THESIS

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Coupled High Solids Fermentation and
Anaerobic Filtration of Cellulosic Residues

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COUPLED HIGH SOLIDS FERMENTATION AND ANAEROBIC FILTRATION OF CELLULOSIC RESIDUES

By

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ABSTRACT

COUPLED HIGH SOLIDS FERMENTATION
AND ANAEROBIC FILTRATON OF CELLULOSIC RESIDUES

By

Yow-Ming Lin

A coupled high solids fermentation and attached-growth anaerobic filtration process to produce methane from cellulosic residues was developed and successfully operated for 18 months using wheat straw as the substrate. The process was conducted in eight 600 ml packed reactors and two anaerobic filters connected in series allowing semi-continuous feeding of straw at a solids concentration of 34%. A mobil liquid phase was circulated at a constant rate to carry COD from the packed reactors to the anaerobic filters where 85% of the total methane was generated.

The major functions of the packed reactors were the hydrolysis of the solid substrate and the production of organic acids. The volatile fatty acid COD, composed mostly by acetic, propionic, and butyric acids, was produced at a slower rate than soluble COD in the packed reactors. The initial soluble COD, 65% of the COD produced, was contributed by leaching while the subsequent slow substrate degradation was attributed to microbial hydrolysis.

Specific methane production rates as high as 2.1 liter CH_4 per day per liter reactor volume and a volatile fatty acid COD removal efficiency of 98% were obtained from the anaerobic filters at loadings of 297 to 594 lb soluble COD/day/10^3 ft^3 with a hydraulic retention time

of 34 hours. Methane contributed 73% to 79% of the total gas production.

Total methane production per unit weight of substrate input was 104.3 ml CH_4 per gram of straw input with 40 days substrate solids retention time and 76.3 ml CH_4/g straw with 18 days solids retention time. At a 40 days solids retention time overall degradation of the un-pretreated straw was 30% with 43% and 41% degradation of cellulose and hemi-cellulose respectively. At an 18 days soids retention time degradations were 20% overall, 26% for cellulose and 31% for hemi-cellulose.

During most of the study, a liquid reservoir served as an equalization basin preventing shock loading to the anaerobic filters. Results of a direct input study, without the liquid reservoir, suggested that the methanogenic bacteria in the anaerobic filters could sustain methane production during transient loading, although the suddenly increased substrate could not be completely utilized on the first pass. A mathematical model of solid substrate degradation in the packed reactor was developed. The curves computed from the model agreed closely with the experimental data. Biological hydrolysis of the un-pretreated wheat straw was found to be the rate limiting step in the system.

DEDICATED

TO MY BELOVED MOTHER

AND

TO THE MEMORY OF MY FATHER

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TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

FIGURE Page

CHAPTER ONE

INTRODUCTION

Anaerobic digestion (anaerobic methane fermentation) is a common and successfully used process in wastewater treatment. Methane and carbon dioxide are the final end products of this process resulting from microbial decomposition of organic matter in the absence of molecular oxygen. Although methane gas is a useful fuel that some treatment plants recover and use for heating the digesters or driving pumps, anaerobic digestion has been mainly used for sludge and wastewater stabilization; the methane gas has usually been treated as a by-product. However, in recent years, the increasing interest in alternative energy sources has altered the traditional role of anaerobic digestion. In addition to waste stabilization, anaerobic digestion has been considered as an energy production process and organic waste as the alternative energy source.

Among organic wastes, cellulosic agricultural residues are most abundant. It is estimated that, considering only food crop residues, over 290 million tons are produced every year in the United State (Benson, 1977). Table 1-1 shows the quantity of some agricultural residues produced in the United States. Most of these cellulosic residues exist in dry form or at very high solids concentration. In order to utilize these natural products to produce methane by anaerobic fermentation, a new process needs to be developed to overcome difficulties of mixing and pumping slurries with high solids content in the reactor and associated piping as well as the dificulties of substrate input to and

removal from the reactor. The current anaerobic fermentation processes require relatively low solids concentration for operation. Sawyer (1960) suggested that the optimum solids concentration in the conventional digester should not exceed six to eight percent. If conventional digesters were to be used for the fermentation of high solids cellulosic material, a large quantity of water would have to be added to reduce the solids concentration resulting in a greater reactor volume and larger residual sludge volume. This would make the process uneconomical.

Table 1-1. U. S. Food Crop Residue Generation

Crop	10^6 Tons/Year
Corn	156
Wheat	49
Soy Bean	40
Sorghum Grain	24
Oats	12
Barley	11
Rice Straw	4.4
Peanut	1.6
Rye	0.9

* After Jewell (1980).

This research investigates a new process that consists of fixed-film anaerobic filters and packed bed reactors which contain a high solids concentration of stationary phase and a mobil liquid phase. The special configuration of the packed bed reactors enable this process to have semi-continuous input of high solids cellulosic substrate. The anaerobic filters convert the fatty acids produced in the packed reactors to methane and carbon dioxide after being transported by the mobil liquid phase. Because the required reactor volume is inversely proportion to solids concentration, increasing the substrate concentration would allow a reduction in the total system volume and system cost, making this process economically competitive. Detailed presentation of this process and the experimental system used to conduct the process will be given in the following chapters.

The objectives of this research were to:

1. Design an anaerobic fermentation system which would allow semi-continuous feeding of high solids substrate without causing retardation of methane production.
2. Evaluate the performance of the high solids packed reactor and to investigate the behavior of cellulosic substrate degradation, total soluble COD production and volatile fatty acid production.
3. Evaluate the performance of the anaerobic filters including methane production and COD removal efficiency.
4. Evaluate the operational parameters, include liquid flow rate, hydraulic retention time, substrate input interval, and the percent of substrate solids concentration.

5. Determine the degradation rate of a cellulosic substrate without pretreatment.
6. Develop a mathematical model for the liquid soluble COD production by solid substrate in the packed reactor.

CHAPTER TWO

THEORETICAL BACKGROUND AND LITERATURE REVIEW

Methane fermentation is a complex biological process in which a mixed culture of microorganisms decomposes organic matter to gaseous end products, methane and carbon dioxide, in the absence of exogenous electron acceptors other than carbon dioxide (McInerney et al., 1981a). One distinct characteristic of this process is that only a small portion of the chemical energy from the decomposition of organic substrate is used for bacterial cell growth and about 90% of the energy is retained in the methane produced. The advantages of low biological growth and the production of an energy rich gas have made anaerobic digestion a favorite treatment method for sludges and strong organic wastes for a long time.

The mechanism of anaerobic fermentation was not clearly understood until the 1950's, although it had been successfully operated for many years. After Barker (1956) and Buswell et al. (1952), reported their studies of methane fermentation, extensive research was conducted by many investigators. Those studies have provided a better understanding of the complicated anaerobic fermentation process. This chapter will review the literature regarding important chemical, microbiological, engineering process control prameters, and other related concepts of methane fermentation as well as recent developments of this process.

2.1 Chemical and Microbiological Background

Anaerobic fermentation is carried out mainly by a diverse group of bacteria. The microbial population in the anaerobic fermentation ecosystem is composed of obligate and facultative anaerobic bacteria. Obligate anaerobes (aerophobic anaerobes) can only survive in the strict anaerobic condition, while the facultative anaerobes (aerotolerent anaerobes) can also use molecular oxygen during metabolism. According to current knowledge, the bacterial population involved in anaerobic fermentation can be classified into four different groups, namely, (1) fermentative bacteria, (2) obiligate hydrogen producing acetogenic bacteria, (3) methanogenic bacteria, and (4) homoacetogenic bacteria. The following paragraphs will discuss the microbiological functions of these bacteria and chemical reactions that exist in the system during microbial decomposition of organic substances.

2.1.1 Metabolic Stages of Anaerobic Fermentation

Traditionally, methane fermentation has been considered to have only two metabolic stages, an acid forming stage and a methane forming stage. In the first stage, a complex of fermentative acid forming bacteria degrade high molecular weight organic compounds such as polysaccharides and protein to volatile fatty acids, hydrogen, carbon dioxide, ammonia and sulfide. The second, or methane forming stage, involves a complex group of strict anaerobic methane bacteria. These methane bacteria convert the products from the first stage to methane and carbon dioxide.

Recently, a three stage scheme, first proposed by Bryant et al.

(1967), has become widely accepted (McCarty, 1981) and further expanded by microbiologists (Bryant, 1976, 1979; Kasper and Wuhrmann, 1978; Boone and Bryant, 1980; McInerney et al., 1981a, 1981b). The establishment of the three stage scheme (Figure 2-1) was based on the finding that fatty acids other than formate and acetate are degraded by syntrophic association of hydrogen-producing acetogenic bacteria and hydrogen-utilizing methanogens, and not by methanogens alone.

The first stage of the three-stage scheme is the same as in the two stage scheme model; the fermentative bacteria hydrolize polysaccharides to smaller organic sugars and degrade these products to fatty acids, alcohols, hydrogen, and carbon dioxide. The second stage involves hydrogen-producing acetogenic bacteria which are involved in (1) -oxidation of fatty acids of even numbered carbon to acetate and hydrogen and odd-numbered fatty acids to acetate, propionate, hydrogen; (2) oxidation of alcohols such as ethanol to acetate and hydrogen; and (3) decarboxylation of propionate to acetate, hydrogen and carbon dioxide (McInerney et al., 1981b).

The chemical reactions for the conversion of longer-chain fatty acids to acetic acid by acetogenic bacteria are shown in Equations 2-1 to 2-4 in the Table 2-1. Evidence of the second metabolic stage in anaerobic methane fermentation was obtained by the successful isolation of two fatty acid oxidizing acetogenic bacteria, Syntrophomonas wolfeii and Syntrophobacter wolinii, via coculture with hydrogen-utilizing bacteria. The microbiological characteristics of these two bacteria have also been studied (McInerney et al., 1981b; Boone and Bryant, 1980).

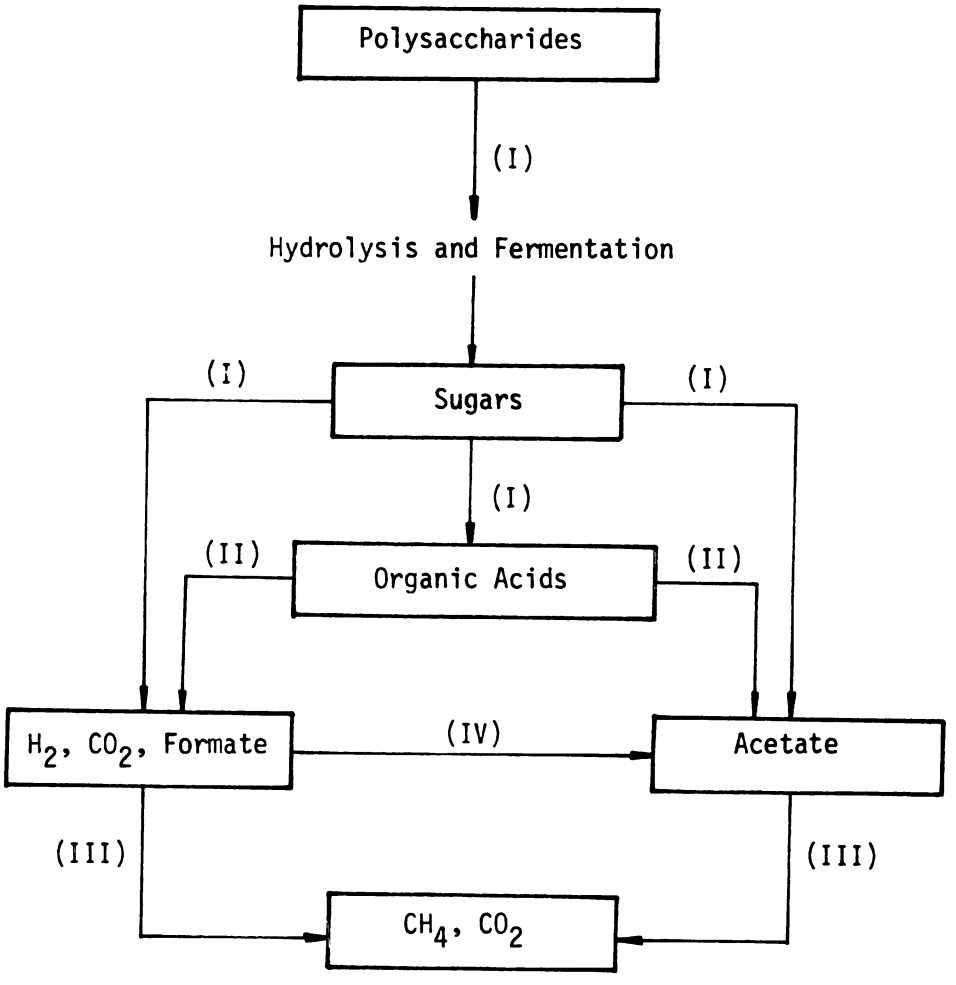


Figure 2-1 Three stage scheme of anaerobic methane fermentation. Involves four groups of microorganisms, (I) fermentative bacteria; (II) obiligate H_2-production acetogenic bacteria; (III) methane production bacteria; (IV) homoacetogenic bacteria.

Table 2-1 Metabolic Function Of H_2-producing Acetogenic Bacteria

---

A. β - oxidation of longer-chain fatty acids

a. Even-numbered carbon to acetate and hydrogen

$$CH_3CH_2CH_2COO^- + 2\ H_2O \rightleftharpoons 2\ CH_3COO^- + 2\ H_2 + H^+ \qquad (2\text{-}1)$$

butyrate $\Delta G_o' = +\ 11.5$ Kcal/reaction

b. Odd-numbered carbon to acetate, propionate, and hydrogen

$$CH_3CH_2CH_2CH_2COO^- + 2\ H_2O \rightleftharpoons CH_3COO^- + CH_3CH_2COO^- + 2\ H_2 + H^+ \qquad (2\text{-}2)$$

valerate

$\Delta G_o' = +\ 11.5$ Kcal/reaction

B. Decarboxylation of propionate to acetate, CO_2, H_2

$$CH_3CH_2COO^- + 3\ H_2O \rightleftharpoons CH_3COO^- + HCO_3^- + 3\ H_2 + H^+ \qquad (2\text{-}3)$$

propionate $\Delta G_o' = +\ 18.2$ Kcal/reaction

C. Oxidation of alcohols to acetate and hydrogen

$$CH_3CH_2OH + H_2O \rightleftharpoons CH_3COO^- + H^+ + 2\ H_2 \qquad (2\text{-}4)$$

ethonal $\Delta G_o' = +\ 2.3$ Kcal/reaction

In the third, or terminal, stage of methane fermentation, methanogenic bacteria split acetate to methane and carbon dioxide, and use hydrogen to reduce carbon dioxide to methane.

An additional metabolic group, homoacetogenic bacteria, which is capable of oxidizing hydrogen anaerobicly with the reduction of carbon dioxide to acetate, was discovered in an anaerobic fermentation ecosystem (Zeikus, 1979; Wolfe and Higgins, 1979). So far, Acetobacterium woodii is the only physiologically well characterized

hydrogen-consuming homoacetogenic bacterium (Balch et al., 1979).

Table 2-2 taken from Zeikus (1979) shows the physiological characteristics of four groups of bacteria that are often isolated from anaerobic sludge digesters and have been discussed in the previous paragraphs.

In a mixed culture anaerobic fermentation ecosystem, the effective metabolism of one group of bacteria is closely related to interaction with other groups of bacteria. Therefore, their metabolism may not be separated into distinct steps for metabolic optimization (McInerney and Byrant, 1981a; Zeikus, 1979).

