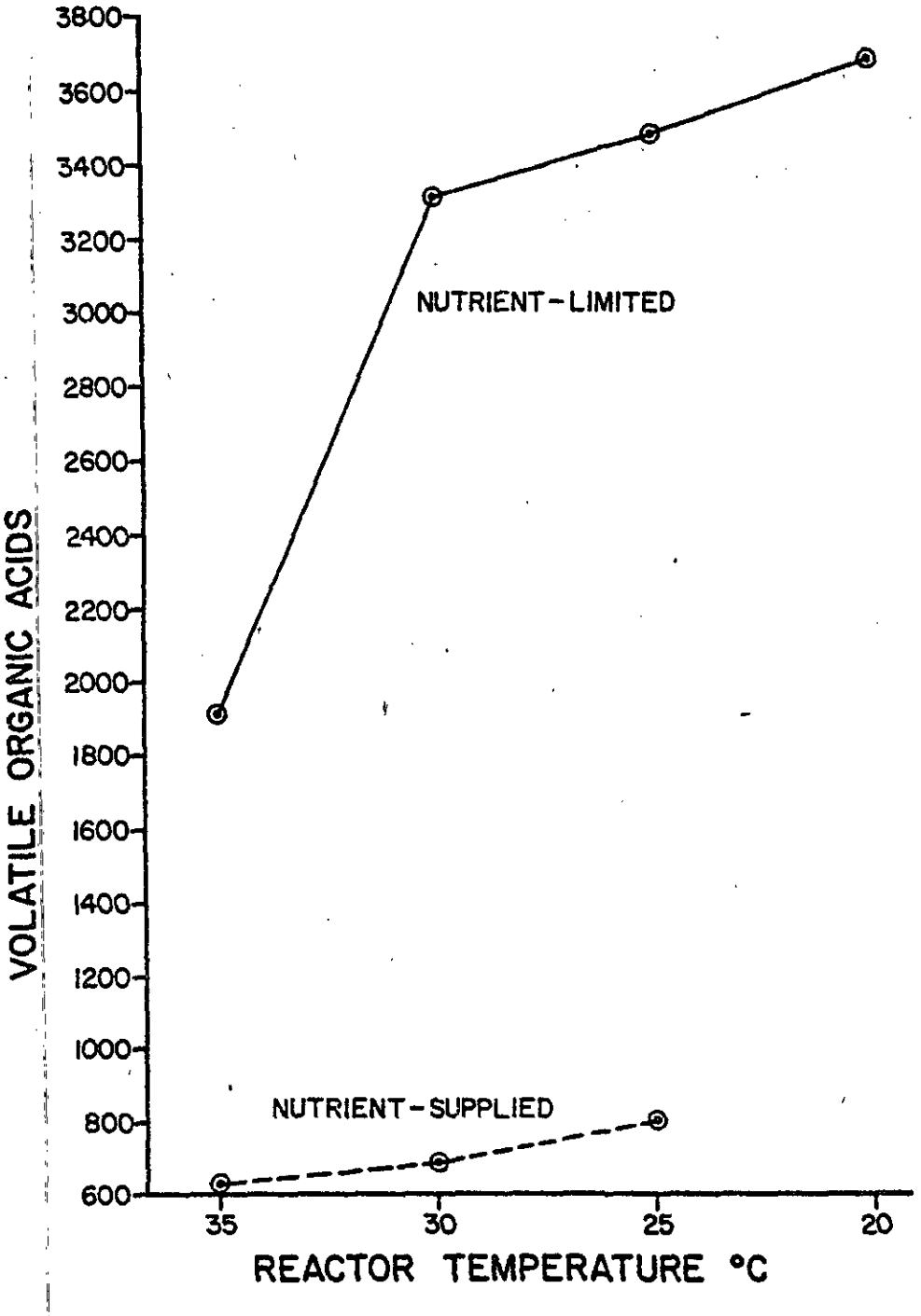

Figure 15. Influence of temperature on volatile organic acids production.

the experiments. At 35°C, the nutrient-limited VOA concentration was 1911 mg/l (as acetic acid) which was three times the VOA concentration of the nutrient-supplied experiment. At 25°C, the VOA concentration of the nutrient limited-experiment was 3,487 mg/l which was more than five times the VOA concentration of the nutrient-supplied experiment.

4.1.4. Influence of temperature on suspended solids. Figure 16 shows the relationship of effluent suspended solids with reactor temperature. Effluent suspended solids increased with decreasing reactor temperature for both the nutrient-limited and the nutrient-supplied experiments. An important result is that the suspended solids for the nutrient-limited experiment was more than twice that of the nutrient-supplied experiment at each of the temperatures evaluated.

4.1.5. Influence of temperature on volatile suspended solids. Figure 17 shows the relationship of effluent volatile suspended solids (VSS) to reactor temperature. Like the suspended solids concentration, the VSS for the nutrient-limited experiment were always greater than those for the nutrient-supplied experiment.

4.1.6. pH. The pH values measured during both the nutrient-limited and the nutrient-supplied experiments are listed in Tables 12 and 13. The effect of reactor temperature on pH is not readily apparent from the experiments as, when necessary, a 25 percent NaOH solution was added to the reactor to maintain the pH within sutiable levels for methanogensis. Nevertheless, as can be seen from Tables 12 and 13, the addition of sodium hydroxide solution was not necessary for either of the two experiments until the temperature was lowered to 25°C.

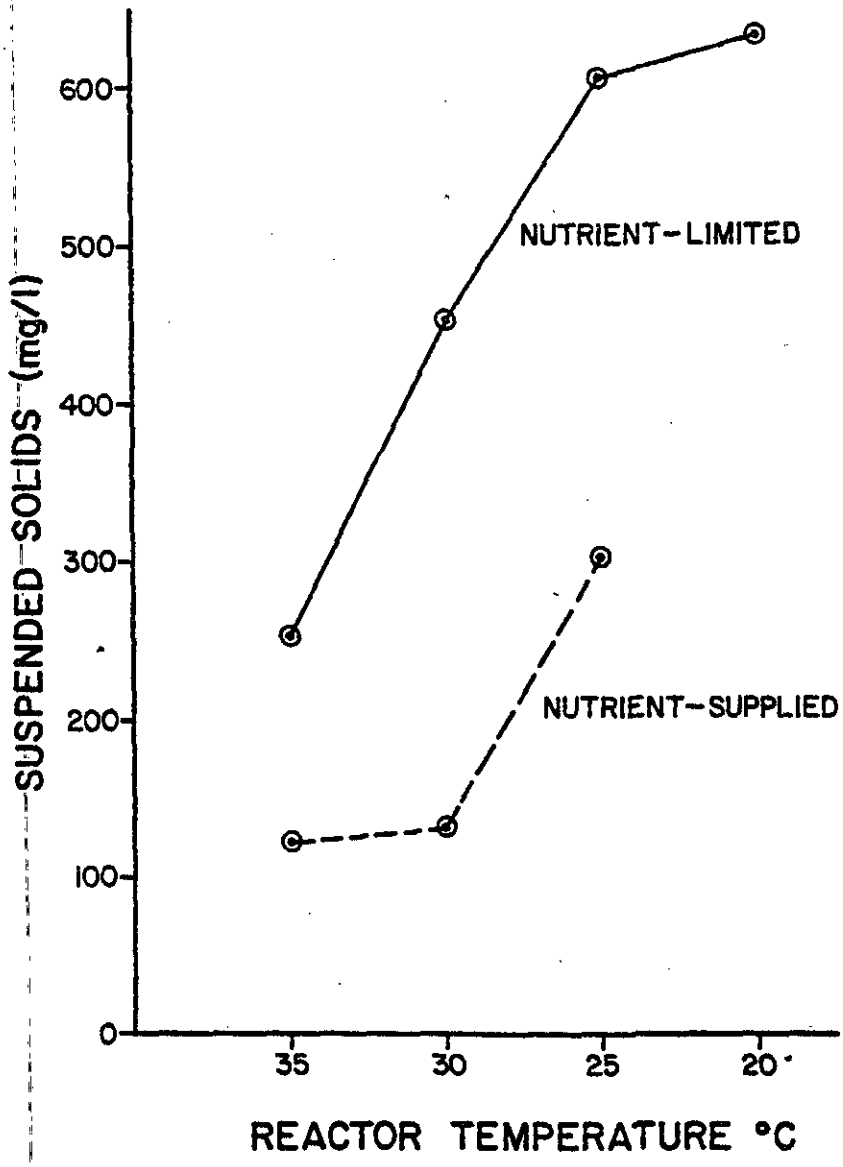

Figure 16. Influence of temperature on suspended solids.