2.1.2 Methanogenic Habitats

Several different types of methanogenic habitats can be found in the ecosystem, and they may be classified into three types (Wolfe and Higgins, 1979). Type A habitat shown in Figure 2-1, includes aquatic sediments, anaerobic sludge digesters and marsh; it is a complete anaerobic fermentation system which involves all four groups of bacteria. Animal tracts such as rumen and caecum are Type B habitats (Figure 2-2). In a Type B habitat, only fermentative bacteria (Group I) and hydrogen-utilizing bacteria (Group III) are involved in the ecosystem. Fatty acids are the major end products, and longer-chain acids are not converted to acetic acid but are absorbed into the bloodstream where they serve as the major energy source for the ruminant. Another special methanogenic habitat, Type C (Figure 2-3), only involves methanogens that utilize acetate, hydrogen and carbon dioxide present in the system to produce methane. Some thermal springs in the

Table 2-2 Physiological Characteristics of Four Groups of Bacteria

Organism	Metabolic Group	Catabolic Substrate	Doublicg Time (hr)	Fermentation Products
Clostridium thermocellum	Fermentative bacteria	Cellulose	7	H_2/CO_2, Ethanol.
		Cellobiose	2	Acetic, Lactic.
'S organism'	H_2-producing acetogenic bacteria	Pyruvate	-	H_2/CO_2, Ethanol, Acetic.
		Ethanol	-	'H_2', Acetic.
Acetobacterium woodii	Homoacetogenic bacteria	Fructose, Lactic	6	Acetic.
		H_2/CO_2	24	Acetic.
Methanobacterium thermoautotrophicum	Methanogenic bacteria	H_2/CO_2	2-4	CH_4, CO_2
Methanosarcina barkeri	Methanogenic bacteria	H_2/CO_2, CH_3OH, CH_3NH_2	10-12	CH_4, CO_2
		CH_3COOH	> 24	CH_4, CO_2

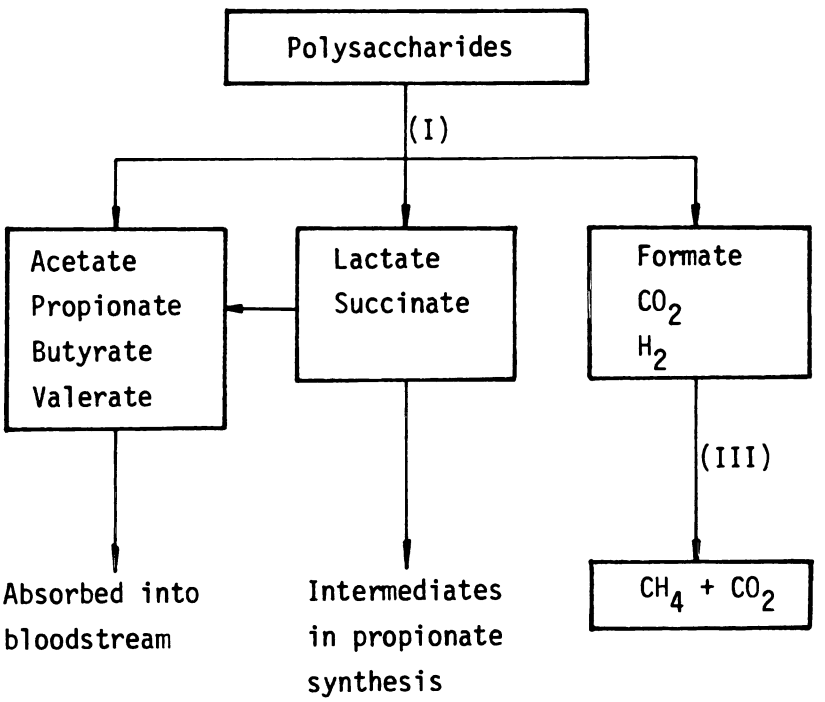


Figure 2-2 Type B Habitat. Rumen, caecum, intestine.

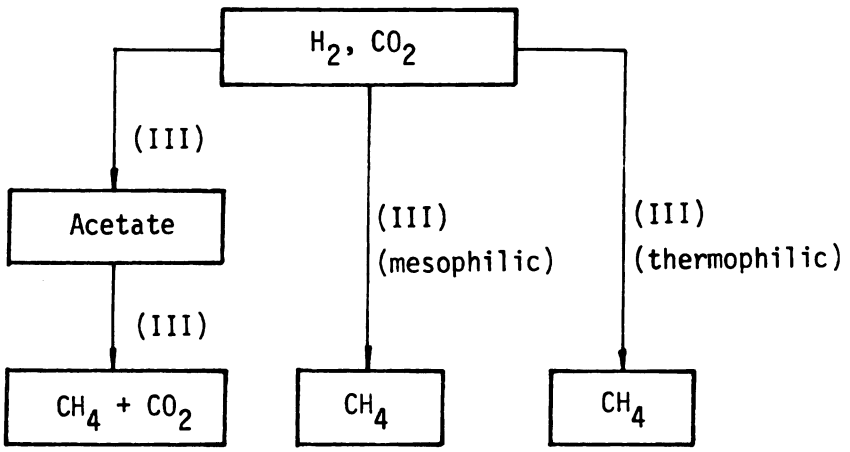


Figure 2-3 Type C Habitat. Thermal Spring.

Yellow Stone National Park and Lake Kivu (in Africa) (Wolfe and Higgins, 1979) have been found belonging to this type of habitat. Thermal springs contain hydrogen, carbon dioxide, sulfide, and mineral salts. Some methanogens have been isolated from several thermal springs in Yellow Stone Park. In Lake Kivu, methanogens reduce carbon dioxide by using volcanic hydrogen to produce methane (Deuser et al., 1973). Among all the methanogenic habitats mentioned above, the rumen is the most studied anaerobic fermentation ecosystem (Hungate, 1975; Hobson, 1971, 1974, 1982; Prins and Clarke, 1979).

2.1.3 Production of Fatty Acids in Anaerobic Methane Fermentation

Organic substrates that are subject to anaerobic degradation are mostly carbohydrate, protein, and lipids. Agricultural residues contain mainly polysaccharides, such as cellulose, hemi-cellulose, and pectin. As described in the Section 2.2.1, these materials are first hydrolized by extracellular enzymes secreted by fermentative bacteria into lower molecular weight compounds, such as monosaccharides (glucose, fructose, xylose), and oligosaccharides (sucrose, cellobiose, short-chain fructosans). These smaller organic compounds can be transported through the fermentative bacterial cell wall and further degraded to fatty acids and other organic acids, alcohols, hydrogen, and carbon dioxide.

Figure 2-4 shows the pathway of hydrolysis and fermentation of cellulose, hemi-cellulose, and pectin to monosaccharides and to pyruvate via the Embden-Meyerhof-Parnas pathway. Pyruvate is the key intermediate product in the first metabolic stage of anaerobic fermen-

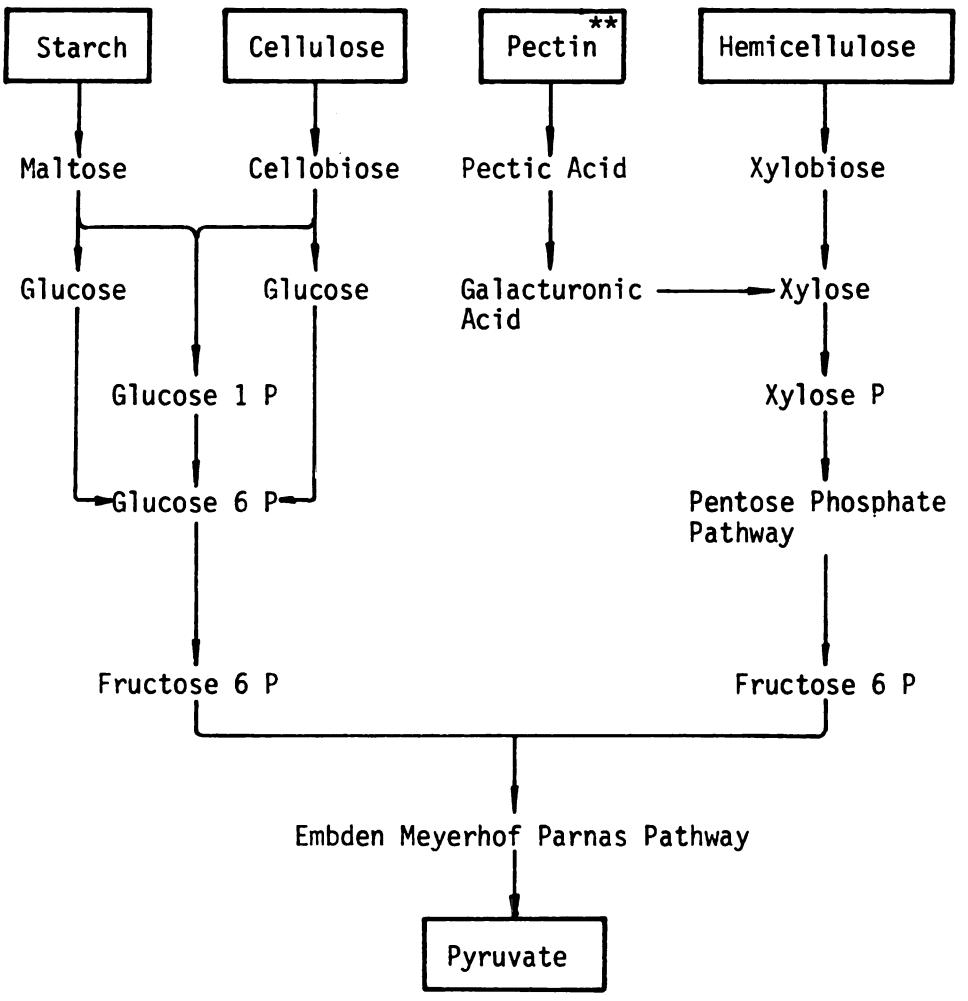


Figure 2-4 Pathways in the rumen fermentation of the major insoluble carbohydrates present in plants.

** Pectic substances consist of D-galacturonic acid and its methyl ester, D-galactose and L-arabionse. The details of its structure are not completely known, but the major part consists of (1-4)-linked α-D-galacturonic acid residues (Wood, 1970).

tation, because most of the important organic acids produced in this stage are obtained from pyruvate. Figure 2-5 gives the identified pathways of microbial fermentation of organic acids from pyruvic acid in pure culture. From Figure 2-5, it can be seen that a wide variety of acids and alcohols can be produced from pyruvic acid depending on the particular type of microorganism involved. In mixed cultures, such as Type A ecosystem, the major fermentation products in the first metabolic stage are acetic acid, propionic acid, butyric acid, hydrogen, and carbon dioxide. Certain fermentation products of monosaccharides may involve reaction of the Pentose Phosphate Pathway and Entner-Doudoroff Pathway (Lehninger, 1975).

In a normal fermentation system, acetic acid is the predominant acid while in a stressed system, propionic and butyric acids may have higher concentrations (Hobson et al., 1981).

2.1.3.1 Physical and Chemical Properties of Fatty Acids

Saturated fatty acids are single lipids with the general formula,

$$CH_3 - (CH_2)_n - COOH \qquad n \geq 0 \qquad (2\text{-}5)$$

The terminal carboxyl group of the fatty acid is very hydrophilic and the hydrogen carbon chain, constructed from two identical carbon monomers, is almost insoluble in water. The hydrophilic-hydrophobic character gives fatty acids a polar carboxyl head and a non-polar hydrocarbon tail. Table 2-3 gives the physical and chemical characteristics of some major fatty acids commonly found in the complete

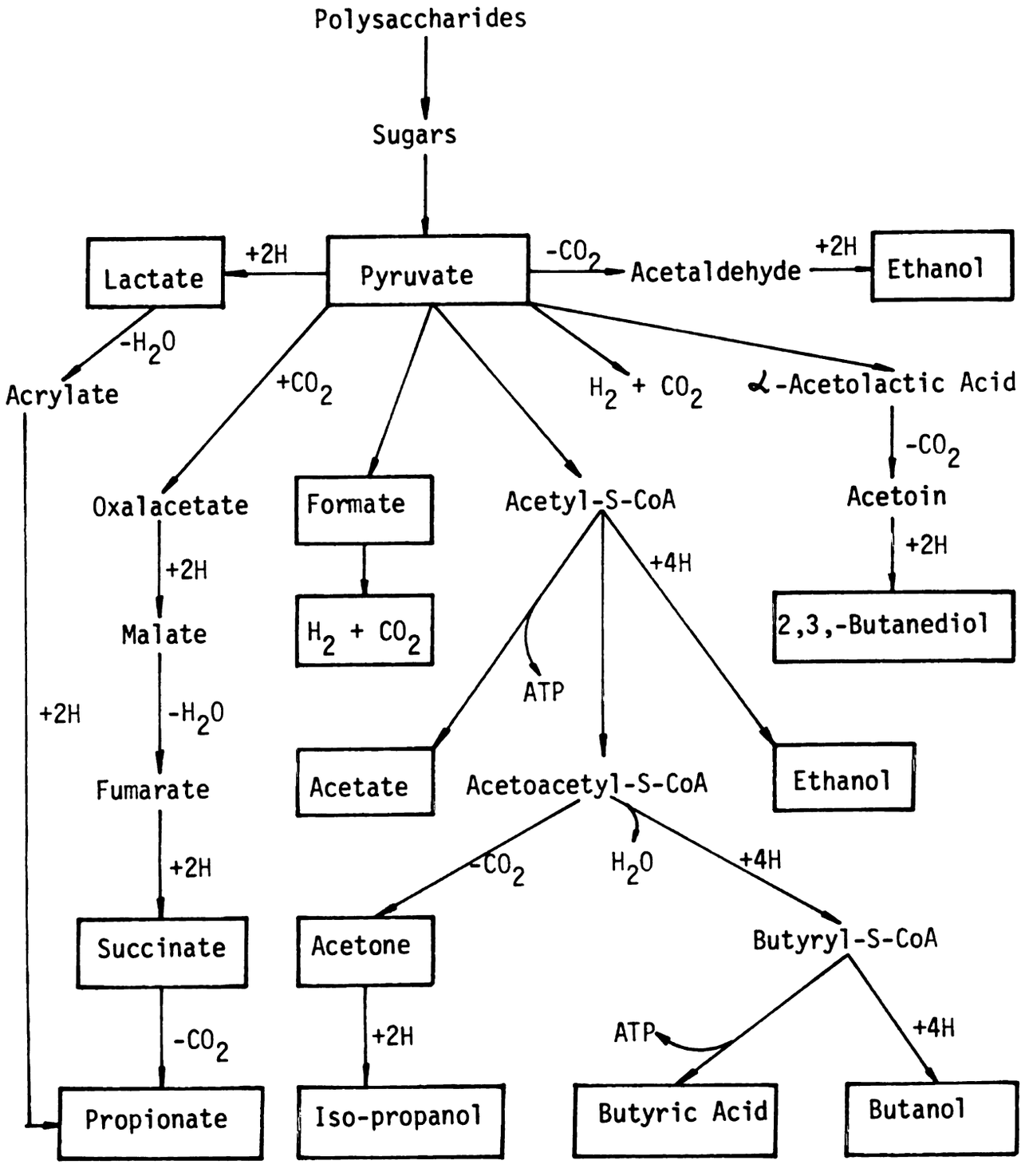


Figure 2-5 Some major end products of the microbial fermentations of sugars from pyruvic acid. 2H represents two hydrogen atoms being donated in a reductive step. Reduced form products have more hydrogens an electrons per carbon atom. (after Lynch, 1979)

Table 2-3 Physical and Chemical Properities of Volatile Fatty Acids

No. of Carbon	Name	Formula	M.W.	Boiling Point, °C	Specific Gravity	Solub. in H_2O *	g COD/g
1	Formic Acid	HCOOH	46.03	100.7	1.220	∞	0.3476
2	Acetic Acid	CH_3COOH	60.05	117.9	1.0492	∞	1.066
3	Propionic Acid	CH_3CH_2COOH	74.08	140.99	0.9930	∞	1.512
4	Iso-Butyric Acid	$(CH_3)_2CHCOOH$	88.11	153.2	0.9681	20.0	1.816
4	Butyric Acid	$CH_3CH_2CH_2COOH$	88.12	163.53	0.9577	5.62	1.816
5	Iso-Valeric Acid	$(CH_3)_2CHCH_2COOH$	102.13	176.0	0.9310	4.2	2.037
5	Valeric Acid	$CH_3(CH_2)_3COOH$	102.13	187.0	0.939	3.76	2.037
6	Iso-Caproic Acid	$(CH_3)_2CH(CH_2)_2COOH$	116.16	207.7	0.925	slightly	2.204
6	Caproic Acid	$CH_3(CH_2)_4COOH$	116.16	202.0	0.922	0.4	2.204

* gram per 100 ml water

Source: Handbook of Chemistry and Physis

anaerobic methane fermentation system.

2.1.4 Methanogens and Methanogenesis

Methane bacteria are a unique group of microorganisms involved in the terminal stage of anaerobic fermentation to produce methane. This section will review some of the current knowledge of this special group of bacteria and the mechanism of methane formation.

2.1.4.1 Physiology of Methane Bacteria

Although methanogens are a morphologically diverse group of bacteria, varying from short, lancet-shaped cocci to long, filamentous rods, they share some common physiological properties that are not found in any other group of bacteria. They require strict anaerobic conditions and a very low redox potential (-330mv) for growth (Zehnder, 1978). Studies of methane bacterial cells, both gram-negative and gram-positive types have failed to find muramic acid and peptidoglycan which are present in all other bacterial cells. Fox et al. (1977) found that both transfer RNA and ribosomal RNA oligonucleotide sequences of methanogens are different from typical bacteria. They also indicated that methanogens are one of the most ancient groups of organisms (Fox et al., 1977; Balch et al., 1979). Woese (1978, 1981) declared that methanogens are neither prokaryotes nor eukaryotes but are the largest group of archebacteria. Balch et al. (1979) presented a new taxonomic scheme for the methanogens based on the relationship of oligonucleotide sequences of the 16 S ribobomal RNA. Table 2-4 gives the new taxonomic scheme as well as some characteristics of methanogens

Table 2-4 Methanogenic Species in Pure Culture

Species	Morphology	Moltility	Gram Reaction	Cell Wall	Substrate
Methanobacterium					
formicicum	stright to curved rod; filaments	none	+	pseudomurein	H_2/CO_2, formate
bryantii	same as M. F.	none	+	pseudomurein	H_2/CO_2
thermoautotrophicum	same as M. F.	none	+	pseudomurein	H_2/CO_2
Methanobreibacter					
ruminantium	lancet-shaped; cocci or short rods; no flagellum	none	+	pseudomurein	H_2/CO_2, HCOOH
arboriphilus	short rods; single flagellum	none	+	pseudomurein	H_2/CO_2
smithii	same as M. ruminantium with single flagellum	none	+	pseudomurein	H_2/CO_2, HCOOH
Methanococcus					
Vannielii	coccus	motile	-	protein subunits with trace glucosamine	H_2/CO_2, HCOOH
volate	coccus	motile	-	same as M. vannielii	H_2/CO_2, HCOOH

Table 2-4 Continued

Species	Morphology	Moltility	Gram Reaction	Cell Wall	Substrate
Methanomicrobium mobile	short, curved rod	motile	-	protein subnuits	H_2/CO_2, HCOOH
Methanogenium cariaci	irregular coccoid cells	motile	-	protein subunits	H_2/CO_2, HCOOH
marisnigri	irregular coccus	motile	-	protein subunits	H_2/CO_2, HCOOH
Methanosprillum hungatei	long, curved rods; spirillum; filaments	motile	-	protein subunits with external sheath	H_2/CO_2, HCOOH
Methanosarcina barkeri	irregular coccus in packets	none	+	heteropoly-saccharide	H_2/CO_2, CH_3OH methyl amine acetate

that have been isolated in pure culture.

From Table 2-4, it can be seen that methane bacteria are diverse in morphology. Some species are motile while others are not, and it is interesting to note that nonmotile species are gram-positive and motile species are gram-negative. All species share the common metabolic capacity to produce methane from hydrogen and carbon dioxide. Several species can utilize formate but only one is able to use acetate as a substrate.

Most methanogens are most active in the temperature range from 33°C to 45°C. Methanobacterium thermoautotrophicum is the only known thermophilic methane bacterium with an optimum temperature of 65°C to 70°C (Zehnder, 1979). Methane bacteria are very sensitive to pH changes, growing best in the pH range from 6.5 to 7.7 (Smith and Hungate, 1958), with the optimum being 7.05 to 7.20 (Harmeer and Borchardt, 1969). It has also been found that important hydrogen-producing and hydrogen consuming anaerobes do not grow at pH values below 6.0 (Weimer and Zeikus, 1977). Although some non-methanogenic anaerobes can grow at lower pH values (Cohen et al., 1979; Eastman, 1981), even as low as 2.0 (Canale-Parola, 1970), no methanogens can grow well at pH values less than 6.0 or above 8.0. Zeikus (1979) indicated that the inhibition of hydrogen oxidizing methanogens by high proton concentration may be related to thermodynamic regulation; at lower pH conditions, proton reduction to hydrogen become the thermodynamically favored process rather than the normal oxidation of hydrogen to proton.

All methanogenic bacteria contain several types of special coenzymes (Bryant, 1979), such as coenzyme M, coenzyme 420, and coenzyme

factor B. Coenzyme M is a methyl carrier and participates in methanogenesis from methanol and acetate (Smith and Mah, 1978; Taylor and Wolfe, 1974). Coenzyme 420 is involves in the electron transfer and serves as an electron carrier similar to the function of ferredoxin (Tzeng et al., 1975). Coenzyme factor 420 is a low molecular weight, heat stable cofactor and is believed to be involved in the enzymatic formation of methane from methyl coenzyme M (Gunsalus and Wolfe, 1976). Two other cofactors; F_{430} and F_{342} were also discovered but their functions are still not known (Gunsalus and Wolf, 1978).

2.1.4.2 Methane Formation From Hydrogen And Carbon Dioxide

As indicated in Table 2-4, the only known methanogenic substrates are H_2/CO_2, formate, methylamine, and acetate. In spite of different morphologies, all known pure cultures of methane bacteria can use hydrogen as the electron source to reduce carbon dioxide, to methane according to Equation 2-6.

$$4\ H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3\ H_2O \qquad (2\text{-}6)$$

$$\Delta G_o' = -32.7\ \text{Kcal/reaction}$$

Equation 2-6 shows that eight electrons, derived from four moles of hydrogen, were used to reduce one mole of carbon dioxide. The carbon dioxide utilized by methane bacteria is partly reduced to methane and partly metabolized and fixed for cell material. This character is different from other autotrophs that just use carbon dioxide as the single carbon source for growth. It is also noted that the standard free energy change of Equation 2-6 is very negative, indicating that methane

bacteria have a very strong affinity for hydrogen gas. The special ability of methanogens to consume hydrogen is the major factor maintaining very low hydrogen concentration in anaerobic environment. Hungate (1970) reported that the K_m for the utilization of hydrogen in the rumen was only 10^{-6} M.

2.1.4.3 Methane Production From Acetate

It has been reported that about 70 - 73 % of methane produced is from the decarboxylation of acetate (Jeris and McCarty, 1965; Smith and Mah, 1966). A few species of bacteria have been reported to utilize acetate as an energy source and to produce methane (Barker, 1936; Bryant, 1974). However, up to the present time, only one species of acetate utilizing methanogen, Methanosarcina barkeri, has been isolated in pure culture (see Table 2-4).

Stadtman and Barker (1949) performed a series of experiments by using ^{14}C-methyl or ^{14}C-carboxyl labeled acetate and formate and reported that methane was derived from the methyl group of acetate and carbon dioxide was derived from the carboxyl group of acetate as Equation 2-7 shows:

$$^{*}CH_3{}^{o}COO^{-} + H_2O \rightleftharpoons {}^{*}CH_4 + H{}^{o}CO_3^{-} \qquad (2\text{-}7)$$

$$\Delta G_o' = -7.4 \text{ Kcal/mole}$$

The hydrogen atoms on the methane were obtained from the methyl group of acetate plus the fourth hydrogen atom contributed by water (Pine and Barker, 1956). These observations cleared up the earlier controversy

of methane formation from acetate, i.e., whether acetate is completely oxidized to hydrogen and carbon dioxide and then the carbon dioxide is reduced with hydrogen to methane versus the direct conversion of the methyl group of acetate to methane (Smith and Mah, 1980).

In addition to acetate, Methanesarcina barkeri can also utilize CH_3OH, CH_3NH_2 and H_2/CO_2 as the substrate to produce methane. The chemical reactions as well as the standard free energy changes for these reactions are shown in Table 2-5. Weimer and Zeikus (1979) reported that M. barkeri grow about four times faster on H_2/CO_2 or methanol than on acetate.

Equation 2-7 shows that the standard free energy change is a small negative value (-7.4 Kcal/mole) that is insufficient to produce one ATP, since values for the free energy change of ATP hydrolysis have been estimated to range from -8.5 Kcal/mole to -12.5 Kcal/mole (Decker et al., 1970). Normal efficiency of energy transfer in bacteria are 30% to 70% (McCarty, 1975; Decker et al., 1970), and, therefore, minimun energy required to produce one ATP would be 11.1 Kcal/mol. As mentioned earlier, the growth rate of Methanosarcina on acetate is very slow (doubling time greater than 24 hours), and its growth yield is only 1.6 - 3.0 mg dry weight/m. mol CH_4 (Smith and Mah, 1978). This slow growth rate may be related to the small energy yield. However the actual mechanisms of methane production from acetate and its energy production mechanisms are not fully understood today.

Table 2-5 Transformations of Methanosarcina

Equations	$\Delta G_o'$ Kcal/reaction
A. Methanol	
1. $4\ CH_3OH \rightleftharpoons 3\ CH_4 + HCO_3^- + H^+ + H_2O$	- 75.2
2. $4\ CH_3OH + CH_3COO^- \rightleftharpoons 4\ CH_4 + 2\ HCO_3^- + H^+$	- 82.6
3. $CH_3OH + H_2 \rightleftharpoons CH_4 + H_2O$	- 26.9
B. Methylamine	
1. $4\ CH_3NH_3^+ + 3\ H_2O \rightleftharpoons 3\ CH_4 + HCO_3^- + 4\ NH_4^+ + H^+$	- 53.8
2. $2(CH_3)_2NH_2^+ + 3\ H_2O \rightleftharpoons 3\ CH_4 + HCO_3^- + 2\ NH_4^+ + H^+$	- 52.5
3. $4(CH_3)_3NH^+ + 9\ H_2O \rightleftharpoons 9\ CH_4 + 3\ HCO_3^- + 4\ NH_4^+ + 3\ H^+$	-159.8
C. Carbon Dioxide	
1. $4\ H_2 + H^+ + HCO_3^- \rightleftharpoons CH_4 + 3\ H_2O$	- 32.4
D. Acetate	
1. $CH_3COO^- + H_2O \rightleftharpoons CH_4 + HCO_3^-$	- 7.4

* Source: Smith and Mah, 1980

2.1.4.4 Biochemical Pathway of Methane Formation

Barker (1956) first introduced a scheme to explain the possible pathways of carbon in methane formation from various sources as shown in Figure 2-6. Barker's scheme suggests that an unidentified one-carbon carrier, R, is bound to various substrates which are reduced to methane with the regeneration of carrier. McBride and Wolfe (1971) discovered coenzyme M ($HS-CH_2-CH_2-SO_3H$, 2-mercaptoethan sulfonic acid,

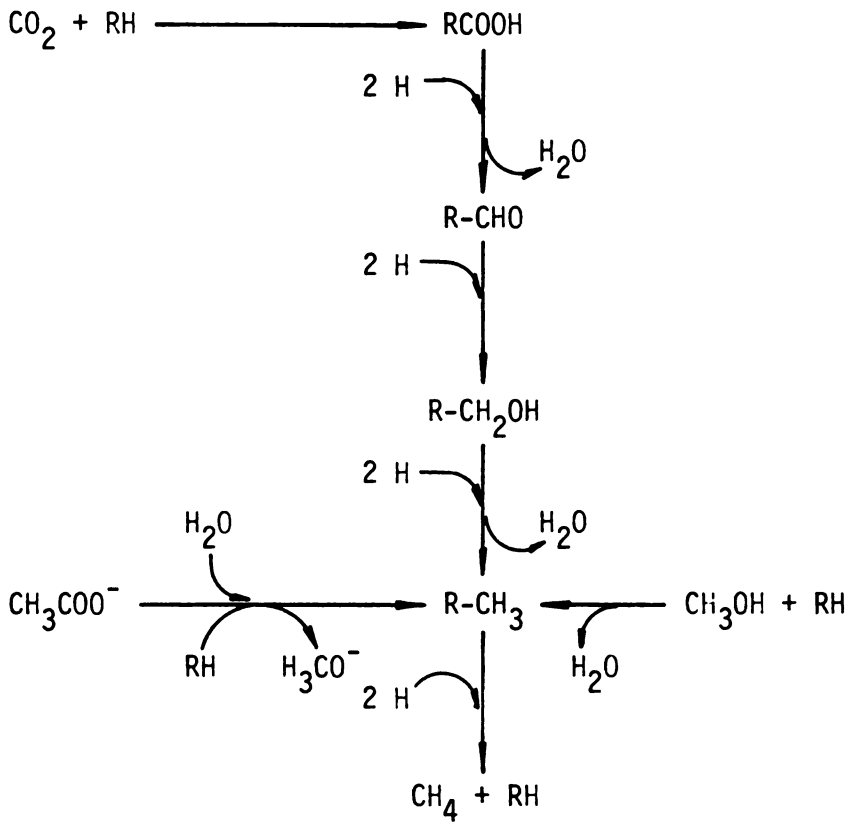


Figure 2-6 Barker's scheme of methane formation.

abbreviated as HS-CoM) which was later shown to be one of the unknown carriers in Barker's scheme. It is now widely accepted that HS-CoM is a necessary methyl carrier for methyl transfer in methanogesesis.

Barker's scheme was modified by Wolfe (1979) to a cyclic pathway (Figure 2-7) by the findings that the methyl-reductase reaction was coupled to the activation and reduction of carbon dioxide, and that an intermediate, involved in the primary step of CO_2 activation, is generated from the terminal reaction in Barker's scheme,

$$CH_3\text{-S-CoM} \xrightarrow[\text{methylreductase}]{H_2,\ Mg^{+2},\ ATP} CH_4 + \text{H-S-CoM} \qquad (2\text{-}8)$$

2.1.4.5 Unique Properties Of Methanogens

Reviewing the material given above, some of the unique properties found in the methane bacteria can be summarized as follows:

1. Methane is the common metabolic product for all methanogens and only for methanogens.
2. Methanogens can use only a narrow range of substrates; hydrogen/carbon dioxide, formate, methanol, methylamine, and Acetate.
3. Methanogens require strict anaerobic conditions and an extreme low redox potential (-330 mV) for growth.
4. Methanogens contain unique coenzymes and cofactors: CoM, F420, F430, F342.
5. The 16 S rRNA sequences are unique.
6. Cell walls contain no D-amino acids or muramic acid.
7. Cytochromes (electron transferring proteins containing an

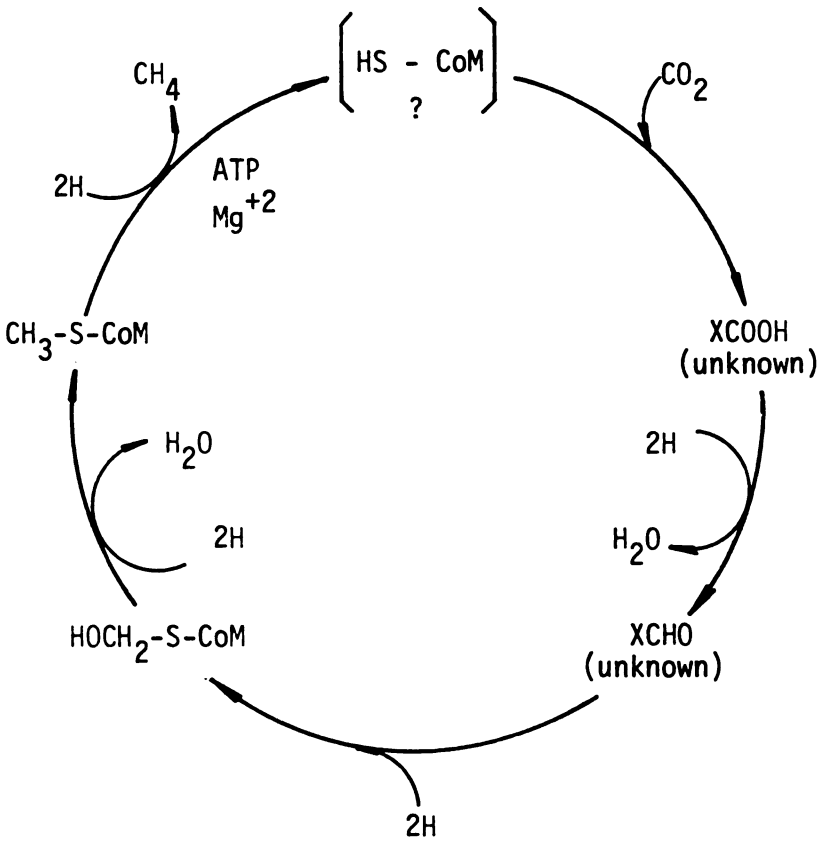


Figure 2-7 Modification of Barker's scheme for CO_2 reduction to methane to emphasize a cycle where the unknown activated-intermediate produced by the methylreductase is involved in CO_2 activation. (after Wolfe, 1979)

iron-porphyrin group) and quinones (electron carrying coenzymes) are absent.

8. Unique carbon dioxide fixation reactions are involved in cell systhesis.

2.1.5 Role of Hydrogen in Anaerobic Fermentation

Hydrogen is an intermediate product in the anaerobic fermentation of organic matter. However, it is rarely detectable in a normal digester, because it is consumed by the hydrogen utilizing methanogens as rapidly as it is formed.

It has already been stated that hydrogen utilizing methane bacteria have a great affinity for hydrogen (Equation 2-6). From Table 2-1, the standard free energy change for the reactions in Equations 2-1 to 2-4 all show positive values which indicate that the degradation of butyrate, valerate, propionate, and ethanol to acetate are thermodynamically unfavorable, unless the partial pressure of hydrogen is maintained at a very low level. One way to achieve low hydrogen concentrations is the consumption of hydrogen by hydrogen utilizing methanogens. As has mentioned before, H_2-producing acetogenic bacteria are syntrophicly associated with hydrogen utilizing methane bacteria and can not survive if separated from hydrogen utilizing bacteria. By combining Equation 2-6 and Equations 2-1 to 2-4, as shown in Table 2-6, the result of the syntrophic association is obvious, the free energy changes for Equations 2-9 to 2-12 all having negative values. Therefore the degradation of the above substrates becomes energetically favorable.