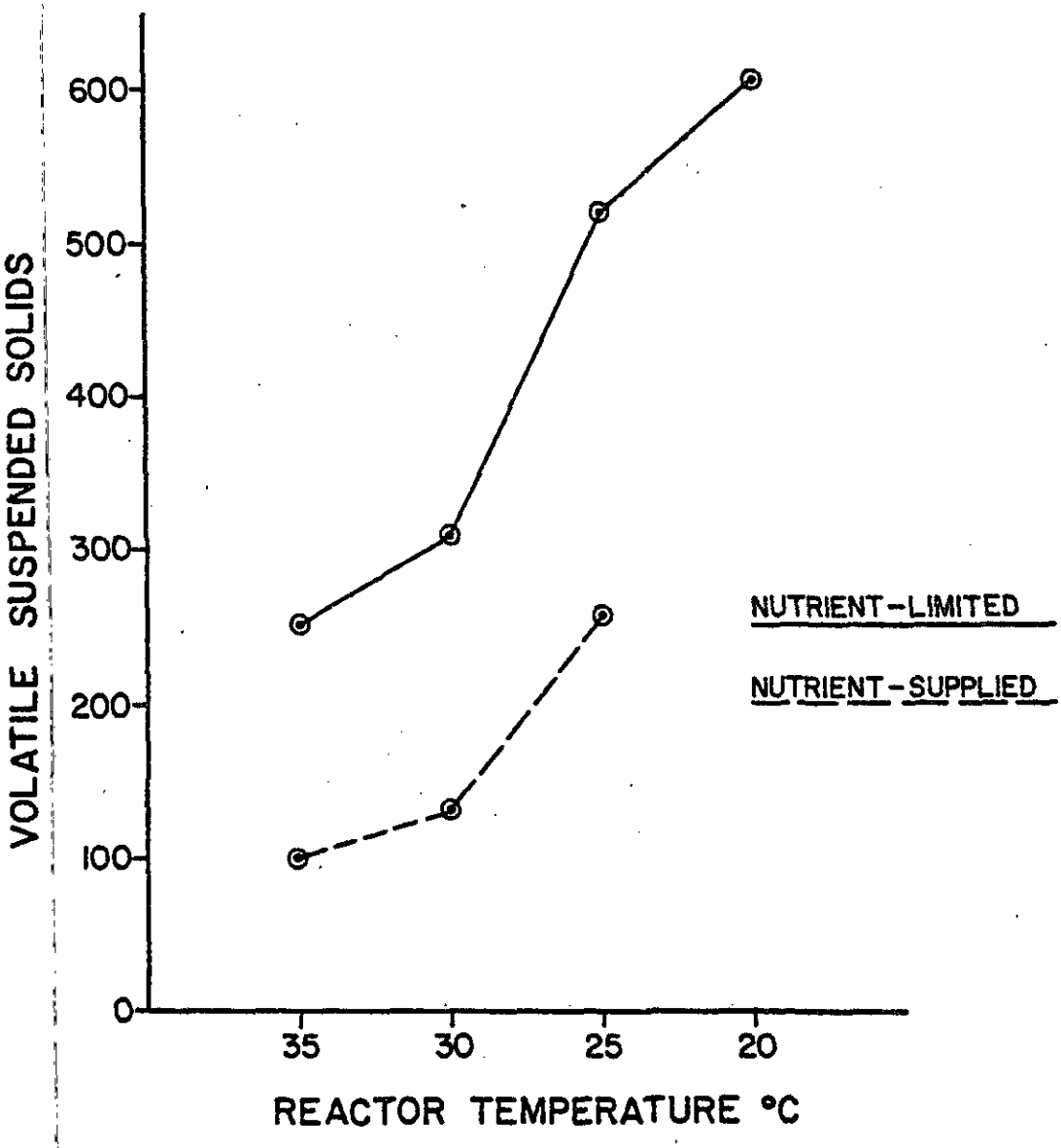

Figure 17. Influence of temperature on volatile suspended solids.

Biofilm Composition

4.2.1. Biofilm carbon, hydrogen, and nitrogen composition. Carbon, hydrogen, and nitrogen (CHN) concentrations of the biofilm attached to the aluminum oxide support material are listed in Table 14. CHN values were determined with a Perkin-Elmer 240 Elemental Analyzer for both the sample of interest and for virgin support material. The values listed in Table 14 are blank corrected. Biofilm CHN concentrations showed little correlation with temperature for the two experiments. In the nutrient-limited experiment, carbon concentration showed an increase with decreasing temperature but hydrogen flucuated up and down and nitrogen values were all less than 0.10 percent. In the nutrient-supplied experiment, carbon concentration fluctuated with decreasing temperature while hydrogen and nitrogen increased.

4.2.2. Biofilm volatile organic matter. Tables 12 and 13 list the biofilm volatile organic matter (BVOM) for the nutrient-limited and the nutrient-supplied experiments, respectively. The value given in Tables 12 and 13 are blank corrected and represent the percent of BVOM contained in a sample of dried biofilm. The BVOM was determined by ashing a dried sample of support material with attached biofilm and subtracting the percentage contribution of a blank (see Section 3.5.5). As can be seen from Figure 18, decrease in reactor temperature caused the BVOM in the nutrient-limited experiment to first increase and then decrease considerably. The BVOM for the nutrient-limited experiment was 3.7 percent at 35°C and increased to 5.0 percent at