Table 2-6 Chemical Reactions For The Syntrophic Association of Bacteria and H_2-utilizing Methanogen

---

(2-6) + (2-1)

$$2\ CH_3CH_2OH + HCO_3^- \rightleftharpoons 2\ CH_3COO^- + CH_4 + H_2O + H^+ \qquad (2\text{-}9)$$

$\Delta G_o' = -27.8$ Kcal/reaction

(2-6) + (2-2)

$$2\ CH_3CH_2CH_2CH_2COO^- + HCO_3^- + H_2O \rightleftharpoons 2\ CH_3CH_2COO^- + 2\ CH_3COO^- + CH_4 + H^+ \qquad (2\text{-}10)$$

$\Delta G_o' = -9.4$ Kcal/reaction

(2-6) + (2-3)

$$4\ CH_3CH_2COO^- + 3\ H_2O \rightleftharpoons 4\ CH_3COO^- + 3\ CH_4 + H^+ \qquad (2\text{-}11)$$

$\Delta G_o' = -24.5$ Kcal/reaction

(2-6) + (2-4)

$$2\ CH_3CH_2CH_2COO^- + HCO_3^- + H_2O \rightleftharpoons 4\ CH_3COO^- + CH_4 + H^+ \qquad (2\text{-}12)$$

$\Delta G_o' = -9.4$ Kcal/reaction

The effect of hydrogen partial pressure on the free energy change for the degradation of fatty acids can best be described by Figure 2-8 (Zeikus, 1979, McInery and Bryant, 1980). This shows that hydrogen partial pressure has to be lowered to 2×10^{-3} atm for the degradation of butyric acid and 9×10^{-5} atm for the degradation of propionate. Methane formation from hydrogen and carbon dioxide is energetically favorable at hydrogen partial pressures greater than 2×10^{-6} atm. When a reactor is stressed, such as shortened retention time or transient organic

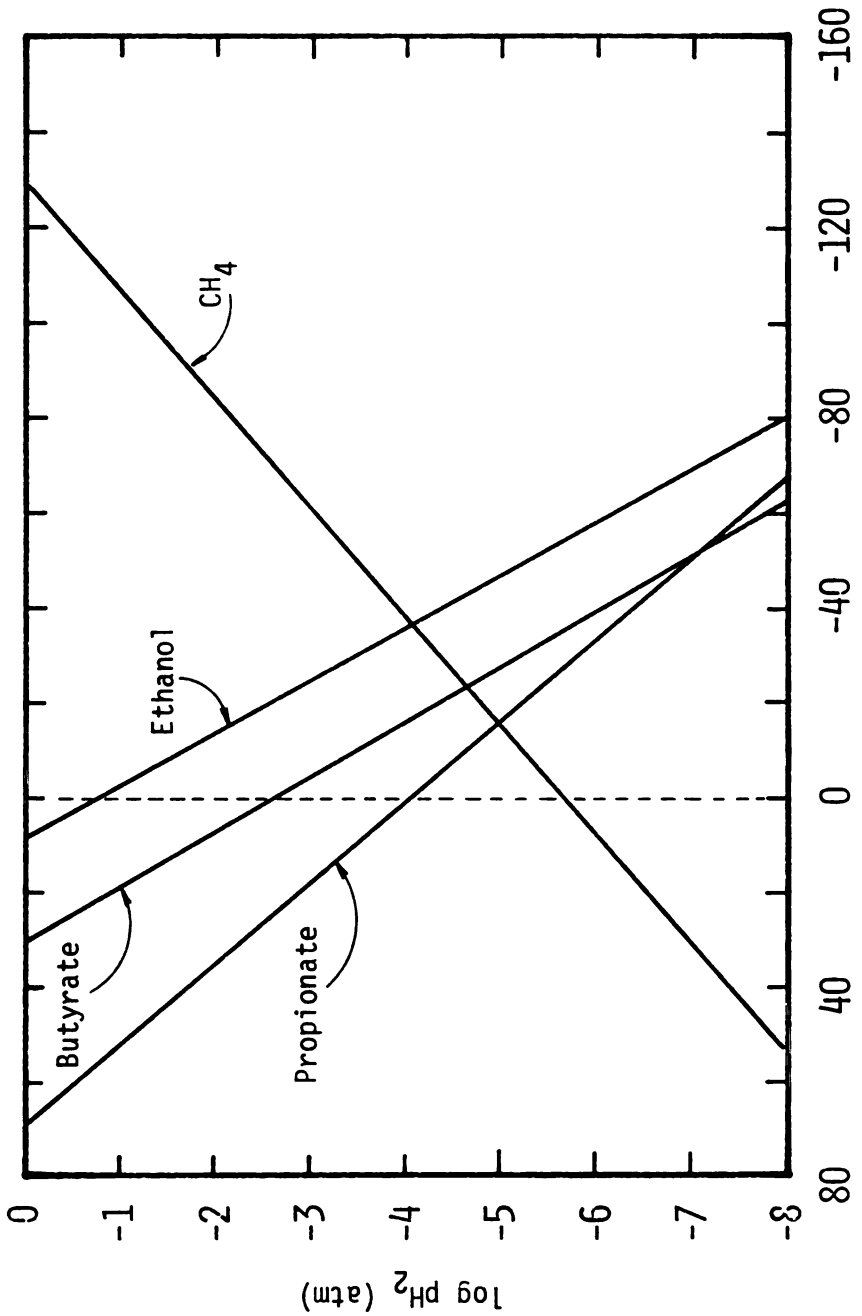


Figure 2-8 Effect of hydrogen partial pressure (pH_2) on the free energy change for the oxidation of ethanol, propionate, and butyrate. (after Bryant, 1980)

loading, hydrogen produced from fermentation of organic substrate can not be effectively consumed by methanogens causing the hydrogen concentration to increase which results in a supression of fatty acid degradation. Figure 2-8 shows that a slight increase of hydrogen partial pressure above 10^{-5} will cause propionate degradation to become unfavorable, while further increases of H_2 partial pressure will cause other acids to accumulate in the system.

2.1.5.1 Effects Of Hydrogen Concentration To The Fermentative Products

Hydrogen concentration in the anaerobic fermentation system also plays an important role in regulating the quantity and types of organic products formed by the fermentative bacteria by interspecies hydrogen transfer (Wolin, 1974).

Production of molecular hydrogen by fermentative bacteria is through the reoxidizing of the reduced NAD^+ (NADH, diphosphopyridine nucleotide) generated in the glycolysis pathway as Equation 2-13 shows:

$$NADH + H^+ \rightleftharpoons NAD^+ + H_2 \qquad (2\text{-}13)$$

$$\Delta G_o' = +4.3 \text{ Kcal/reaction}$$

The above equation is thermodynamically unfavorable at hydrogen partial pressure above 10^{-3} to 10^{-4} atm as indicated by Wolin (1974). The effects of hydrogen partial pressure on the free energy change for Equation 2-13 are shown in Table 2-7. Several investigators (Kaspar and Wuhrmann, 1978; Wolin 1974) have reported that when hydrogen is effectively consumed by methanogens, the oxidized fermentation products

such as acetate and carbon dioxide will increase and the reduced (electron sink) products such as propionate, ethanol will decrease, and an increase of hydrogen production which in turn is used by hydrogen-utilizing methanogens. This phenomenon can be seen from the experimental results performed by Wolin (1974) as shown in Figure 2-9 and Table 2-8.

Table 2-7 Effects of Hydrogen Partial Pressure on Free Energy Change

H_2 (atm)	$\Delta G_o'$
10^{o}	+ 4.33
10^{-1}	+ 2.97
10^{-2}	+ 1.61
10^{-3}	+ 0.25
10^{-4}	- 1.11
10^{-5}	- 2.47
10^{-6}	- 3.83

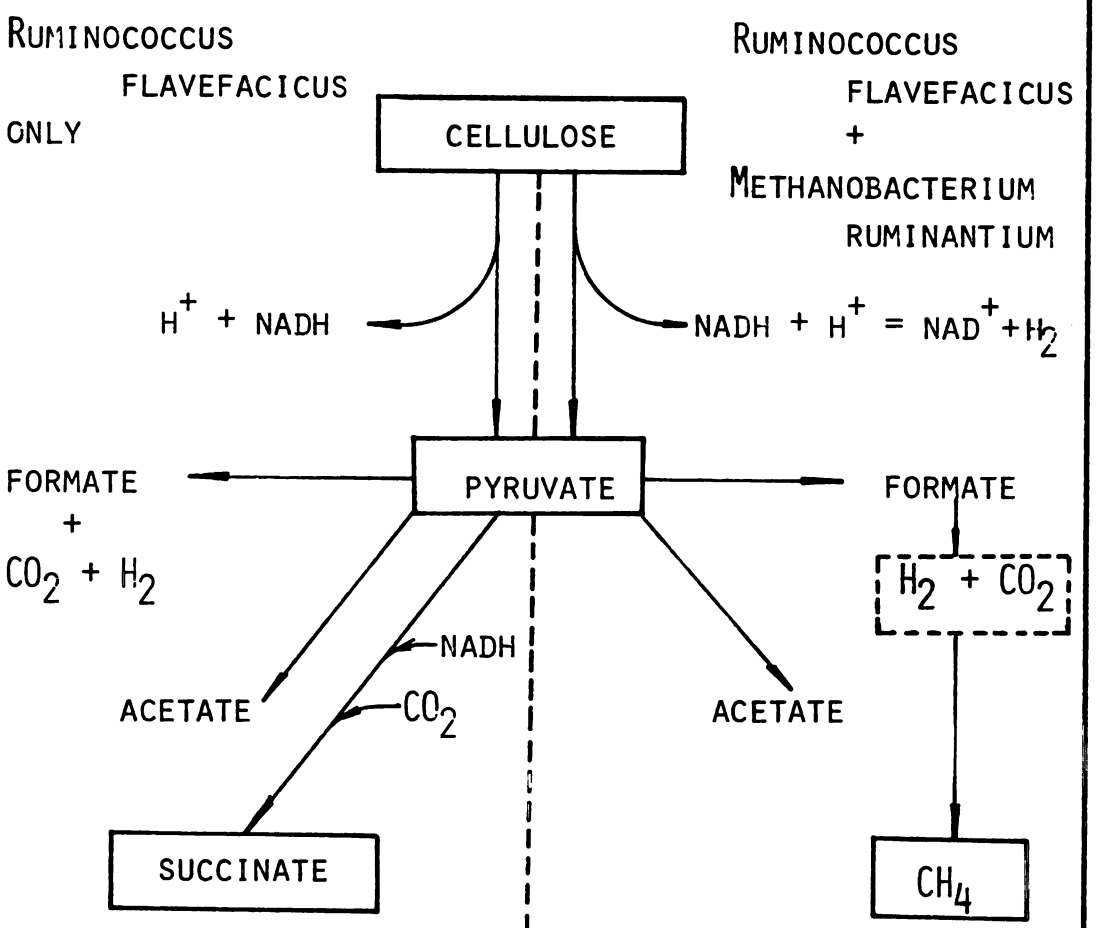


Figure 2-9 Fermentation Interactions Between Ruminococcus flavefacius And H_2-utilizing Methanogens.

Table 2-8 Fermentation Of Cellulose By R. flavefacicus And R. flavefacicus Plus M. ruminantium

Products	moles/100 moles Cellulose	
	R. flavefacicus	R. flavefacicus + M. ruminatium
Acetate	74	145
Formate	35	3
Succinate	94	25
Hydrogen	33	0
Carbon Dioxide	37	79
Methane	0	63

* Source: Wolin (1974)

Ruminococcus flavefacicus is an important celluloytic species found in the rumen. Figure 2-9 shows that when R. flavefacicus grows alone on cellulose, the main products are succinate and acetate with small amounts of carbon dioxide and hydrogen; but no methane is found. When a coculture of R. flavefacicus and Methanobacterium ruminantium is grown, the main products are acetate, carbon dioxide and methane. In the coculture environment, hydrogen concentration is maintained at a low level, shifting electron flow from the production of succinate to the regeneration of NAD^+ and hydrogen. Pyruvate metabolism is shifted from succinate to more acetate formation. Therefore, if the hydrogen concentration is high in the system the reduced fermention products and hydrogen will accumulate and substrate utilization may be inhibited (Mah et al., 1977).

The results of the interaction between hydrogen utilizing methanogens and nonmethanogens in anaerobic fermentation may be summarized as follows: (1) increase substrate utilization; (2) different proportions

of reduced end products; (3) more ATP systhesized by the nonmethanogens; (4) increased growth of both organisms (Wolin, 1974).

2.1.6 Role Of Nitrate And Sulfate In Anaerobic Methane Fermentation

If sulfate and nitrate are present in the system, methane fermentation will be inhibited because nitrate and sulfate have higher electron affinity than carbon dioxide and will compete seriously with carbon dioxide for electrons. Table 2-9 gives the redox potential of some redox pairs. Figure 2-10 illustrates the relationship of four electron acceptors, O_2, NO_3^-, $SO_4^=$, and CO_2, according to the order of magnitude of their redox potential.

From Table 2-9, it can be seen that hydrogen has the greatest tendency to donate electrons and oxygen has the greatest tendency of accepting electrons. In natural ecosystem, nitrate is first reduced, followed by the reduction of sulfate and finally the formation of methane (Figure 2-10). Therefore, methane can only be formed in the absence of nitrate and sulfate. If nitrate exists in the system, methane is produced only after all the nitrate is reduced to nitrogen.

Table 2-9 The Oxidation-reduction Potentials Of Some Redox Pairs

Redox Pair	Redox Potential , E_o' (Volt)
$2\ H^+/H_2$	- 0.41
$NAD^+/NADH$	- 0.32
CO_2/Acetate	- 0.29
CO_2/CH_4	- 0.24
$SO_4^=/H_2S$	- 0.22
Fumarate/Succinate	+ 0.03
NO_2^-/NO	+ 0.36
NO_3^-/NO_2^-	+ 0.43
Fe^{+3}/Fe^{+2}	+ 0.77
$1/2\ O_2/H_2O$	+ 0.82

* Source: Brock, 1979

Most of the nitrate reducing bacteria are facultative anaerobes, they can transfer electrons to oxygen or to nitrate when oxygen is absent. Since sulfate reducing bacteria are obligate anaerobes, they can use hydrogen as the major electron donor;

$$4\ H_2 + SO_4^= \rightleftharpoons H_2S + 2\ H_2O + 2\ OH^- \qquad (2\text{-}14)$$

$$\Delta G'_o = -36.4 \text{ Kcal/reaction}$$

$$4\ H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3\ H_2O \qquad (2\text{-}15)$$

$$\Delta G'_o = -32.4 \text{ Kcal/reaction}$$

It appears that sulfate-reducing bacteria can successfully compete with

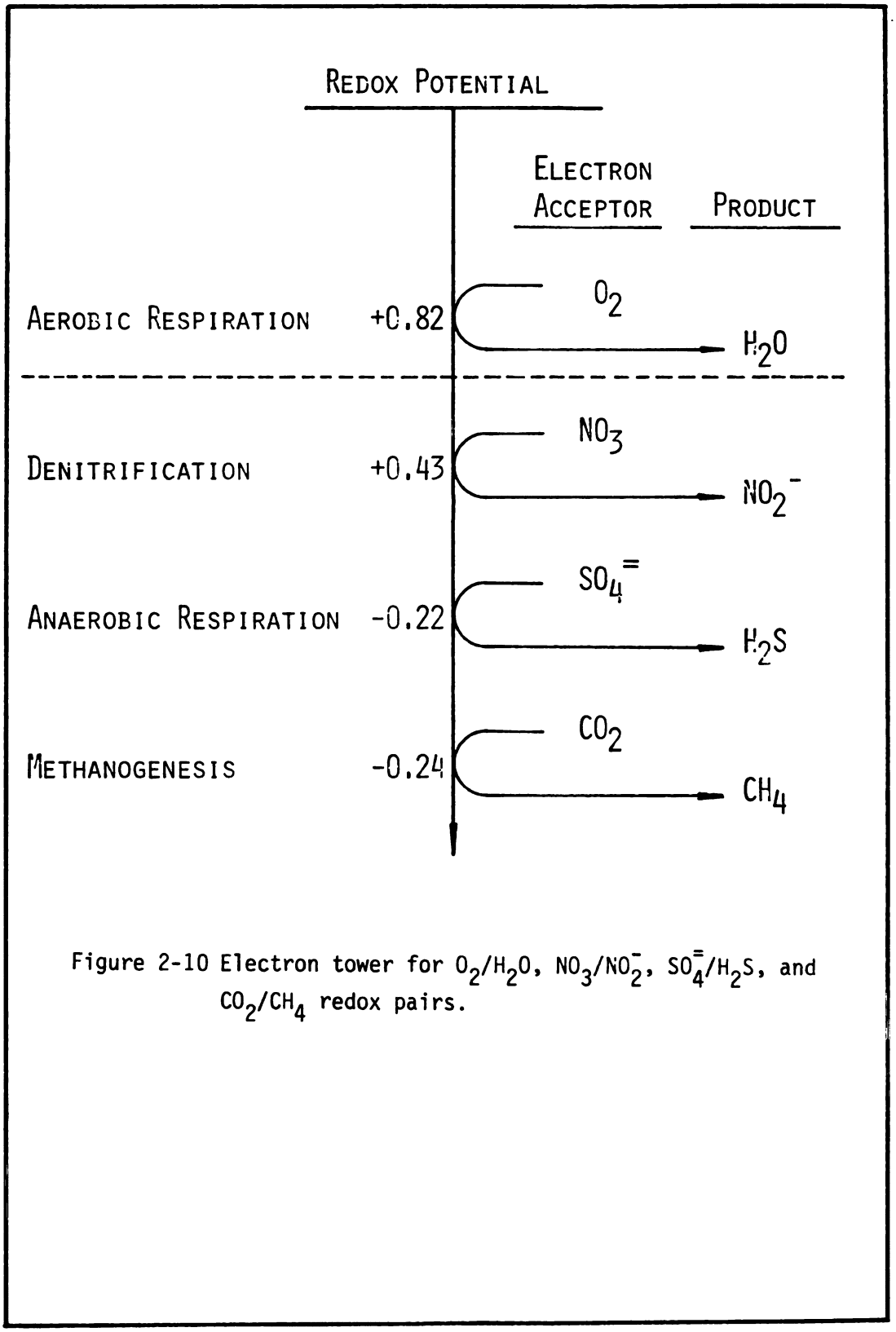


Figure 2-10 Electron tower for O_2/H_2O, NO_3/NO_2^-, $SO_4^=/H_2S$, and CO_2/CH_4 redox pairs.

hydrogen utilizing methanogens for hydrogen. If sulfate-reducing anaerobes are present in the system, electron flow is diverted from methane formation to H_2S production.