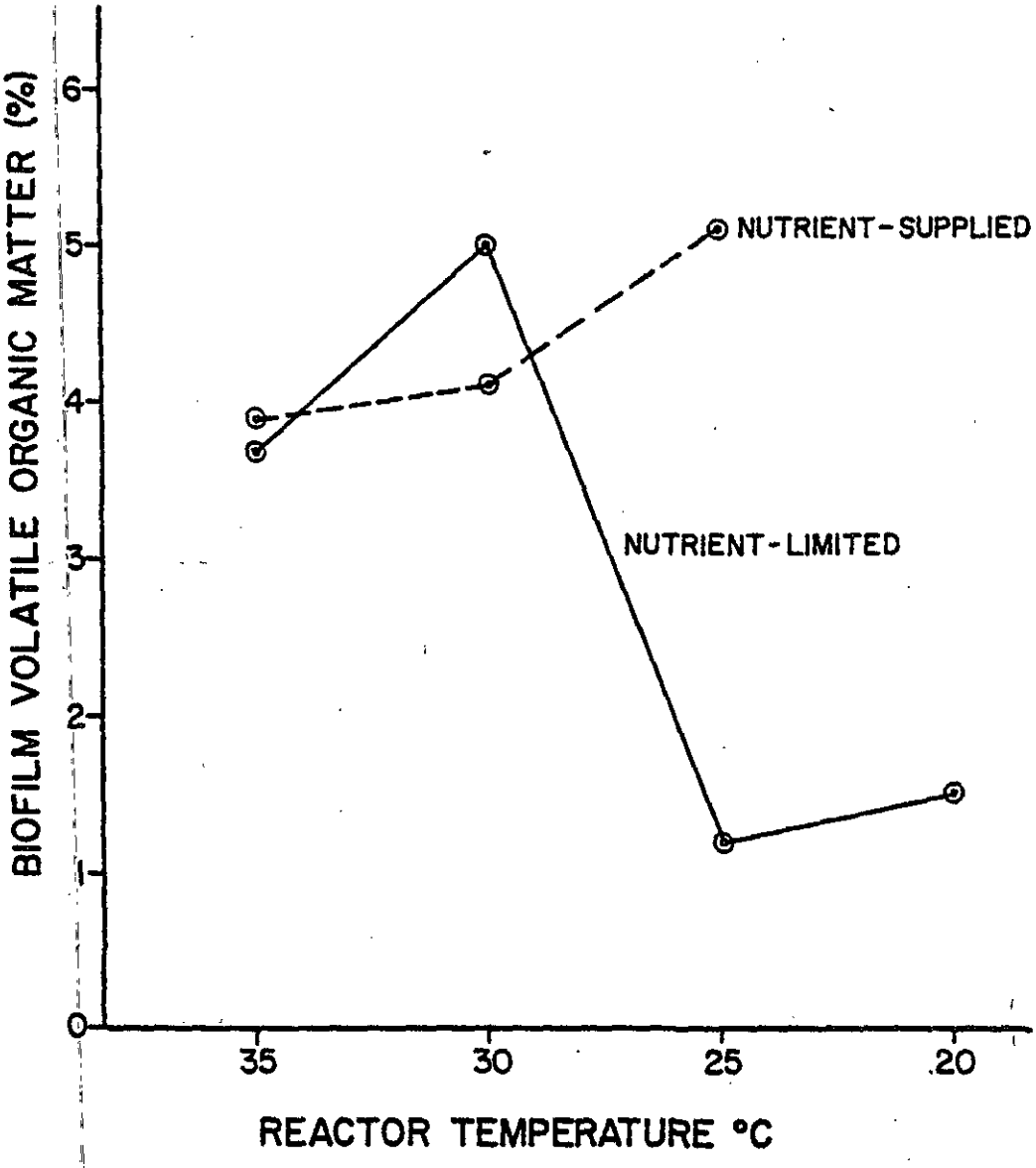

Figure 18. Influence of temperature on biofilm volatile organic matter.

Table 14. Biofilm Carbon, Hydrogen, and Nitrogen Composition

Nutrient-Limited Experiment				
Reactor Temperature	35°C	30°C	25°C	20°C
% C	0.55	0.79	0.71	1.42
% H	0.32	0.45	0.31	0.42
%N	<0.10	<0.10	<0.10	<0.10

Nutrient-Supplied Experiment			
Reactor Temperature	35°C	30°C	25°C
% C	1.43	0.65	0.72
% H	0.13	0.30	0.40
%N	<0.10	0.11	0.15

30°C then decreased to 1.2 percent at 25°C and 1.5 percent at 20°C.

The BVOM increased with decreasing reactor temperature in the nutrient-supplied experiment. At 35°C the BVOM was 3.9 percent and increased to 4.1 percent at 30°C and 5.1 percent at 25°C.

4.3. Scanning electron photomicrographs. Scanning electron photomicrophs of the aluminum oxide support material with attached biofilm are shown in Figure 19. The photomicrographs are from a sample of media support material taken at the completion of the 25°C nutrient-supplied temperature evaluation, which was the last temperature evaluation of the study. Both Series A and Series B are a series of photomicrographs of a different individual support particle.

Photo A-1 was taken at 100X and shows the relationship of several support particles. The particle of interest is slightly above and left of center. Photo A-2 was taken at 300X and shows biofilm attached within and around a triangular shaped hole. Photo A-3 was taken at 1500X and is a closer examination of the biofilm within the triangular hole. Note that there is a horizontal ledge-shaped structure at the bottom of Photo A-3. Photo A-4 was taken at 4,500X and shows the bacteria within the biofilm attached to the ledge-shaped structure at the bottom of Photo A-3.

Series B is a microscopic examination of the biofilm attached to another support particle. Photo B-1 was taken at 450X and shows the particle and part of the biofilm that the series develops from. The area of interest is the biofilm attached to the ragged edge of the particle shown in Photo B-1 which is just left of center. Photo B-2

was taken at 7000X and is a closer examination of the biofilm described in Photo B-1. Both Photos B-3 and B-4 were taken at 20,000X and show the bacteria within the biofilm.

No attempt was made to identify the bacterial organisms or determine the composition of the biofilms.

SERIES A

Photo A-1
100X

Photo A-2
300X

Figure 19. Scanning electron photomicrographs.

SERIES A

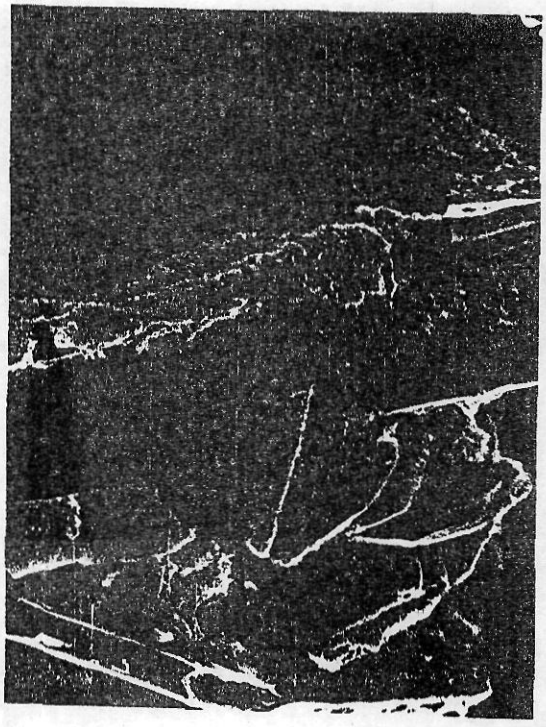

Photo A-3
1500X

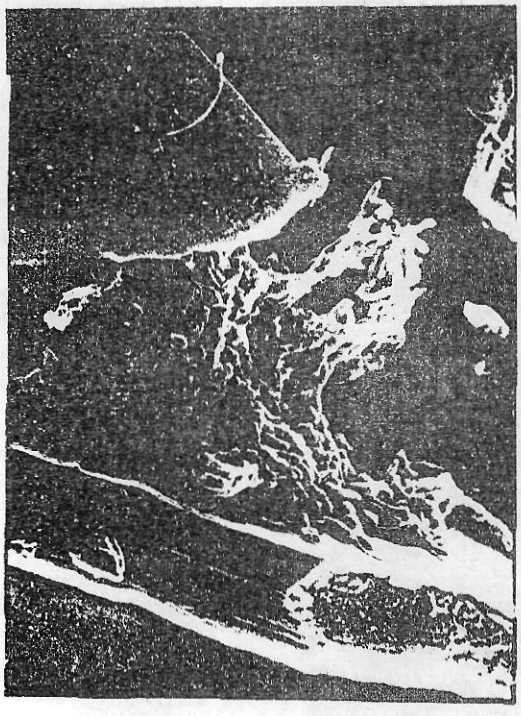

Photo A-4
4500X

Figure 19 continued.

SERIES B

Photo B-1
450X

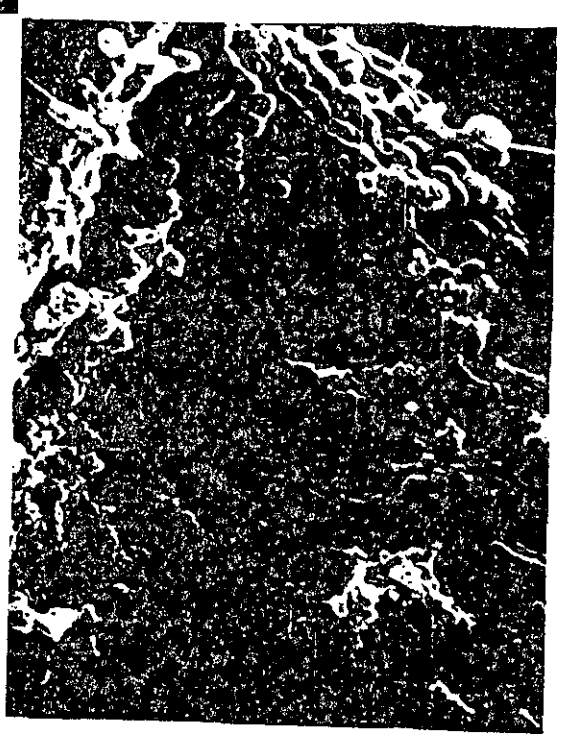

Photo B-2
7000X

Figure 19 continued.