2.2 Anaerobic Fermentation Process Stability

In view of the previous discussion, a well operated anaerobic fermentor must be low in hydrogen concentration, have near neutral pH, and balanced production and utilization of volatile fatty acids. In other words, the stability of an anaerobic fermentation system may be distorted by inproper pH, high hydrogen partial pressure, and high fatty acid concentration. These three parameters are actually closely related, variation of one factor causing other parameters to be affected. For instance, when fatty acids begin to accumulate in the system, the pH value will drop and inhibit the activity of hydrogen utilizing methanogens. Therefore the hydrogen concentration will increase, which in turn will supress the degradation of volatile fatty acids, resulting in a further pH decrease. In addition to these three factors, process instability may be also caused by sudden changes of environmental and operational conditions, such as a sudden change in temperature , organic loading, and hydraulic loading. Several organic and inorganic compounds, such as ammonia and heavy metals, also play a significant role in process instability. Further discussion of some of these factors will be given in the following sections.

2.2.1 The Effects of Volatile Fatty Acids And pH

Accumulation of fatty acids and reduction of pH in a reactor are two common signs of a failing anaerobic fermentation system. Inhibition resulting from high concentrations of fatty acids has been studied by several investigators. Two major conclusions may be drawn from their studies which conflict with each other. One group of researchers (McCarty and McKinney, 1961a; Cassell and Sawyer, 1959; Sawyer et al., 1954; Kaplonsky, 1951) believed that methane bacteria were inhibited because of the drop of pH value caused by high fatty acid concentration in the system, and that this inhibition may be removed by the addition of buffering chemicals to raise the pH value. Another group (Buswell, 1939; Schulze and Raju, 1958; Mueller et al., 1959) argued that fatty acids themselves were directly toxic to methane bacteria at concentrations above 2000 mg/l regardless of the pH maintained, and the toxic condition can be released only by diluting the reactor substrate or reducing the substrate loading rate. Buswell and Mogan (1962) further reported that propionic acid would inhibit the methane bacteria. However, studies by McCarty et al. (1964) found another controversial result that propionic acid had little effect on methane bacteria but did inhibit the acid forming bacteria. Andrews (1969) tried to solve the conflicting ideas about fatty acid toxicity and reported that the toxicities were caused by the non-ionized portion of volatile acids. Thus toxicity is directly related to both the pH value and the acid concentration because the relative concentrations of ionized and un-ionized fatty acids are affected by hydrogen ion concentration, for instance,

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+ \qquad (2\text{-}16)$$

When pH decreases, equilibuium shifts to the left hand side and causes the un-ionized acid concentration to increase. Krocker (1979) also reported the same results that toxicity would increase when the pH dropped and un-ionized volatile acid concentration increased.

Because non-methanogenic bacteria can grow in a low pH environment, an unbalanced reactor with a lower pH value would favor rapid growth of non-methane bacteria and faster production of fatty acids. This will result in a further pH drop, increasing inhibition of methanogenic activity and causing the accumulation of hydrogen and fatty acids. The result of this adverse cyclic interaction between pH, acid concentration, and hydrogen concentration is the total failure of fermentation system.

2.2.2 Ammonia Toxicity

Ammonia may be present in the anaerobic fermentation system in the form of ammonium ion (NH_4^+) or free ammonia (NH_3). Concentration of these two forms of ammonia are affected by the hydrogen ion concentration in the system; low pH favors the formation of ammonium ion (NH_4^+) and high pH favors free ammonia (NH_3) production (Equation 2-17).

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad (2\text{-}17)$$

The dissociation constant for ammonia at 35° C is 1.849×10^{-5}, or $pK_a = 4.733$.

Ammonia serves as the nitrogen source for the microbial growth in fermentation systems. However, it can also be a toxic agent if excess concentration is present in the system. McCarty (1964) reported that ammonia nitrogen concentrations of 150 to 300 mg/l are inhibitory to the system at pH values greater than 7.4 to 7.6, and, if the concentration exceeds 3000 mg/l, ammonium ion itself becomes very toxic regardless of the pH. However, Krocker (1979) reported that process inhibition by ammonia was the result of excessive concentration of free ammonia rather than ammonium ion.

2.2.3 Salt Toxicity

A number of earth-metal salts such as sodium, potassium, calcium, and magnesium may be associated with the substrate and may be introduced into the system. The presence of these substances may inhibit process opeeration if high concenetrations is present. McCarty and McKinney (1961b) performed a series of experiments and found the process instability due to metal salts was associated with the metal cations rather than volatile acid anions. They also reported various cation concentrations that would cause inhibition, as shown in the Table 2-10.

Table 2-10 Concentration For Salt Toxicity (mg/l)

Cation	Moderately Inhibitory	Strongly Inhibitory
Sodium	3500 - 5500	8000
Potassium	2500 - 4500	12000
Calcium	2500 - 4500	8000
Magnesium	1000 - 1500	3000

* Source: McCarty, 1964

In the laboratory, the existance of these salts is mostly contributed by the agents used for pH control. Therefore, concentration of these substances are usually fairly low and do not cause inhibition effects unless large amounts of chemicals are added.

2.2.4 Heavy Metal Toxicity

Several heavy metals such as copper, nickel, zinc, and chromium are frequently toxic to microbial activity in many biological processes. The maximum allowable concentrations of these heavy metals vary as shown in Table 2-11 which summarizes the results reported by previous investigators.

Table 2-11 Toxic Concentrations of Some Heavy Metals in Anaerobic Digesters

Metal	Toxic Concen. (mg/l)	Reference
Copper	150 - 250	Rudgel, 1941
	500	Rudgel, 1946
	1000	Barnes & Braidech, 1942
Nickel	200	Barnes & Braidech, 1942
	1000	Wischmeyer & Chapman, 1947
Zinc	1000	Rudolphs & Zeller, 1932
	350	McDermott et al., 1963
Chromium*	2000	Barnes & Braidech, 1942
	200	Pagano et al., 1950

Source: Kugelman and Chin, 1970

* At normal pH levels, chromium normally reduces to the trivalent form which is very insoluble and consequently is not as toxic as the hexavalent chromium.

Heavy metal toxicity may be released by precipitation of the metals by adding sulfides such as sodium sulfide into the reactor. The solubility product of heavy metal sulfides range from 3.7×10^{-19} for FeS to 8.5×10^{-45} for CuS (McCarty et al., 1964; Lawrence and McCarty, 1965). At pH values higher than 7.6, concentrations of zinc greater than 1000 mg/l, can be precipitated out as zinc carbonate (Mosey et al, 1971, 1975).

Heavy metals do not exist or are only present in trace amounts in cellulosic agricultural residues. Therefore, they are not considered as potential toxicants in this research.

2.3 Anaerobic Methane Fermentation Process Control

In order to maintain stable process operation and to obtain optimum efficiency, it is important to understand the controlling parameters. Some biological and chemical factors that directly and indirectly influence process stability have been discussed in the previous sections. Application of these concepts and other factors required for effective operation will be disscussed in this section. Important operating variables include,

1. pH
2. Alkalinity
3. Volatile fatty acid concentration
4. Temperature
5. Absence of toxic material
6. Nutrient availability
7. Retention time
8. Degree of mixing

2.3.1 Alkalinity, pH and Fatty Acid Concentration

A complete anaerobic fermentation system (type A habitat) terminates with the formation of methane. Because methanogens are more sensitive to pH changes than other groups of bacteria (see Section 2.1.4.1), the optimum pH range for methanogens (6.5 - 7.7) automatically becomes the optimum pH range for the entire system. The pH value of a digester is a function of three parameters: alkalinity, volatile fatty acid concentration, and the fraction of carbon dioxide in the reactor's gas phase. Alkalinity is the measurement of carbonate and bicarbonate concentration in the reactor and it acts to buffer against pH fluctuation due to changing acid concentrations. Under normal conditions, pH in the reactor is maintained in the proper range by the destruction of fatty acids and formation of bicarbonate buffering.

The main buffering substance in most anaerobic digesters is NH_4HCO_3. A suitable ammonia nitrogen concentration, 50 - 200 mg/l (McCarty, 1964), can provide both the nutritional requirement for microbial growth and the necessary bicarbonate buffering. Ammonium ion (NH_4^+) does not provide bicarbonate buffering directly but only through:

$$NH_3 + CO_2 + H_2O \longrightarrow NH_4^+ + HCO_3^-$$

In the anaerobic fermentation system, total alkalinity is composed of both bicarbonate alkalinity and fatty acid alkalinity and has the relationship expressed in Equation 2-18,

$$TA = BA + (0.85 \times 0.833)(TFA) \qquad (2\text{-}18)$$

where TA = total alkalinity, mg/l as $CaCO_3$

BA = bicarbonate alkalinity, mg/l as $CaCO_3$

TFA = total fatty acid concentration, mg/l as acetic acid

Acetic acid is converted to the equivalent alkalinity as $CaCO_3$ by a factor of 0.833. The factor of 0.85 in Equation 2-18 is an adjust factor because 85% of the volatile acid alkalinity is measured by titration of total alkalinity to pH 4 (McCarty, 1964). To ensure a sufficient buffering capacity, a bicarbonate alkalinity in the range of 1000 - 5000 mg/l at pH range of 6.6 to 7.6 must be maintained. According to the relationship in Equation 2-18, bicarbonate alkalinity will be decreased due to the increased concentration of total volatile fatty acid. One control parameter often used by anaerobic digester operators is the total volatile fatty acid concentration (mg/l as acetic acid) to total alkalinity (mg/l as $CaCO_3$) ratio. If the value of this ratio drops lower than 0.8 the reactor becomes unbalanced.

Low pH reactor may be restored by reducing the substrate feeding rate or adjusting pH by the addition of chemical reagents such as bicarbonate, phosphate, lime, sodash etc.. Among those buffering reagents, lime is the most popular chemical being used by many wastewater treatment plants for pH control. However, lime is good only for completely mixed reactors where the pH has dropped below 6.5. Also, the amount of lime dosage must be carefully controlled; lime should be added only to raise the pH to about 6.7 (McCarty, 1964). Over dose of lime will cause excessive consuming of CO_2 and resulting in high pH (about 8.0).

$$Ca(OH)_2 + 2\ CO_2 \longrightarrow Ca(HCO_3)_2 \qquad (2\text{-}19)$$

$$Ca(OH)_2 + CO_2 \longrightarrow CaCO_3 + H_2O \qquad (2\text{-}20)$$

Equation 2-19 shows that lime initially reacts with CO_2 to form calcium bicarbonate. When the bicarbonate alkalinity reaches some point between 500 and 1000 mg/l, and the pH is about 6.7, additional lime will result in the formation of insoluble calcium carbonate (Equation 2-20) without increasing the pH or alkalinity until the CO_2 in the gas phase is depleted. Sodium bicarbonate is also a good pH control agent because it can provide 5000 - 6000 mg/l of alkalinity without causing toxic effects.

2.3.2 Effect of Temperature

The chemical composition of a microbial cell, the activities of cellular enzymes, and bacterial nutrition are all influenced by the temperature at which a bacterium is grown. Therefore, the growth rate of microorganisms is a function of temperature. Conceptually, temperature ranges for the optimal growth of microorganisms can be divided into three temperature regions: a thermophilic zone (above 45 ^{o}C) , a mesophilic zone (20 - 45 ^{o}C), and a psychrophilic zone (below 20 ^{o}C). The effect of temperature on anaerobic fermentation has been intensively studied by many investigators (Golueke, 1958; Malina, 1962; Farrel et al., 1967; Speece et al., 1970; Maly and Fadrus, 1971; Pfeffer, 1974; van Velsen et al., 1979). The recommended temperature range for efficient anaerobic sludge digestion is between 30^{o}C and 35^{o}C for the mesophilic digesters (Malina, 1964).

It is a common understanding that a mesophilic organism operating optimally at 30°C should not be expected to function well at an elevated temperature of 60°C. Therefore, a system normally operating at 30°C could be upset if the temperature is raised to 45°C. Buswell (1952) reported that in a sudden change of temperature of as little as one or two degrees (centigrade), inhibited methane formation and volatile fatty acids accumulate.

A temperature change also affects the CO_2 concentration in both liquid and gas phases. The solubility of carbon dioxide decreases with increasing temperature. Thus, the CO_2 concentration will decrease in the aqueous phase and increase in the gas phase at higher temperatures. The carbonate equilibrium constants are also affected by temperature change; pK_{a1}, for $H_2CO_3 = HCO_3^- + H^+$, decreases from 6.52 at 5°C to 6.30 at 60°C, and pK_{a2}, for $HCO_3^- = CO_3^- + H^+$, decreases from 10.56 at 5°C to 10.14 at 65°C (Snoeyink & Jenkins, 1980). Therefore bicarbonate concentration will be decreasing with increasing of temperature.

2.3.3 Absence Of Toxic Material

If toxic materials are presence in the reactor, two signals of inhibition may be exhibited: (1) a decrease in methane gas production; (2) a decrease in volatile fatty acid concentration. In a mixed culture ecosystem with mixed substrates, it is difficult to obtain a definite concentration at which a component becomes toxic. The magnitude of a toxic effect may be relieved or enhanced by complex interactions, known as antagonism (a reduction of the toxic effect of one substance by the presence of another) and synergism (an increase of

the toxic effect of one substance by the present of another). Microbial cultures may also become acclimated to the toxic substances. For instance, McCarty (1964) indicated that an anaerobic digester is inhibited by un-ionized ammonia nitrogen at a concentration greater than 150 mg/l as NH_3-N. However, Krocker et al. (1975) performed a successful anaerobic digestion experiment with swine manure at un-ionized ammonia concentrations of 500 mg/l as NH_3-N. The degree of acclimation may also explain the variability in toxic concentrations reported by various invistigators (Table 2-11).

Parkin et al. (1983) studied the response of methane fermentation to several toxicants (including ammonia-nitrogen, copper, nickel, chloroform, formaldehyde, hydrazine) and reported that the system could recover after extended periods of zero gas production, provided the microbial solids retentation time is long enough. Therefore, those processes with high solids retention time and short hydraulic retention time, such as anaerobic filters and anaerobic biological rotating disks, should have the highest potential for recovery from toxic inhibition.

2.3.4 Effect Of Retention Time

Hydraulic retention time and microbial solids retention time are the two most important control parameters for process design and operation. Hydraulic retention time (HRT) is defined as the ratio of effective reactor volume to the flow rate of substrate stream passing through the reactor and can be expressed as:

$$HRT = \frac{V}{Q} \qquad (2\text{-}21)$$

where V = effective reactor volume, (L^3)

Q = liquid substrate flow rate, (L^3/T)

Solid retention time (SRT) is defined as the total active microbial mass in the system divided by the total quantity of active microbial mass that is withdrawn from the system per unit of time and can be expressed as:

$$\phi_c = \frac{X_t}{(\Delta X / \Delta T)} \qquad (2\text{-}22)$$

where ϕ_c = microbial solids retention time, (time)
or sludge age, or mean cell residence time

X_t = total active microbial mass in system, (mass)

$(\Delta X / \Delta T)$ = total quantity of active biomass withdrawn per time, (mass/time).

Biological solids retention time is numerically equal to the hydraulic retention time for a steady state, completely mixed reactor without recycle. Adequately long solids retention time is crucial for effective operation of anaerobic fermentation processes; low solids retention time will cause washout of the microbial mass from the reactor resulting in system failure. A summary of minimum solids retention times for anaerobic digestion of various substrates are shown in Table 2-12.

Table 2-12 Minimun Solids Retention Time For Anaerobic Methane Fermentation

Temperature, °C	Substrate	θ_c^m (day)	Reference
15	Municipal sludge	60[a]	O'Rourke, 1968
20	Acetic acid	7.8	O'Rourke, 1968
	Stearic & palimitic acid	7.2	O'Rourke, 1968
	Mixed acids	7.2	O'Rourke, 1968
	Municipal sludge	10[a]	O'Rourke, 1968
25	Acetic acid	4.2	Lawrence & McCarty, 1969
	Propionic acid	2.8	Lawrence & McCarty, 1969
	Stearic & palimitic acids	5.9	O'Rourke, 1968
	Mixed acids	5.9	O'Rourke, 1968
	Municipal sludge	7.5[a]	O'Rourke, 1968
30	Acetic acid	4.2	Lawrence & McCarty, 1969
35	Acetic acid	3.1	Lawrence & McCarty, 1969
	Propionic acid	3.2	Lawrence & McCarty, 1969
	Butyric acid	2.7	Lawrence & McCarty, 1969
	Stearic & palimitic acid	4.0	O'Rourke, 1968
	Mixed acids	4.0	O'Rourke, 1968
	Municipal sludge		O'Rourke, 1968
	Municipal sludge	2.6[a]	Torpey, 1955

Source: Lawrence and McCarty, 1970

θ_c^m = limiting minimum solids retention time, determined by calculation from experimental data except as noted.

a = θ_c^m determined by washout.