SERIES B

Photo B-3
20000X

Photo B-4
20000X

Figure 19 continued.

CHAPTER V

Discussion

The results presented in Chapter IV indicate that the AFEB process is affected by both temperature and nutrient addition. Highest COD removal efficiencies were observed at 35°C and process efficiency was influenced by the addition of nutrient salts. This chapter will discuss the results presented in Chapter IV and compare those results with other data found in the literature.

Also included in this chapter are results from a previous study by Switzenbaum and Jewell (83) which involved the effects of temperature and organic volumetric loading rates on an AFEB reactor treating dilute organic wastes. The results are presented in Section 5.2 and are provided for comparing specific removal rates and activation energies.

5.1. Comparison of the effects of temperature on gas production rates.

Little correlation can be made relating the effects of temperature and nutrient addition on gas production in this study. As presented in Section 4.1.1, the average daily gas production rate decreased with decreasing temperature for the nutrient-limited experiment. However, average daily gas production first decreased, and then increased with decreasing temperature in the nutrient-supplied experiment. From the data, it appears that the addition of nutrient salts had a much greater effect on gas production than did decreasing temperature. Figure 8 shows that the average daily gas production rate for the nutrient-supplied experiment was always greater than the gas production rate for the nutrient-limited experiment. However, the methane composition for the nutrient-supplied experiment was less than the

nutrient-limited experiment for each of the temperatures evaluated (Figure 10).

The theoretical amount of methane produced per gram of COD removed can be determined from the relationship that at 0°C and one atmosphere, one gram of COD is equivalent to 350 ml of methane (58). Table 15 lists the theoretical amount of methane that could be produced per gram of total COD removed and the percent of the theoretical methane actually produced for each of the temperatures evaluated in both of the experiments. As can be seen from Table 15, low percentages of the theoretical methane production rate were observed in both the nutrient-limited and the nutrient-supplied experiments. The highest percentage for the nutrient-limited study was 66.8 percent at 30°C and the lowest was 46.7 percent at 20°C. The highest percent of the theoretical value for the nutrient-supplied experiment was 81.6 percent at 20°C and the lowest was 36.4 percent at 30°C.

Normally gas production rates, and in particular methane production rates, are a good indicator of reactor performance. Switzenbaum and Danskin (81) were able to obtain up to 95.3 percent of the theoretical methane production value with an associated solubable COD removal of 92.3 percent. Hickey and Owens (31) were able to obtain an average of 92 percent of the theoretical methane production value with a concomitant COD removal of 94 percent. Kugelman and Jeris (42) report that a decrease in the fraction of methane in daily gas production can indicate an upset digester, and Graef and Andrews (28) state that methane production is directly related to the metabolic activity of methanogenic bacteria.

Table 15. Theoretical Methane Production

	Nutrient-Limited Study	Nutrient-Supplied Study
Total COD Removed/Day (mg/d)		
35°C	3515	4183
30°C	2691	4313
25°C	2226	3373
20°C	2038	-
Theoretical CH_4 Production (ml/d)		
25°C	1230	1464
30°C	942	1510
25°C	779	1181
20°C	713	-
Measured CH_4 Production (ml/d)		
35°C	635	822
30°C	629	549
25°C	411	964
20°C	333	-
Percent of Theoretical Production Rate		
35°C	51.6	56.1
30°C	66.8	36.4
25°C	52.8	81.6
20°C	46.7	-

There are several reasons which may explain the low methane production rates observed in this experiment. One reason may be due to the scale of the experiment. Small gas leaks may add up to a very large error in gas measurement. Also, in larger systems operating for extended time periods, it is much easier to normalize values and obtain a more representative number. Another reason is that some surging in the liquid level of the reactor did exist and small amounts of digester gas may have escaped through the liquid effluent line. In addition, the variation in the feed pump rate could have affected the growth rate of the microorganisms thus introducing variability in gas production. However, other parameters correlated well with each other and with environmental conditions to indicate that the reactor was not upset and that the population of microorganisms was viable.

For the above reasons, COD removal rates were considered to be a better gauge of reactor performance and are used in subsequent process evaluations.

5.2. Temperature effects on biomass concentration. The effects of temperature on biomass concentration in this experiment were difficult to determine due to the small scale of the reactor. As presented in Tables 12 and 13, unattached biomass (measured as volatile suspended solids) ranged from a low of 99.7 mg/l in the nutrient-supplied experiment to a high of 604 mg/l in the nutrient-limited experiment. The attached biomass (measured as biofilm volatile organic matter) ranged from 37 to 57 mg/g of support material in the nutrient-limited

experiment and from 39 to 51 mg/g of support material in the nutrient-supplied experiment.

Figure 16 shows that suspended solids increased with decreasing temperature for both the nutrient-limited and the nutrient-supplied experiments. Likewise, Figure 18 shows that the attached biomass concentration increased with decreasing temperature for the nutrient-supplied experiment but the effect of decreasing temperature on the attached biomass of the nutrient-limited experiment is unclear. In the nutrient-limited experiment, the attached biomass first increased with decreasing temperature, then decreased sharply, and then increased again. Though the probable cause for such an erratic response to temperature decrease is nutrient limitation, it is difficult to say exactly what caused the BVOM to fluctuate so greatly.

The low biomass concentrations found in this study and the photomicrographs presented in Section 4.3 show that a very small amount of biofilm formed on the support particles. The low biomass formation has been attributed to high shear from the recycle flow, and abrasion from particle to particle contact, or possibly to sample preparation (82).

The concentration of volatile suspended solids from this study compare well with results obtained by Switzenbaum and Danskin (81). In their whey treatability study, they found VSS to vary from 197 mg/l to 880 mg/l. As expected, the VSS in this study were very much higher than those obtained by Switzenbaum and Jewell (83). They found VSS to vary from a low of 6.6 to a high of 42 mg/l over the range of conditions examined. The large differences between the VSS values is

undoubtedly due to the much higher substrate concentration used in this study.

But, the values for the biofilm volatile organic matter obtained in this study are very much lower than those found in previous work. The maximum attached biofilm (measured as biofilm volatile organic matter) in this study was 57 mg/g of support material. Switzenbaum and Jewell (83) found attached biofilm to exceed 95 mg/g of support material.

Nevertheless, the trends observed for biomass concentration as a result of decreasing temperature, agree with the trends observed in previous work. The unattached biomass (measured as VSS) for both the nutrient-limited and the nutrient-supplied studies, and the attached biomass (measured as BVOM) for the nutrient-supplied study increased with decreasing temperature. Similarly, Switzenbaum and Jewell (83) found that biofilm thickness and associated biomass concentrations tend to increase with decreasing temperature.

5.3. Temperature effects on volatile organic acids concentration.

Volatile organic acids (VOA) concentration has proven to be a good indicator of the condition of an anaerobic reactor as changes in the VOA concentration reflect changes in the bacterial population of the anaerobic process. In this experiment, the VOA were measured as acetic acid and their concentrations, as affected by reactor temperature, are shown in Figure 15. The VOA concentration for both the nutrient-limited and nutrient-supplied experiments increased with decreasing reactor temperature. The VOA concentration of the nutrient-limited

experiment ranged from 1900 mg/l at 35°C to 3,407 mg/l at 25°C which represents more than an 83 percent increase. Over the same temperature range the VOA concentration in the nutrient-supplied experiment went from 627 mg/l to 823 mg/l which is equal to a 31 percent increase.

The results from the nutrient-supplied experiment are within the range of VOA concentrations found in other studies. Switzenbaum and Danskin (81) measured 141 mg VOA/l in their whey treatability study with similar reactor conditions. Hickey and Owens (31) measured 970 mg VOA/l at similar reactor conditions treating one percent acid whey in a fluidized bed process.

In contrast, the VOA concentration in the nutrient-limited experiment increased 1.9 times when react or temperature was lowered from 35°C to 20°C. And at similar reactor conditions, the VOA concentration for the nutrient-limited experiment was nearly 14 times greater than that found in the Switzenbaum and Danskin (81) study and nearly twice that observed in the Hickey and Owens (31) study.

The literature shows that the production of VOA in the AFEB process to be largely dependent upon the organic loading rate and only slightly effected by temperature (31, 81, 83). In anaerobic sludge digestion VOA content usually runs in the range of 50 to 300 mg/l (93). But more important than any given value for volatile organic acids concentration is its rate of change (27, 51). A sharp rise in VOA content indicates that something has happened either to retard the methanogenic bacteria or to stimulate the acidogenic population (27). Thus, as shown earlier, decreasing reactor temperature caused a greater change in VOA concentration (which indicates a greater effect

on the microbial population) for the nutrient-limited experiment than for the nutrient-supplied experiment.

5.4. Comparison of Arrhenius temperature dependence plots. The effects of temperature on reaction rates of the AFEB process can be evaluated by developing a temperature dependence plot from the Arrhenius expression. The Arrhenius expression (1) has been widely used for relating reaction rates to temperature dependence over limited temperature ranges, and has been especially useful for describing temperature dependence in biological and microbiological processes. In chemical engineering, the Arrhenius law has been strongly suggested from various standpoints as being a very good approximation to true temperature dependency (46).

The Arrhenius equation may be expressed as:

$$K = K_o \exp(-E/RT) \qquad (5.1)$$

where

K = reaction velocity

K_o = frequency factor

E = activation energy (calories/mole)

R = gas constant (1.98 cal/mole °K)

T = absolute temperature, °K

K_o, the frequency factor, is assumed to be a constant independent of temperature and has the same units as K, the reaction velocity.

Taking the natural logarithm of Equation (5.1) yields

$$\ln K = \ln K_o - \frac{E}{R}\frac{1}{T} \qquad (5.2)$$

Equation (5.2) has the form of a straight line when the natural logarithms of the reaction velocities are plotted against the reciprocals of absolute temperature. The slope of the line is equal to -E/R.

Molecular collision and transition theories have aided in elucidating the meaning of K_o and E in the interpretation of the Arrhenius equation. Levenspiel (46) provides the following guides for interpreting an Arrhenius temperature dependence plot:

1. From Arrhenius' law the plot of ln K versus $\frac{1}{T}$ gives a straight line with;
   a) large slope for large E,
   b) small slope for small E.
2. Reactions with high activation energies are very temperature-sensitive. Reactions with low activation energies are relatively temperature insensitive.
3. From the Arrhenius law the frequency factor K_o does not affect the temperature sensitivity of the reaction.
4. A given reaction is much more temperature-sensitive at low temperature than at high temperature.
5. A change in activation energy indicates a shift in the controlling mechanism of the reaction.

In this study, K, the reaction velocity was calculated as a soluble chemical oxygen demand (SCOD) removal rate based on the specific

surface area of the biofilm support material. The surface area of the support material was determined from a previous study to be 45,216 cm^2/l (assuming spherical particles) (83). The unexpanded bed volume in this study was 368 cm^3. Thus the total surface area of the biofilm support material in this study was 16,639 square centimeters. The specific SCOD removal rates for each of the temperatures evaluated in both the nutrient-limited and nutrient-supplied experiments are listed in Tables 16 and 17. Summary data and average specific removal rates from Switzenbaum and Jewell's study (83) are listed in Tables 18, 19, and 20.

Arrhenius temperature dependence plots for the nutrient-limited, nutrient-supplied, and low strength experiments are shown in Figure 20. A least-squares line of best fit is also shown for each of the experiments in Figure 20. The slope of the line of best fit is equal to -E/R and from that relationship, E, the activation energy, was calculated for each of the experiments. The activation energies for the high strength nutrient-supplied experiment, the high strength nutrient-limited experiment, and the low-strength study were 3099, 2267, and 1875 calories per mole, respectively. Corresponding equations for the individual best fit lines are given in Table 21.

Caution must be exercised when comparing data from different experiments as the physical characteristics of a given experimental system may influence the effects of temperature on intrinsic growth characteristics (59). In addition, the temperature response of a biological process is affected by substrate composition and concentration, and the predominant population of microorganisms (11, 63). The

Table 16. Nutrient-limited Experiment - Specific Removal Rates

Temperature	35°C	30°C	25°C	20°C
Influent COD (mg/l)	9580	9231	7920	9719
Avg. effluent SCOD (mg/l)	3800	4385	4927	5040
SCOD removed (mg/l)	5780	4846	4793	4679
Avg. flow rate (cm^3/day)	614	614	614	614
Surface area* (cm^2)	16,639	16,639	16,639	16,639
Specific removal rate**	0.213	0.179	0.177	0.173
ln (specific removal rate)	-1.55	-1.72	-1.73	-1.76
°K	308	303	298	293
1/°K	.003247	.003300	.003356	.003413

* Surface Area = surface area of biofilm support material.

** Specific Removal Rate = mg COD removed/cm^2/day.

Table 17. Nutrient-Supplied Experiment - Specific Removal Rates

Temperature	35°C	30°C	25°C
Influence COD (mg/1)	9780	9820	10160
Avg. effluent SCOD (mg/1)	1995	2410	3590
SCOD removed (mg/1)	7785	7410	6570
Avg. flow rate (cm^3/1)	614	614	614
Surface area* (cm^2)	16,639	16,639	16,639
Specific removal rate**	.287	.273	.242
ln (specific removal rate)	-1.25	-1.30	-1.42
°K	308	303	295
1/°K	.003247	.003300	.003356

* Surface area = surface of biofilm support material.

** Specific removal rate = mg SCOD removed/cm^2/day.

Table 19. Summary Data - Low Strength Study, 20°C (83)

Flow Rate (1/day)	Influent COD (mg/1)	Effluent SCOD (mg/1)	SCOD Removed (mg/1)	Surface Area* (cm^2)	Specific Removal** Rate
2	200	34.1	165.9	18086	0.018
2	400	48.6	351.4	"	0.039
2	800	79.5	520.5	"	0.055
3	200	51.8	148.2	"	0.025
3	400	55.1	344.9	"	0.057
3	600	101.7	498.3	"	0.083
6	200	55.4	144.6	"	0.048
6	400	76.4	323.6	"	0.107
6	600	124.0	476.0	"	0.158
12	200	86.4	113.6	"	0.075
12	400	131.3	268.7	"	0.178
12	600	204.8	395.2	"	0.262
18	200	108.0	92.0	"	0.092
18	400	172.0	228.0	"	0.227
18	600	268.0	332.0	"	0.330
36	200	124.0	76.0	"	0.151
36	400	232.0	168.0	"	0.334
36	600	340.0	260.0	"	0.518
Average specific removal rate				=	0.153
ln[specific removal rate]				=	-1.88

* Surface area = surface area of biofilm support material.
** Specific removal rate - mg SCOD removed/cm^2.day.

Table 20. Summary Data - Low Strength Study, 10°C (83)

Flow Rate (l/day)	Influent COD (mg/l)	Effluent SCOD (mg/l)	SCOD Removed (mg/l)	Surface Area* (cm^2)	Specific Removal** Rate
2	200	54.3	145.7	18086	0.016
2	700	66.9	333.1	"	0.037
2	600	136.6	463.4	"	0.051
3	200	60.2	139.8	"	0.023
3	400	77.2	322.8	"	0.054
3	600	142.7	457.3	"	0.076
6	200	90.5	109.5	"	0.036
6	400	139.1	260.9	"	0.087
6	600	240.3	359.7	"	0.119
12	200	99.1	100.9	"	0.067
12	400	105.1	214.9	"	0.143
12	600	321.0	279.0	"	0.185
18	200	110.0	90.0	"	0.090
18	400	812.2	187.8	"	0.187
18	600	345.8	254.2	"	0.253
36	200	129.6	70.4	"	0.140
36	400	243.5	156.5	"	0.312
36	600	385.1	214.9	"	0.428
Average specific removal rate				=	0.128
ln [specific removal rate]				=	-2.06

* Surface area = surface area of biofilm support material.
** Specific removal rate = mg SCOD removed/cm^2/day.