The limiting minimum solids retention time is defined as the value of θ_c^m which occurs when influent substrate concentration is much greater than the half velocity coefficient, K_s (Lawrence & McCarty, 1970). If the following assumptions hold: (1) A constant proportion of the organisms are viable; (2) The primary substrate serves as the essential limiting nutrient; (3) Microbial growth can be expressed by Monod's model, the solids retention time for a steady state, completely mixed, single reactor without recycle can be expressed as:

$$\theta_c = \frac{K_s/S + 1}{YK - b(K_s/S + 1)} \qquad (2\text{-}23)$$

where K_s = half velocity coefficient, equal to the substrate concentration when dF/dt = 1/2 (K), in which dF/dt = rate of microbial substrate utilization per unit volume, K = maximum rate of substrate utilization per unit weight of microorganism; S = substrate concentration; b = microorganism decay coefficient, $time^{-1}$; Y = growth yield coefficient, mass of organism formed per mass of substrate utilized. When the influent substrate concentration, S, is much greater than the half velocity coefficient, K_s, then Equation 2-23 can be simplified as,

$$\theta_c^m = \frac{1}{YK - b} \qquad (2\text{-}24)$$

The limiting minimum solids retention time listed in Table 2-12 were calculated using Equation 2-24.

In general, solids retention times of 10 to 30 days at 35^oC are employed by many anaerobic sludge digesters: these retention times are 3 to 10 times greater than the limiting values. McCarty (1970) sug-

gested that a safety factor of about 3 to 10 should be applied to the minimum solids retention for operation and design of anaerobic digesters.

Another control strategy occasionally used is the volumetric organic loading rate. It is defined as the rate per unit volume at which organic substrate is fed into the reactor, and can be expressed as:

$$\text{Organic Loading Rate} = \frac{(\text{organic concentration}) \times Q}{V}$$

$$= \frac{\text{organic substrate concentration}}{\text{HRT}} \qquad (2\text{-}25)$$

Therefore organic loading rate is related both to the hydraulic retention time (HRT) and the percentage of organic contents in the influent substrate. The values of loading rate can be changed by changing substrate BOD or HRT at a given substrate concentration. At lower organic loading rate and when substrate concentration does not change, higher percent of organic substrate will be degraded but less CH_4 will be produced per volume of reactor as compared to that at the higher organic loading rate.

2.3.5 Effect Of Mixing

For many conventional anaerobic fermentation processes, mixing is an important operational parameter to achieve satisfactory treatment efficiency. Sufficient mixing of the reactor can provide the following benefits: (1) uniform distribution of substrate, microorganisms, and temperature; (2) substrate is kept in continuous contact with the

microorganism; (3) biological intermediates and end-products are uniformly distributed; and (4) prevention of a scum blanket.

Finney and Evans (1975) hypothesized that methane production is influenced by the phase transfer rate and suggested that vigorous agitation, low pressure (vacuum), and high temperature would increase the rate of phase transfer resulting in higher methane production rates. However, Coppinger et al. (1979) reported no decrease in gas production when mixing was discontinued. They indicated that the gas bubbling and thermal convection currents provided sufficient mixing for the reactor. Hashimoto (1982) reported that although a continuous mixed fermentor produced significantly higher methane than the fermentors mixed only two hours per day, the methane production rate from the continuously mixed fermentor was only slightly higher than the rate produced from another fermentor with intermittent mixing. Therefore, he concluded that there is little potential for increasing fermentation rates by excess increased mixing, and that phase transfer controling mechanisms have minimal effect on the CH_4 production rate.

2.3.6 Nutrient Requirements

Microorganisms require a variety of substances for synthesis of cell material and for generation of energy. Since microorganisms are extremely diverse in their physiological properties, nutrient requirements for each species of bacteria are not identical. The chemical composition of cell material gives the basic idea of the major material that are required for cell growth. The approximate elementary composition of a microbial cell is given in Table 2-13.

Table 2-13 Approximate Elementary Composition of Microbial Cells

Element	Percent of Dry Weight
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All others	0.3

* Adapted from Stanier et al., 1976

The major components of a microbial cell are hydrogen, oxygen, carbon, nitrogen, phosphorus, and sulfur, these elements accounting for about 95% of the total cellular dry weight. The function of these material as well as other nutrients are summarized in Table 2-14.

Table 2-14 General Physiological Functions Of The Principal Elements

Element	Physiological Functions
Hydrogen	Constituent of cellular water, organic cell materials.
Oxygen	Constituent of cellular water, organic cell materials; electron acceptor in aerobes.
Carbon	Component of organic cell material.
Nitrogen	Component of proteins, nucleic acids, coenzymes.
Sulfur	Component of proteins and coenzymes.
Phosphorus	Constituent of nucleic acids, phospholipids, and coenzymes.
Potassium	Principal inorganic cation in cells, cofactor for some enzymes.
Magnesium	Cofactor for enzymatic reactions; functions in binding enzymes to substrate; component of chlorophylls.
Calcium	Cofactor of some enzymes.
Iron	Constituent of cytochromes and heme or nonheme proteins; cofactor for some enzymes.
Cobalt	Component of Vitamin B_{12}
Copper, Zinc	Inorganic constituents of special enzymes.

Source: Stanier et al., 1976

Most of the wastewaters treated by anaerobic fermentation processes contain sufficient nutrients for microbial growth although certain types of substrates such as cellulosic residues may be deficient in some nutrients such as nitrogen, phosphorous, sulfide, and iron. These deficient materials may be supplied as inorganic salts. Ammonium car-

bonate or hydroxide and anhydrous ammonia have been found as suitable nitrogen sources for methane fermentation. Other inorganic salts such as $(NH_4)_2HPO_4$, $NaHCO_3$ may also be used to meet the nutrients requirements. Micronutrients such as manganese, cobalt, copper, molybdenum, and zinc are required in very small quantities. They are usually present in adequate amounts in tap water or as contaminants of the major inorganic constituents in the growth media or influent substrate.

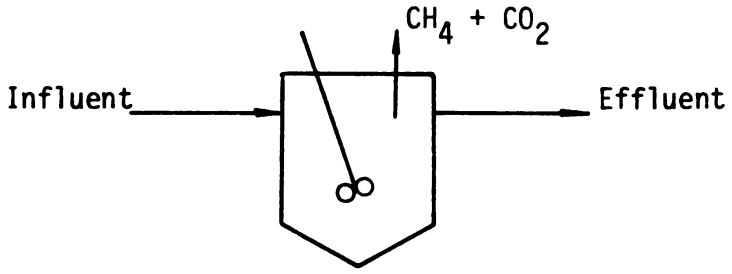
2.4. Anaerobic Fermentation Process Models

Anaerobic fermentation processes that have been developed so far may be summarized into seven different configurations (Figure 2-11): (1) conventional - completely mixed without solids recycle, (2) anaerobic contact - completely mixed with solids recycle, (3) batch-load, (4) plug flow with solids recycle, (5) anaerobic expanded bed, (6) high solids or dry anaerobic fermentation, (7) anaerobic filtration. Brief descriptions of each process will be given in the following paragraphs.

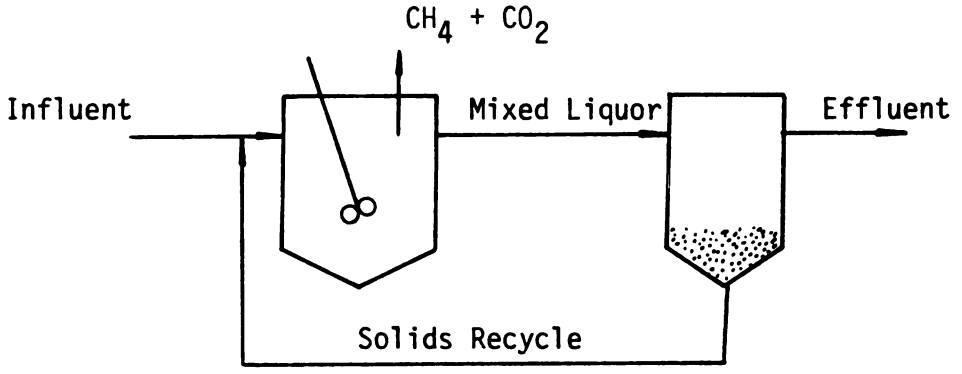
2.4.1 Conventional Anaerobic Digester

Most of the early anaerobic digesters were designed without maintaining a high microbial population in the system and belong to the flow-through type reactor without sludge recycle. The term "conventional" was used to describe this type of anaerobic digestor. Conventional digester has been used mostly in municipal sewage treatment plants for sludge stabilization. Sludge and microorganisms are uniformly distributed in the entire reactor and the digested effluent

(1) Conventional-Completely mixed, no solids recycle



(2) Anaerobic Contact-Completely mixed with solids recycle



(3) Batch-Load

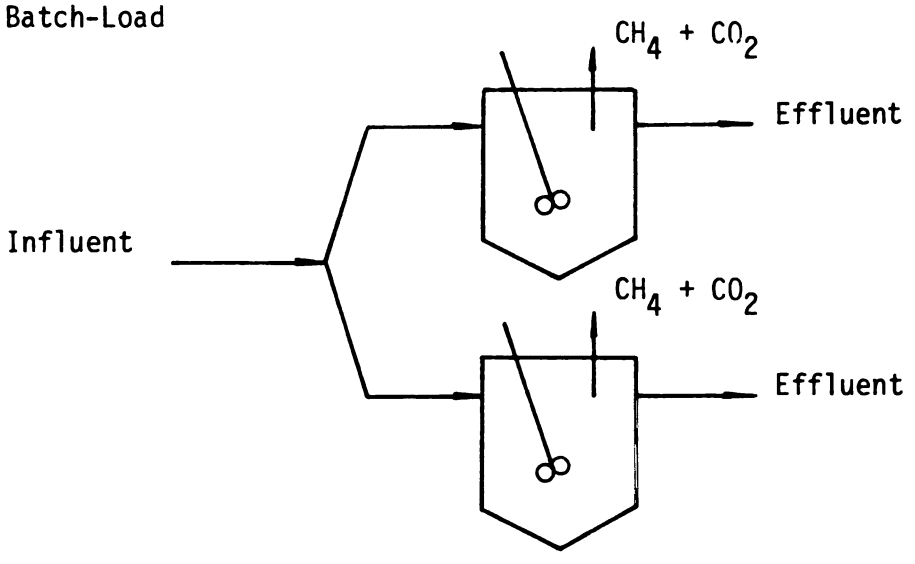
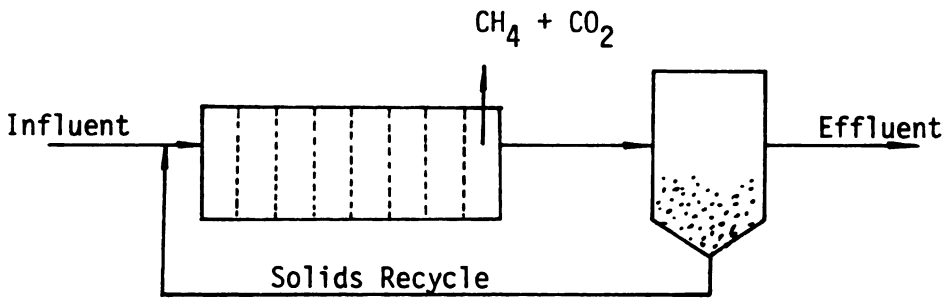
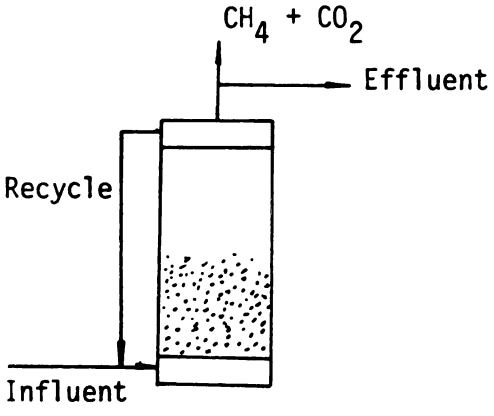


Figure 2-11 Schematic Diagrams of Anaerobic Fermentation Processes.

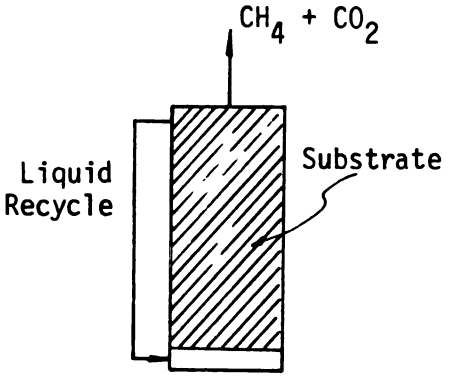
(4) Plug Flow - with solids recycle



(5) Anaerobic Expanded Bed



(6) Dry Fermentation



(7) Anaerobic Filter

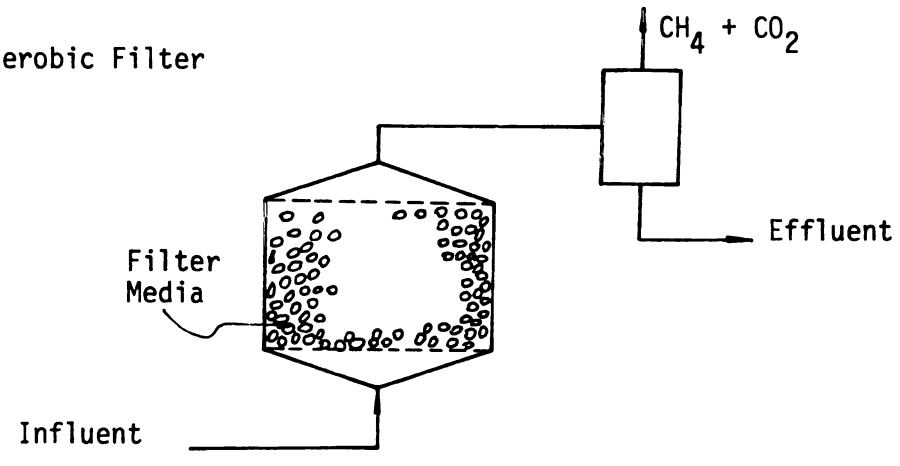


Figure 2-11 Continued

is withdrawn at the same rate as the influent sludge to maintain a constant reactor volume. This process assumes that the microbial concentration in the reactor and in the effluent are equal.

2.4.2 Anaerobic Contact Reactor

Anaerobic contact process, or anaerobic activated sludge process, is a modified form of the conventional anaerobic digester. This process employs a settler to separate the microbial solids from the effluent liquid and mix the recycled sludge with raw waste to maintain a high microbial concentration in the main reactor for more efficient and rapid treatment. This process assumes that all substrate stabilization occurs in the main reactor and the total biological mass in the system is equal to the biological mass in the main reactor. This process has been applied to treat medium strength industrial wastes.

2.4.3 Batch Load Model

The batch load model is composed of two identical completely mixed anaerobic digesters. When the first one is fermenting the waste as a batch reactor, the second one is receiving raw sludge. When the second reactor is full of substrate, and begins its digestion period, the first reactor is emptied to receive incoming sludge. This process has been found to have a higher treatment efficiency than that obtained from conventional reactors.

2.4.4 Plug Flow With Recycle

The plug flow model applies a longitudinal reactor without longitudinal mixing during the passage of substrate through the reactor. Similar to the contact process, this model has a settling tank to recycle active anaerobic sludge to mix with raw waste. The biodegradable organics decrease along the tank while the microbial concentration increases. The plug flow anaerobic reactor has been applied for animal waste treatment both in pilot scale and full scale (Hutchinson, 1972; Bell, 1973; Jewell, 1976). Jewell (1976) indicated that with the absence of internal mixing and at lower operating temperatures (22.5 ^{o}C), a successful plug flow anaerobic digester could be obtained which would be economically competitive with the conventional process.

2.4.5 Anaerobic Expended Bed

Anaerobic expended bed, or anaerobic fluidized bed, are newly developed fixed-film anaerobic processes. These closely related processes consist of a column packed with an inert material, often send, which will expand as the waste flows upward through the column. The particles serve as a support surface for microorganisms to attach, and provides a large surface area to mass ratio. These processes provide a high degree of contact between the substrate and biomass and therefore result in high treatment efficiency.