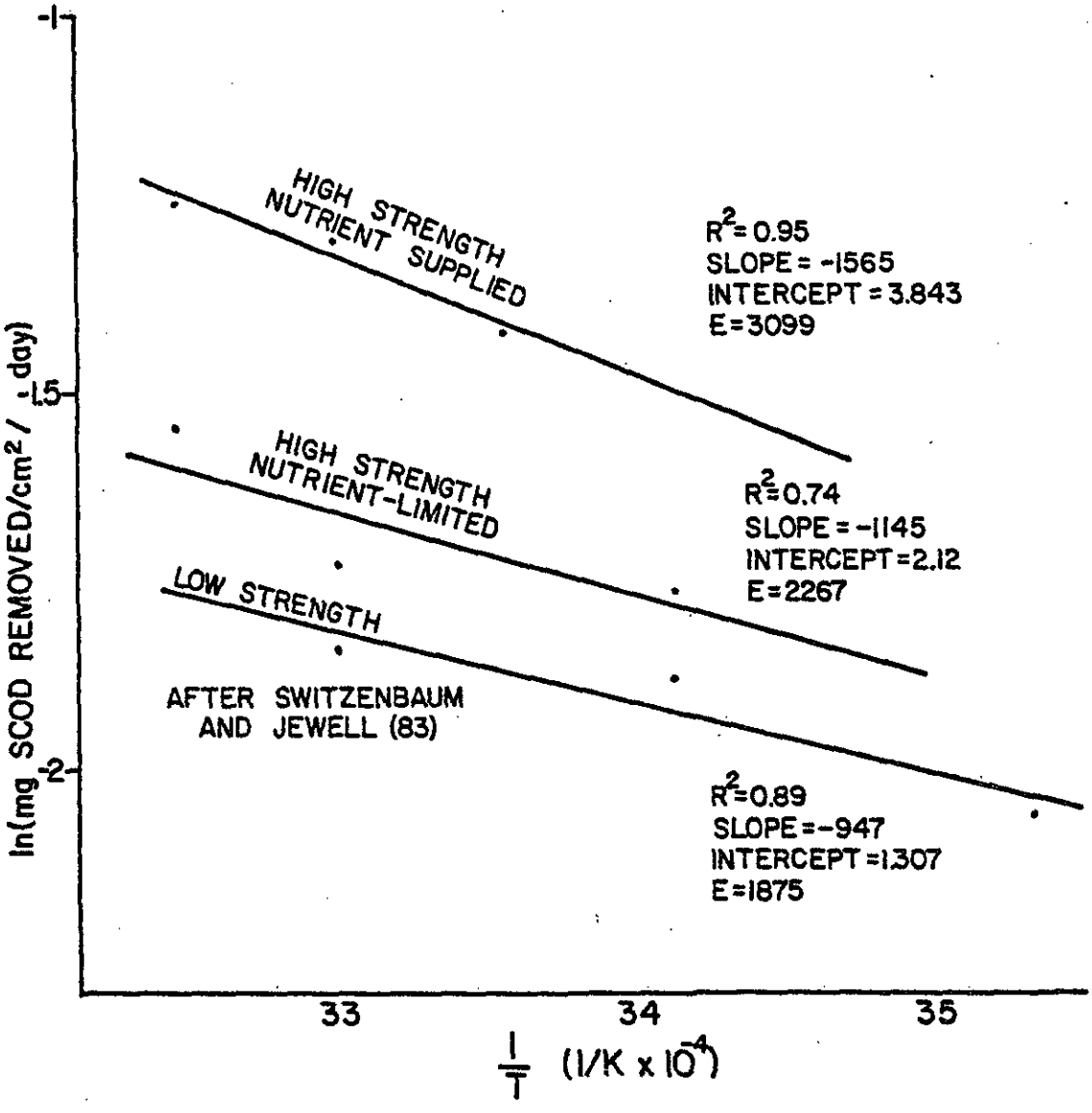

Figure 20. Arrhenius temperature dependence plots.

Table 21. Arrhenius Equations for the Least-Squares Lines of Best Fit

1. High Strength Nutrient-Supplied Study:

$$K = 46.67 \exp[-3099 \text{ cal/mole}/RT]$$

2. High Strength Nutrient-Limited Study:

$$K = 8.331 \exp[-2267 \text{ cal/mole}/RT]$$

3. Low Strength Study:

$$K = 3.695 \exp[-1875 \text{ cal/mole}/RT]$$

where

$R = 1.98$ calories/mole °K

$T = °K$

justification for comparing the data obtained by Switzenbaum and Jewell (83) with the data obtained in this study centers on:

1) the physical characteristics of the two experiments were very similar,
2) the same biofilm support material (with attached biofilm) was used in both experiments, and
3) both experiments used a carbohydrate as a substrate.

(Though whey was used as the substrate in this experiment, its major carbohydrate, lactose, is rapidly hydrolyzed by β-galactosidase to galactose and glucose (6). Glucose was used as a substrate by Switzenbaum and Jewell.)

As previously stated, the extrapolation of data from other studies should be approached with care. Nevertheless, the literature does provide a good idea of the range of values determined for other systems. Johnson et al. (37) statistically analyzed a broad variety of biological processes and found two distinct peaks for the frequency of occurrence of activation energies. They found the first peak to occur between 11,000 and 13,000 cal/mole and the second peak to occur between 15,000 and 18,000 cal/mole. Characklis and Gujer (17) report that microbial growth rates generally have an activation energy of 50 KJ/mole (= 11,950 cal/mole) or more. Muck and Grady (59) list activation energies between 5,100 and 40,000 cal/mole for various microbial heterotrophs grown in both batch and continuous culture in the mesophilic range.

Ashare et al. (2) have compiled studies concerning anaerobic slurry processes, and have determined the activation energy from an

Arrhenius temperature dependence plot to be 15,000 cal/mole. The temperature sensitivity of anaerobic slurry systems is also well documented from other sources. Lawrence (43) states that for a given cell retention time, treatment efficiencies for an anaerobic slurry system decrease as temperature decreases. Speece and Kem (73) report that a drop in temperature from 35°C to 27°C in an anaerobic slurry system decreased the rate of methane formation by 80 percent. In this same study the authors also found that slightly increasing the temperature above 35°C stimulated methane production to a greater extent than it did acid formation. Grady (27) cites temperature change in anaerobic slurry systems as being particularly important because of interacting microbial populations. Dague (21) reported that an anaerobic slurry reactor that has been developed at one temperature is likely to have a different balance of microbial organisms than a reactor developed at another temperature, and that changes of only a few degrees can produce an imbalance between acid forming and methane forming organisms which can lead to process failure. Further, he reported that maintaining a reactor at a uniform temperature is more important than maintenance of an optimum temperature which gives the maximum possible conversion rates.

As shown in Figure 20, the slope of the line of best fit is slightly steeper for the high strength nutrient-supplied experiment which indicates that it was slightly less temperature insensitive than the other two experiments. The activation energies determined for the three experiments are, however, within a relatively narrow range (1875-3099 cal/mole); and, depending on the range of values selected

from the literature, are approximately two to 16 times less than the activation energies determined for other biological treatment systems.

5.5. Comparison of Q_{10} values. A temperature correction factor, Q_{10}, which is a factor indicating how many times an overall reaction rate will increase if the temperature is increased by 10°C, has been useful for comparing tempeature effects in biological systems (63). Q_{10} is defined as:

$$Q_{10} = \frac{\text{rate at } (T°C + 10°C)}{\text{rate at } T°C} \tag{5.3}$$

Q_{10} values have also been used to qualitatively indicate whether a system is biochemically or diffusionally limited. Under optimal conditions, dispersed homogeneous systems of exponentially growing bacteria are usually limited only by their intrinsic growth rate (14). Q_{10} values for such systems range between 1.7 and 2.2 (17, 72). In contrast to a biochemically limited system, Q_{10} values for a diffusionally limited system are in the order of 1.3 (17, 72).

Lawrence and McCarty (44) have determined kinetic coefficients for the fermentation of various fatty acids. From their data on acetic acid, a Q_{10} value of 1.72 was calculated for a laboratory scale anaerobic slurry system over the temperature range of 25-35°C. Q_{10} values reported for aerobic systems are even higher. Wuhrman (91) reported that Q_{10} values for the activated sludge process range between 2.00 and 2.06 for temperatures between 0° and 25°C.