2.4.6 High Solids Anaerobic Fermentation

Previous literature has provided little information about high solids fermentation. Early studies by Buswell et al. (1936), Keefer (1947), and Schulze (1958) showed that anaerobic digestion could be performed at solids concentration of up to 20% , although, higher concentrations of volatile fatty acid would build up in the reactor. Wong-Chong (1975) first studied the "dry anaerobic digestion" of animal wastes (dairy and poultry) at solids concentration greater than 20% in both batch and batch feed reactors. He concluded that anaerobic digestion of waste with high solids concentrations is feasible and provided economies in reduced reactor volume, digested sludge handling, and avoiding treatment of digester supernatant. However, he also pointed out the potential inhibition by ammonia due to high concentrations of nitrogenous waste. In his study, Wong-Chung did not resolve the substrate input and output problems for the semi-continous or continous flow reactors. He also did not solve the possible inhibition problem due to high volatile acid concentration built up in the reactor.

Another "dry anaerobic fermentation" study done by Wujicik and Jewell (1979) dealt with a substrate of mixed dairy cow manure and agricultural residues in batch reactor; the study concluded that at a solids content of 40%, the methane bacteria were inhibited. And at 55% total solids concentration, volatile fatty acids reached a maximum. At higher solids content, acid production declined indicating that the fermentative bacteria were inhibited too. Again, their study of high solids anaerobic fermentation did not provide information about substrate input and output methods, and no information was given about the

possible resolution of inhibition due to high volatile acid concentration.

2.4.7 Anaerobic Filtration Process

Anaerobic filtration is a fairly new process. It was first introduced by Coulter et al. in 1957, but their study did not arose much attention at that time. The real development of this process was started by Young and McCarty in 1967 and subsquently by several other investigators working with a variety of wastes (Table 2-15). Up to the present time, anaerobic filtration has not been coupled with high solids anaerobic fermentation of cellulosic residues.

Anaerobic filtration has several advantages over suspended growth processes. With the suspended growth processes mentioned above, either long hydraulic retention times or solids seperation and solids recycle are required to provide an adequate solids retention time (SRT) for efficient treatment. In anaerobic filtration, the substrate is passed upward through a column reactor which is filled with a medium for microorganisms to attach and grow in a manner similar to the anaerobic expended bed. Because the biological solids affix to the surface of the filter medium or become trapped within the interstices void spaces and are not washed out in the effluent stream, a long solids retention time can be obtained without solids seperation. This process is only suitable for soluble or colloidal wastes.

Table 2-15 Summary of Previous Anaerobic Filter Studies

Type of Waste	COD Loading* lb/day-10^3ft^3	Detention Time (Hr) **	COD Removal Efficiency (%)	References
Volatile Acid and Protein Carbohydrate 375-12000 mg/l COD	26.5 - 212	4.5-72	56 - 98	Young & McCarty, 1968
Food Processing (carbohydrate) 8500 mg/l COD	100 - 640	13 - 83	30 - 86 (55-86 soluble)	Pulmmer & Malina, 1968
Acetic Acid 6400 mg/l COD	370	12	30 - 80	Clark & Speece, 1970
Potato Processing Waste 3000 mg/l COD	33 - 145	13 - 59	41 - 79	Pailthorp et al, 1971
Wheat Starch Waste 5930-13100 mg/l COD	237	22	65 (76 % soluble)	Richter et al 1971 Taylor, 1972
Lab Scale Organic-alcohols aldelydes, acids, amine, glycol, phenol 2000 mg/l COD (20000 mg/l COD system failure)	35 - 130	17 - 46	64 - 76	Hovious et al, 1972
Pilot scale Petrochemical, 2000-8000 mg/l COD	40 - 145	72	10 - 13	
Brewery Press Liquor 6000-27000 mg/l COD	50 - 400	15 - 330	30 - 97	Foree et al, 1972

* Based on total (empty bed) volume
** Based on initial unseeded void volume

Table 2-15 Continued

Type of Waste	COD Loading* lb/day-10^3ft^3	Detention Time (hr) **	COD Removal Efficiency (%)	References
"Metrecal" 11000 mg/l COD	427	18	70 - 95	El-Shafie & Bloodgood, 1973
Dilute Waste Sulfide Liquor 1300-5300 mg/l BOD_5	125 - 375	89 - 95	27 - 58 BOD Removal	Wilson & Timpany, 1973
Pharmaceutical Waste (95 % methanol) 1250-16000 mg/l COD	14 - 220	12 - 48	94 - 98	Dennis & Jennett, 1974
Grain Alcohol Stillage 3000 mg/l COD	31 - 124	36 - 72	74 - 84	Dahab & Young, 1981
Pharmaceutical Waste 2000-6000 mg/l COD	34.9-104.6	36	18 - 80 (25 - 94 BOD)	Sachs, Jennett & Myrton, 1982

* Based on total (empty bed) Volume
** Based on initial Unseeded void volume

2.5 Cellulosic Substrate

Cellulose is an important consitituent of all plant tissue, closely associate with lignin and non-cellulosic polysaccharides such as hemicellulose and pectic substance. To study the microbial degradation of cellulosic material there must first be a clear understanding of the physical and chemical nature of the material involved. This section will discuss the physical and chemical properties of the cellulosic substrate.

2.5.1 Physical And Chemical Properties Of Cellulose

Cellulose is a water insoluble, high molecular weight polymer composed of D-glucose residues jointed by β -1,4-glucoside bonds. Figure 2-12 illustrates the conformation formula (chair form) of cellulose, showing that the hydroxyl groups are located in the equatorial position and the H-atoms are located in the axial position of the (1→4)-linked anhydroglucose units. Every other glucose unit is rotated 180^{o} around the main axis. The number of repeating cellobiose units ranges from 500 to 5,000 giving molecular weights from 100,000 to 1,000,000. The chemical properties of cellulose are mainly determined by the glycosidic linkage and three hydroxyl groups on the glucose unit. Major chemical and enzymatic reactions occur at these locations (Fan et al., 1980).

It has also been reported (Baney, 1968) that the hydroxyl group in the 3-position is bound by intramolecular hydrogen bonding to the ring oxygen atom of the next unit as shown in Figure 2-13. Therefore, cellulose molecules are linked longitudinally to form fibrils, which

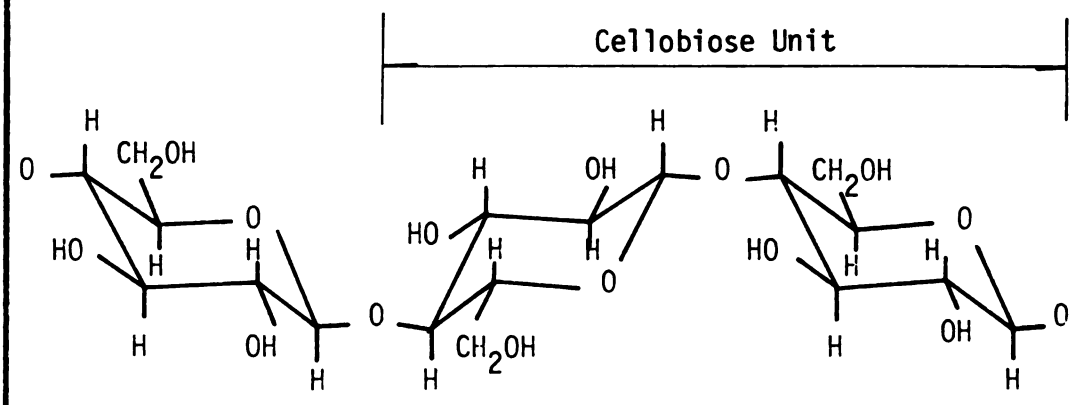


Figure 2-12 Conformation Formula of Cellulose

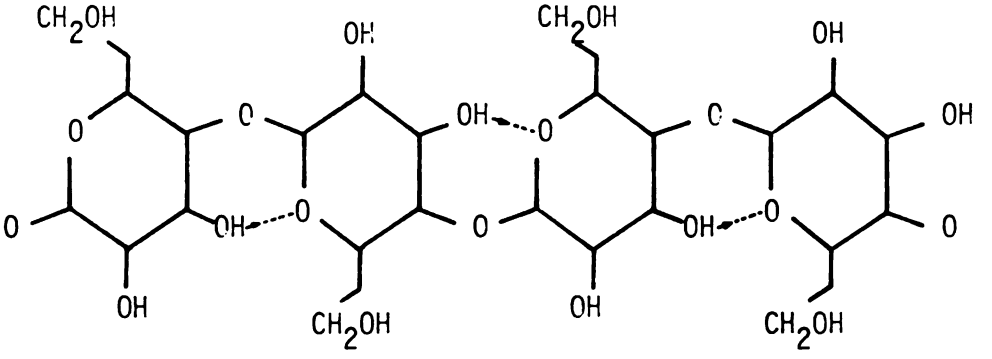


Figure 2-13 Cellulose Molecule With Intrachain Hydrogen Bonds

laterally linked by hydrogen bonding of adjacent fibrous chains. The length of one anhydroglucose unit is about 0.515 nm (5.15 A), and the total length of the native cellulose molecule (10,000 units) is about 5 μm.

The regular cellulose molecule exhibits a crystalline X-ray diffraction pattern. This periodic structure of diffraction repeats itself every 10.3 A in the direction of the fibre axis (Wood, 1970). X-ray diffraction patterns also show that cellulosic fibers contain amorphous areas. The degree of crystallinity varies with the type of cellulosic material. In general, the proportion of crystalline material ranges from 50 to 90%.

2.5.2 Hydrolysis Of Cellulose Material

Unlike starch, which is a poly-α-1,4-D-glucosan, cellulose does not act as a carbonhydrate reservoir which can be readily broken down to glucose whenever necessary. Only a few specialized species of aerobic bacteria and fungi can utilize cellulose. However, many species of facultative and strict anaerobes can secrete extracellular enzymes, called cellulases, to hydrolyze cellulose. The biochemical transformation of cellulose into smaller soluble carbohydrates is known as "cellulolysis".

The degradability of cellulose by microorganisms varies greatly with the nature of the cellulose. The physical and chemical features that may affect biodegradation of cellulose include the following:

1. The degree of crystallinity: cellulose with a higher degree of crystallinity has stronger resistance to enzymatic hydro-

lysis.

2. The unit cell dimension of the cellulose: a smaller unit cell is more easily hydrolyzed.
3. Degree of polymerization of the cellulose: a higher degree of polymerization is expected to have a slower rate of hydrolysis.
4. The nature of the substances with which the cellulose is associated: lignin, hemicellulose, mineral constituents, and trace amount of N and P may associate with cellulose. The higher percentage of lignin associated with the cellulose, the more difficult the enzymatic hydrolysis.

Lignin, one of the most chemically and biologically resistant materials, is a polymer of aromatic compounds. Lignin supplys strength and rigidity to the plant tissues and acts as a physical barrier, minimizing water permeation across the cell walls, and providing protection against infection. The degradability of cellulosic material is inversely correlated to the amount of lignin present in the substrate.

Hemicelluloses are amorphous, non-cellulose, heterogeneous mixtures of linear or branched polymers that may contain D-xylose, D-mannose, D-glucose, D-galactose, and D-glucuronic acid. They can be isolated from the original or delignified tissue by extraction with dilute alkali and acid. The content of hemicellulose in plant tissue ranges from 6 to 40% depending on the type of plant. Hemicelluloses are readily degradable by bacteria.

2.5.3 Properties Of Wheat Straw

Wheat straw was selected as the substrate for this research, because it is a major agricultural residue and it is available in large quantity on the M.S.U. campus and also because it is easy to handle. Wheat straw has been mostly used for animal bedding and has also been used for animal feed, construction material, and paper pulp. Other uses of wheat straw are listed in Table 2-16.

Table 2-16 Usage oF Wheat Straw

Methods	Products
Direct Uses	Fuel, fertilizer, soil conditioner, feed, packing materials, bedding for animals.
Mechanical Conversion	Pulp and paper.
Chemical Conversion	Sugar, alcohol, cellulose derivatives, phenolic compounds, lignin, etc.
Biological Conversion	Sugar, alcohol, enzymes, fermented feed, methane.

Source: Han, 1979

As with many other plant materials, wheat straw is mainly composed of cellulose, hemi-cellulose, and lignin. In addition to three major components, wheat straw also contains trace amounts of protein, calcium, potassium, magnesium, phosphorus, and sulfur.

CHAPTER THREE

EXPERIMENTAL METHODS AND MATERIALS

A specially designed reactor system was used to conduct the experiments of this research. The experimental procedures were designed to verify the feasibility of the proposed process for semi-continuous treatment of cellulosic residues. This chapter will present the experimental system, the experimental procedures, as well as the analytical methods that have been used in this research.

3.1 Description Of The Experimental Apparatus

The reactor system, as shown in Figure 3-1, consists of two fixed-film anaerobic filters, ten high solids packed reactors, a liquid equalization reservoir and a gas collection system. The reactors were connected in series and which was allowed to have a semi-continuous substrate feeding. The packed reactors were designed to have a stationary solid phase and a mobil liquid phase. The liquid phase in the packed reactors was pumped by a constant flow rate pump and was recirculated through two anaerobic filters that served as the methane generators. Figure 3-2 shows the details of the packed reactor and the gas collection system. The packed reactor was made of $^{1}/_{8}$ in. thick acrylic cylinder with the inside diameter of 7.3 cm. ($2\ ^{7}/_{8}$ in.) and the total height of 14.4 cm. (5.7 in.), and which was divided into three sections; a substrate holding section having length of 10.4 cm. in the middle, and two liquid sections with 2 cm. in length for