Q_{10} values for the AFEB process were calculatd from the reaction rate equations listed in Table 21. Q_{10} values for the high strength

nutrient-supplied experiment, the high-strength nutrient-limited experiment and the low-strength study were 1.19, 1.13, and 1.11, respectively.

Thus, based on activation energies and Q_{10} values, the AFEB process is less affected by temperature than either the anaerobic slurry or activated sludge processes. This is a particularly significant point as the activated sludge process is well documented as being relatively insensitive to temperature flucuations (27, 29, 56).

As stated above, microbial growth rates for homogeneous systems generally have a Q_{10} value of about two. The AFEB process was found to have a Q_{10} value between 1.1 and 1.2, which is characteristic for diffusion limited processes. The diffusional limitation in the AFEB reactor is attributed to the concentration gradient in the biofilm. Unlike dispersed growth systems, the AFEB process is a heterogeneous process consisting of at least two boundaries, a substrate-biofilm interface and a biofilm-solid support interface. Since the AFEB reactor is also a completely mixed reactor, no external mass transfer resistances exist, and diffusional limitations involve only the transfer of substrate and metabolic end-products through the biofilm. Several mechanistic models have been developed which utilize Ficks law, the general Monod equation, and flow models for describing mass transfer relationships in a biofilm reactor (57, 60, 67, 89). Currently, however, little information exists concerning many aspects of biofilms (and in particular anaerobic films) among which ecology and density are most significant. Thus these models have served mainly as learning tools.

5.6. Comparison of the effects of nutrient addition on reactor performance. As described in Section 3.3.1, this study originally involved only the examination of the effect of temperature on the AFEB process treating a high strength waste and did not propose to examine the effects of nutrient limitation. It was only due to relatively poor reactor performance and low COD removal rates that nutrient limitations were considered. However, comparison of reactor performance before and after nutrient addition provides information related to successful and efficient reactor operation, and lends support to the literature concerning nutrient requirements in anaerobic digestion.

Tables 7 and 8 list the formulas for the nutrient-limited and the nutrient-supplied substrates. Data were collected concerning the effects of temperature with the reactor first being fed the nutrient-limited substrate and then the nutrient-supplied substrate. Tables 12 and 13 list summary data for each of the experiments.

The operation of the AFEB reactor improved markedly after the addition of the nutrient-supplied substrate. Gas production increased within 24 hours and the effluent appeared darker (probably due to sulfide precipitate). Average daily gas production increased to 1445 cm^3/day (at 0°C and atmospheric pressure) and stayed above the corresponding values for the nutrient-limited experiment. Suspended solids decreased to 121 mg/l and stayed well below the values determined in the nutrient-limited experiment.

More impressive than gas production and suspended solids levels were the increase in COD removal efficiencies and the decrease in volatile organic acids production. Soluble COD removal efficiency increased to nearly 80 percent for the nutrient-supplied experiment

and stayed above 64 percent for all the temperatures evaluated. The soluble COD removal efficiency for the nutrient-limited experiment was comparatively low, the highest value attained was 60.3 percent at 35°C. Figure 15 shows the dramatic effect of nutrient addition on volatile acids (VOA) concentration. The VOA concentration of the nutrient-limited experiment was always at least three t imes higher than the VOA concentration of the nutrient-supplied experiment over the temperature range evaluated. With decreasing temperature, the VOA concentration of the nutrient-limited experiment increased to more than five times the VOA concentration of the nutrient-supplied experiment.

In the studies discussed in Section 2.7.7, the common response of a nutrient limited system to nutrient addition was an increase in substrate utilization. In this study, substrate utilization is synonomous with COD removal, and the addition of nutrient salts to the reactor feed dramatically increased the COD removal rate. As presented earlier, the maximum COD removal efficiency when the reactor was being fed a nutrient-limited feed was 60.3 percent and the concomitant specific removal rate was 0.213 mg SCOD removed/cm^2/day. When the nutrient salts were added to the feed the COD removal efficiency reached 79.6 percent and the concomitant specific removal rate was 0.287 mg SCOD removed/cm^2/day.

Also of particular interest is the volatile organic acids concentration. The VOA concentration for the nutrient-limited experiment was as much as five times the concentration of the nutrient-supplied study. Since VOA are produced by the acid forming

microorganisms, and serve as substrate for methanogens, the high VOA concentration in the nutrient-limited study indicates that the methanogenic bacteria were more severely inhibited by nutrient limitation. Further, the chemical analysis of sweet whey powder (Table 6) indicates that potassium and magnesium were in sufficient quantity as not to be limiting, and improved reactor performance was induced by the addition of either iron, cobalt, or nickel or some combination of those elements.

Again, it is important to stress that the only difference between the substrate used by Switzenbaum and Danskin (81) and the substrate used in the study for the nutrient-limited experiment is the dilution water. It is apparent from the results obtained by Switzenbaum and Danskin (81) that necessary trace nutrient requirements were supplied by the tap water. They were able to attain a 93.1 percent COD removal efficiency treating an influent substrate of 10,000 mg COD/l at an organic loading rate of 8.9 Kg COD/m^3/day. Using the same substrate formula and concentration, and nearly the same organic loading rate, the highest COD removal efficiency attained in this study with the nutrient-limited substrate was 60.3 percent. However, when inorganic nutrient salts were added to the influent, the COD removal efficiency increased to 79.6 percent.

Thus, it must not be assumed that trace nutrient requirements for the anaerobic digestion process will be supplied from the reactor environment. Specific attention must be given to not only the environmental requirements of the microorganisms, but also their nutrient requirements if successful treatment is to be achieved.

C H A P T E R V I

SUMMARY

Seven parameters were monitored on a bench scale AFEB reactor over a four month period to evaluate the effects of temperature and nutrient limitation on the AFEB process. The program of study involved first monitoring the psuedo-steady-state performance of the reactor over the temperature range of 54-20°C. Later, due to poor reactor performance, nutrient salts were added to the influent substrate and reactor performance was monitored over the temperature range of 35- 25°C.

Gas production rates showed little correlation to decreasing temperature for either the nutrient-limited experiment or the nutrient supplied-experiment. However, this was probably due to the scale of the reactor or possibly gas escaping through the liquid effluent line.

Results for the concentration of attached biomass (measured as biofilm volatile organic matter) were low for both of the experiments and indicate that a very small amount of biofilm formed on the support particles. This is supported by scanning electron photomicrographs taken at the end of the study. No direct correlation to decreasing temperature is apparent for the attached biomass in the nutrient-limited experiment. However, attached biomass increased with decreasing temperature in the nutrient-supplied experiment. This trend was also observed in a previous study (83).

Unattached biomass (measured as effluent suspended solids) increased with decreasing temperature for both the nutrient-limited and nutrient-supplied experiments.

The addition of nutrient-salts greatly improved reactor performance. Gas production increased and suspended solids decreased. More importantly, COD removal efficiencies increased and volatile organic acids dramatically decreased. This indicates that the methanogenic bacteria were more severely inhibited by nutrient limitation than were the nonmethanogenic bacteria.

Activation energies were calculated from Arrhenius temperature dependence plots for the nutrient-limited experiment, the nutrient-supplied experiment, and for a previous low strength study. The values are within a rather narrow range (1875-3099 cal/mole) and indicate that the AFEB process is relatively temperature insensitive. This is in contrast to anaerobic slurry systems which are highly temperature sensitive.

Q_{10} values calculated for the above three reactions were 1.19, 1.13, and 1.11, respectively. These values are much lower than those for biochemical intrinsic rate limited systems and indicate that the AFEB process is more restricted by diffusional limitations than by reaction rates.

C H A P T E R V I I

CONCLUSIONS

Based on the results of this study it can be concluded that:

1. The AFEB process is relatively temperature insensitive. Calculated activation energies were low and within an narrow range (1875-3099 cal/mole). These values are much lower than those reported for anaerobic slurry systems. Likewise, calculated Q_{10} values were low (1.11-1.19) and indicate that the AFEB process is even more temperature tolerant than the activated sludge process.
2. The AFEB process is more restricted by diffusional limitations than by biochemical reaction rates. Q_{10} values were found to be on the order of 1.2 which is even less than typical values reported for diffusion limited systems.
3. Supplying external heat to increase the temperature of a waste to 35°C (often advised in anaerobic treatment) may have little advantage in the AFEB process. Since the AFEB process is less temperature sensitive, heating a waste effluent to an optimal temperature may not contribute to increased removal rates as much as in other anaerobic processes.
4. The addition of iron, cobalt, and nickel greatly enhanced reactor performance. COD removal rates increased and VOA dramatically decreased after addition of the above elements to the influent substrate. The higher COD removal rates and

the lower levels of VOA indicate that the methanogenic bacteria were more affected by nutrient-limitation than were the nonmethanogenic bacteria. Micronutrients must not be overlooked and may be a critical requirement for some industrial waste treatment applications.

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A P P E N D I X A

Thermophilic Data

At the beginning of this study the reactor was inadvertently operated in the thermophilic temperature range due to heat input from the recirculation pump. During this period of operation, the influent substrate formula was the same as that used in the nutrient-limited experiment (Table 7) and was supplied at an organic volumetric loading rate of 10 $kg/m^3/day$. Pseudo-steady-state data were collected while the incubator was maintained at 35, 30, and 25°C. However, it was discovered that the reactor temperature was actually in the thermophilic range and the corresponding temperatures were 54, 49, and 44°C. A bimetallic Precision Instrument Thermometer and a high temperature safety shut-off relay were added to the reactor to prevent such problems from reoccurring.

Summary data during the period of thermophilic operation are presented in Table 22. The data show that as the temperature decreased from 54 to 44°C:

a) the gas production and methane composition tended to increase,
b) the SCOD removal efficiency increased from 68.7 to 83.8 percent,
c) the volatile organic acids decreased from 1412 mg/l to 878 mg/l (a decrease of nearly 38 percent),
d) the effluent suspended solids increased from 168 mg/l to 399 mg/l.

Table 22. Thermophilic Summary Data

Temperature	54°C	49°C	44°C
Avg. daily gas prod. (cm^3/day)	1852	2183	1993
% CH_4	67.2	63.9	68.5
% CO_2	32.8	36.1	31.5
CH_4 production (cm^3/day)	1245	1395	1365
CO_2 production (cm^3/day)	607	788	628
Influent COD (mg/l)	9143	9719	10733
Effluent soluable COD (SCOD) (mg/l)	2858	2268	1739
Effluent total COD (TCOD) (mg/l)	3500	2648	2334
SCOD Removed (mg/l)	6285	7451	8994
TCOD Removed (mg/l)	5643	7071	8399
SCOD removal efficiency (%)	68.7	76.7	83.8
TCOD removal efficiency (%)	61.7	72.8	78.3
Effluent suspended solids (mg/l)	168	176	399
Volatile suspended solids (mg/l)	163	172	375
Biofilm volatile organic matter (mg/g (media))	-	38	42
Volatile organic acids (mg/l) (as CH_3COOH)	1412	1193	878
pH	7.3	7.2	7.3

Figure 21 is a graph of the SCOD removal efficiency of the thermophilic data as a function of temperature (also included are the removal efficiencies for the nutrient-limited experiment). As can be seen, the actual results bare no resemblance to the expected results. Two optimal temperature levels for anaerobic treatment have been reported, one in the mesophilic range of 29 to 38°C and the other in the thermophilic range of 49-57°C (26, 49). McCarty (51) has reported that in the thermophilic range, reaction rates proceed faster resulting in more efficient operation. Thus maximum removal efficiency would be expected to occur at 54°C. Also, since the range of 39-48°C lies between the two optimal temperature ranges, a reduction in reactor performance would be expected at 44°C. However, as shown in Figure 21, maximum SCOD removal efficiency occurred and 44°C and reactor performance at 54°C was far less than expected.

The only plausible conclusion that can be made from that data, and it is admittedly qualitative, is that a stable thermophilic bacterial population was not significantly developed while the reactor was at 54°C. And that increased removal efficiencies and gas production rates resulted as the bacterial population grew due to the added time of reactor operation.

In contrast to the above data, Schraa and Jewell (71) found that both medium and high strength wastes could be treated in an AFEB reactor operating in the thermophilic range. They found that at 55°C, anaerobic films were easily and rapidly developed on inert support particles of diatomaceous earth. Further, at 55°C they were able

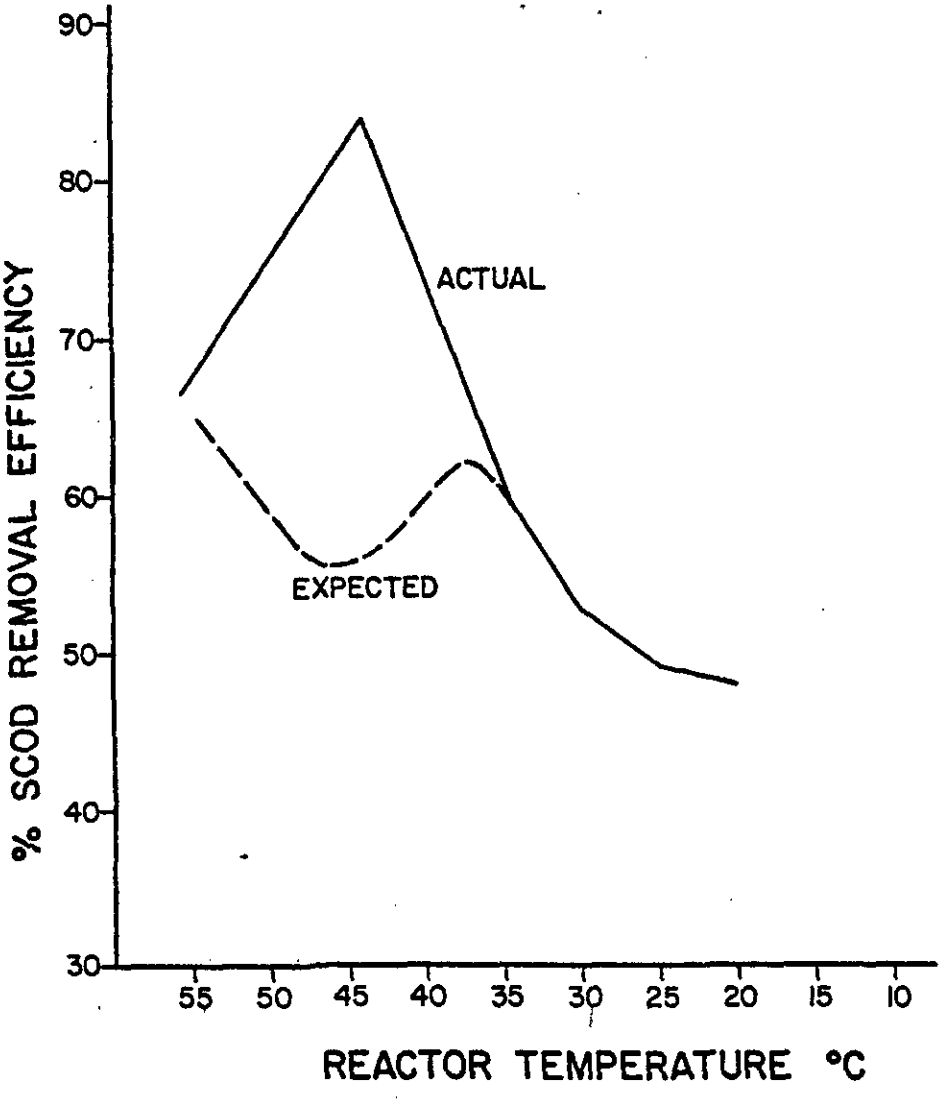

Figure 21. Influence of temperature on thermophilic SCOD removal efficiencies: actual and expected curves

achieve a 70 percent total COD removal efficiency at a volumetric organic loading rate of 30 g COD/l-day.