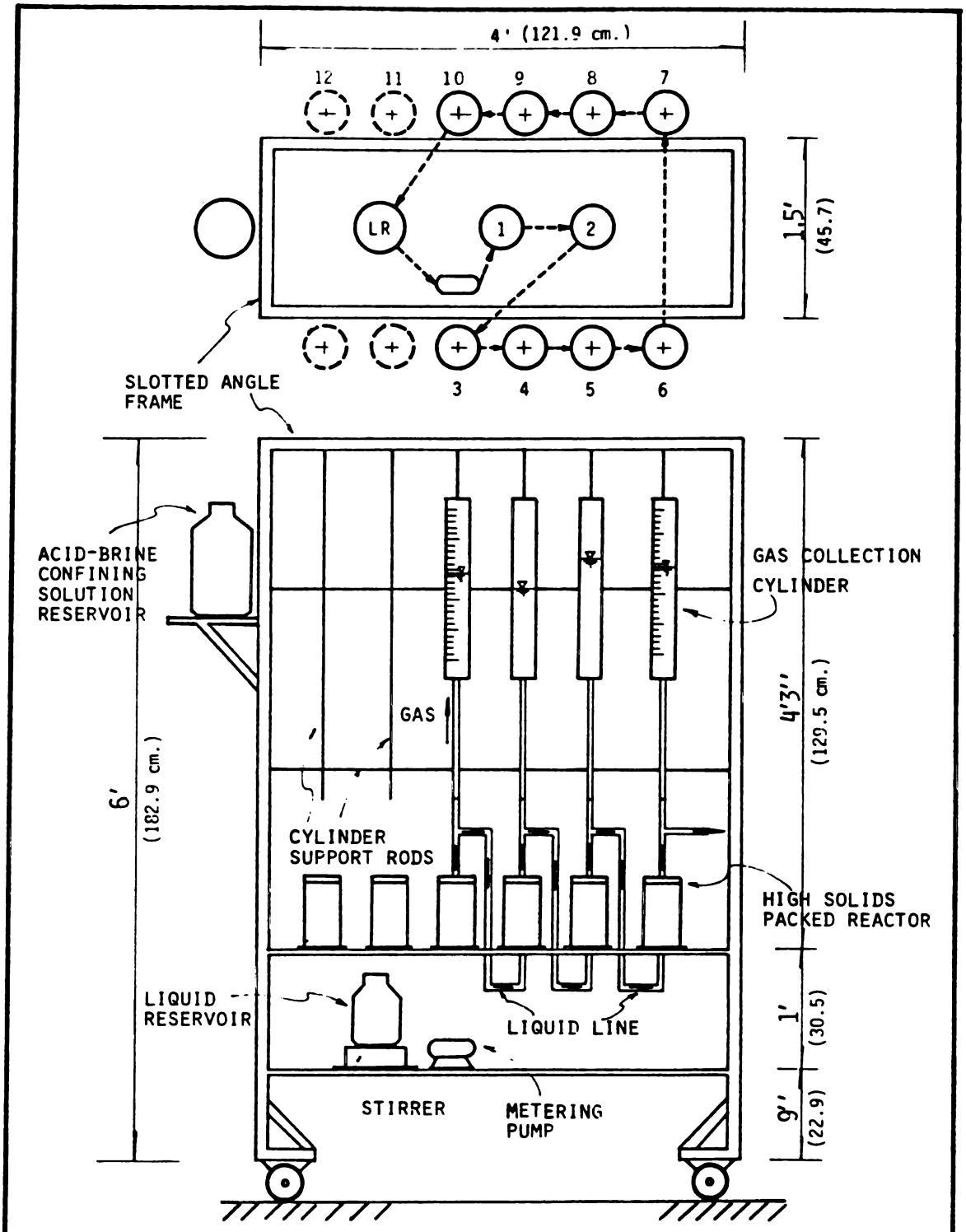


Figure 3-1 Schematic Diagram of Experimental System

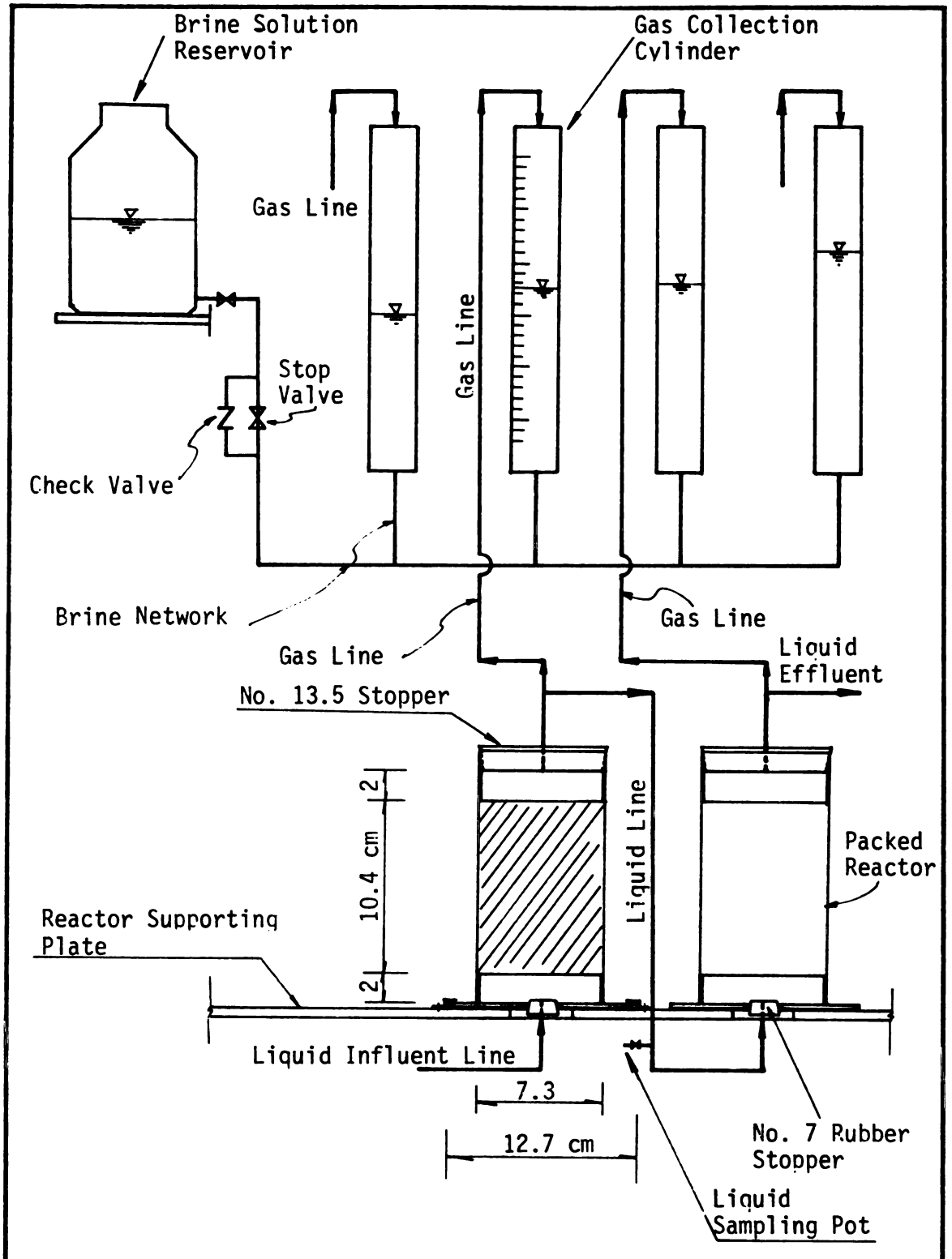


Figure 3-2 Schematic Diagram of Packed Reactor And Gas Collection System.

each section located on the top and the bottom of the reactor. Sixty grams of air dried, chopped wheat straw was placed in the center substrate section for each experimental run. The total volume of one packed reactor was 600 ml and the volume for the center substrate section was 435 ml. The volume occupied by the dry straw was 150 ml; thus, based on the volume to volume ratio, the solids concentration in the packed reactor was: 150/435 = 34.5 % .

The anaerobic filters have the same size and dimensions as the packed reactors, and were constructed by using the same material. Wheat straw was used as the filter media and provided surface area and interstitial void spaces for microorganisms to attach and growth.

Both types of reactor have a 5 inches by 5 inches, 0.375 inches thick acrylic plate base which was designed to support the reactor and for the convenience of installing and removing the reactor. The reactors were sealed using a No. 13 $^{1}/_{2}$ rubber stopper on the top and a No. 7 rubber stopper at the bottom. Tygon tubing (0.375 in. I.D.) was used as the effluent line and also served as the gas vent line. An inverted Y-connector was installed in this gas-liquid line about 10 inches above the top of the reactor to separate the gas and liquid. The effluent liquid line (0.375 in. I.D.) was then connected from the side branch of the Y-connector to the influent of the next reactor. The gas line ($^{3}/_{16}$ in I.D.) was connected from the straight branch of the connector to the gas collection cylinder. The gas volume produced was measured by displacement of an acid brine solution. The brine solution consisted of 10 % NaCl and 2 % H_2SO_4 which has a very low solubility for gases. Another, smaller Y-connector was installed in the effluent line of each reactor so that liquid samples could be with-

drawn easily without disconnecting the tubing between two reactors. A slotted angle frame with dimension of four feet long, 1.5 feet wide, and 6.0 feet high was built to support all experimental reactors. Individual reactors can be easily installed by slipping the base plate into guide rails mounted on the supporting plate.

As shown in Figure 3-2, the reactors designated as No. 1 and No. 2 in the top view are the anaerobic filters. The other reactors surrounding the supporting frame, designated from No. 3 to No. 10, are the high solids, packed reactors. The effluent line of each reactor was connected by a plastic, quick release connector to the influent of the next reactor, so that all reactors were connected in series.

The liquid phase was pumped at a constant rate from the liquid reservoir to the anaerobic filters from which it flowed by gravity through the packed reactors and back to the liquid reservoir. The liquid flow in each reactor was upward. The liquid flow pattern in the reactor system was always from the reactor with the oldest substrate through a series of packed reactors to the one with the newest substrate, and than into the liquid reservoir. Effluent from the liquid reservoir was pumped into Filters No. 1 and No. 2 and the flow circulation pattern was completed by introducing the effluent of Filter No. 2 to the oldest reactor.

The gas collection system was composed of gas cylinders, a brine solution network, and a brine reservoir (Figure 3-1). Ten gas collection cylinders for 10 packed reactors were made from $^1/_8$ inches thick, 2.0 in. I.D. acrylic pipe, each cylinder having an effective volume of 800 ml. A two liter plastic graduated cylinder served for gas collection from Filter No. 1 and a one liter plastic graduated cylinder was

used for Filter No. 2. All gas cylinders and the brine reservoir were connected together by tygon tubing ($^{5}/_{16}$ in.) so that brine displaced from the cylinders could be stored in the reservoir. A protection device, consisting of a check valve (one way flow into the reservoir) and a by-pass tube with a stop valve, was installed at the effluent port of the brine reservoir for the purpose of preventing loss of brine due to possible accidental loosening of the tygon tubing. All gas cylinders were mounted to aluminium supporting rods by moveable clamps. When gas production was measured, the individual gas cylinders were moved so that level of brine inside the cylinder was at the same height as that in the reservoir. Therefore, atmospheric pressure could be maintained every time the gas volume was measured.

A one liter, glass aspirator bottle was used as the liquid equalization reservoir. This reservoir was connected between the newest substrate packed reactor and Filter No. 1. Besides acting as an equalization basin, this bottle held enough volume of liquid for daily sampling. The liquid reservoir sat on a magnetic stirrer to keep the liquid well mixed.

A constant voltage transformer and a variable voltage regulator were used to control the positive displacement piston pump (Model PR-G20 by Fluid Metering, Inc., Oystering Bay, N.Y.) for a desired constant flow rate. A photograph showing the entire experimental apparatus is given in Figure 3-3.

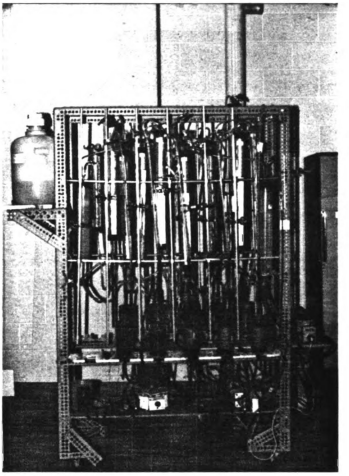


Figure 3-3 Experimental System For High Solids Anaerobic Fermentation And Anaerobic Filtration of Wheat Straw.

3.2 Experimental Procedures

The entire experimental apparatus was kept in a walk-in constant temperature room. The temperature was always maintained at 36 ± 1°C during the entire 18 months of experimental work. The experimental program was divided into three stages. The first stage was the time for conceptual studies, system debugging and modification, and the improvement of analytical techniques, as well as the study of the performance of packed reactors and anaerobic filters. The second stage was the extended study of the performance of packed reactors and anaerobic filters; the results obtained from this period were used for mathematical model development. The third stage was a study of the performance of the anaerobic filter under transient loading. Experimental methods performed in this research will be described in this section.

3.2.1 Substrate Preparation

Baled wheat straw was obtained from the straw storage room belonging to the MSU Department of Animal Science. Straw was first chopped with a communiting machine (Model D, by W. J. Fitzpatric Co.) into pieces about 0.5 inches long and dried at room temperature. The moisture content of the chopped straw was measured by oven drying at 103° C. The average moisture content was found to be 5.67% (S. D. = 0.11).

3.2.2 System Start Up And First Stage Experiment

The experiment was first started with only one reactor (later designated as the No. 1 anaerobic filter), packed with 60 grams of wheat straw, and the liquid reservoir in the system. After connecting the influent line of the reactor, 450 ml of distilled water were added into the reactor, and the top rubber stopper with the effluent line was installed. No nitrogen or other inert gas was applied to try to purge oxygen. The liquid reservoir was filled with 1,000 ml of distilled water. About 50 ml of active anaerobic digester sludge obtained from the Mason Wastewater Treatment Plant, Mason, MI., was injected into the reactor. Liquid was circulated through Reactor 1 and the liquid reservoir. Three days later, the second reactor (anaerobic filter No. 2) was added into the system by using the same procedures. Tubing was reconnected to let the effluent from Reactor 1 flow into the bottom of Reactor 2, and effluent from Reactor 2 went into the liquid reservoir. Another 50 ml of digester sludge was also seeded into Reactor 2. Every three days thereafter, one more reactor was added into the system until Reactor 12 was installed.

Besides daily gas production and pH measurement, no other sample was taken from the system during this period. Several chemical reagents including $NaHCO_3$, NaOH, and NH_4HCO_3 were used to adjust pH value to around 6.8 in the liquid reservoir. After 36 days, Reactors 1 and 2 were moved to the middle part of the supporting frame and stayed in that position serving as the anaerobic filters thereafter. Also, Reactors 11, and 12 were disconnected from the system and acted as long term batch reactors. The position of reactors at that stage is shown

in Figure 3-4.

After Reactors 1 and 2 started to serve as the anaerobic filters, about 100 ml of active digester sludge was injected into these two filters, and the pH of the liquid reservoir was monitored. On the 36th day, a new reactor with 60 grams fresh straw was installed to replace Reactor 3, since, at that time Reactor 3 was the oldest one in the system. The effluent line of Reactor 3 was connected to the liquid reservoir and the effluent line of No.10 was reconnected to the influent line of Reactor 3. Also the effluent of Filter No. 2 was reconnected to the influent line of Reactor 4. In this way, as mentioned earlier, liquid flow was always from the oldest reactor to the newest reactor. Three days later, Reactor 4 was replaced by a new reactor in the same manner.

The substrate input interval of three days was maintained until Reactor 10 (the oldest packed reactor in the system at that time) was replaced by a new substrate reactor. During this period, samples were taken for volatile fatty acids, total soluble COD and pH. However, samples were only taken once a day and the sampling times were not consistent. It was latter found that sampling frequency and sampling time were both crucial for this research. Every time a new reactor was added, about 50 to 100 ml distilled water was added into the liquid reservoir to make up the amount of liquid lost from the system as the result of daily sampling.

After two cycles were completed for a three-day substrate input interval, the substrate input interval was changed to five days until the end of the first stage experiments.

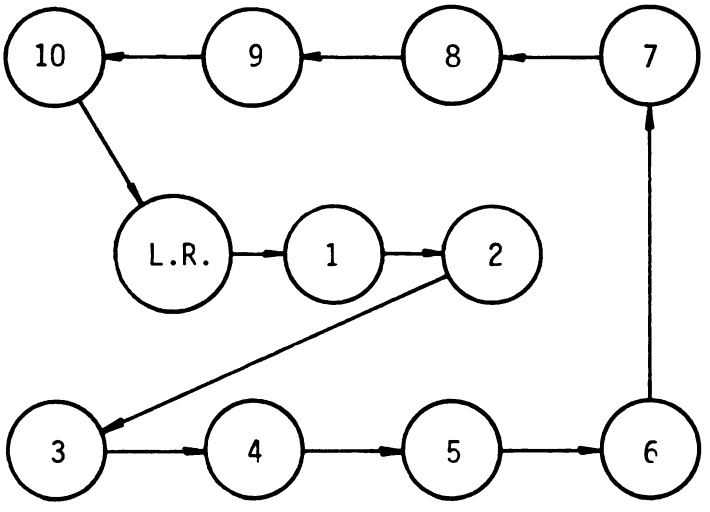


Figure 3-4 Reactor System at Stage I Experiment

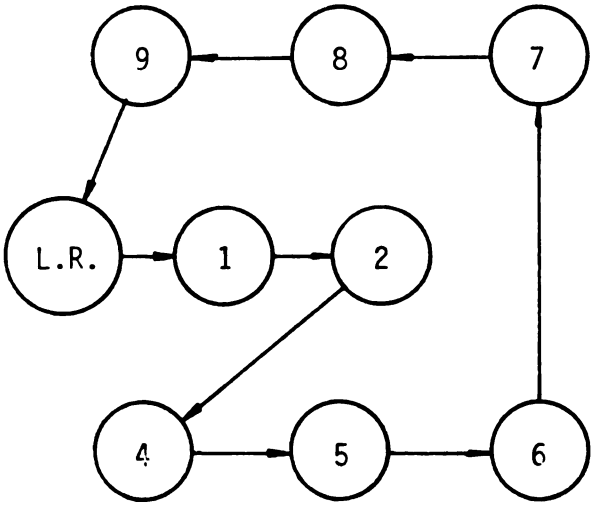


Figure 3-5 Reactor System at Stage II and Stage III Experiments.

The pump flow rate was calibrated from time to time. Unfortunately, however, it was found, after using the first pump for three months, that the flow rate was not constant due to the leaking valves. The actual flow rate in the system was lower because the discharge pressure was higher than when the pump was removed from the system for calibration. A new pump head was then ordered but about two months were lost during the pump failure period. The new pump head provided a fairly constant flow rate and was able to maintain a high discharge pressure. This pump was used continuously until the end of the experimental program.

Liquid samples were first filtered through 0.45 m Millipore filters for soluble COD measurement and for volatile fatty acids. A microscale colorimetric COD method (HACH Co.) was applied for COD measurement (see further discussion in section 3.4.2).

3.2.3 Second Stage Experiment

Six packed reactors and two anaerobic filters were involved in this stage's experiment (Figure 3-5). New substrate was input at the same time at intervals of three days. The substrate input and output, and influent and effluent tubing connection methods were the same as that used in the first stage experiment. The temperature was also kept at $36 \pm 1\ ^{o}C$. Samples were collected two to eight times in a day from the effluent of the packed reactor and anaerobic filters for COD and volatile fatty acid analysis. Gas production and gas composition were measured daily. The fermented straw was dried in the $103^{o}C$ oven and weighed to compare with the initial weight (60 grams). The dried straw

was then stored in a refrigerator for cellulose, hemi-cellulose, and lignin analysis at a later date. A constant voltage transformer and a variable voltage regulator were installed to control the pump flow rate. The pump flow rate was checked daily and it was maintained at a fairly constant flow rate (26 ml/hr). Data were obtained during this stage and a mathematical model for substrate degradation in the packed reactors was developed. The liquid reservoir always stayed in the system serving as the equalization basin for the anaerobic filters and also retaining enough liquid volume in the system for daily sampling Chemical buffering reagents such as NH_4HCO_3 and NaOH were occasionally used when pH adjustment was necessary.

3.2.4 Third Stage Experiment

The liquid reservoir was essential for this research because a certain amount of liquid volume had to be retained in the system for daily sampling. However, in a full scale reactor system, it would be benefical to reduce the overall cost by reducing the system's total volume. This could be partly achieved by excluding the liquid reservoir from the system. Therefore, it is important to examine the performance of anaerobic filters under transient loading, i.e. under the condition when the liquid reservoir is not in the system.

A 20 ml glass U-tube was installed between the metering pump and the effluent line of the newest packed reactor to replace the liquid reservoir. The reason for installing the glass U-tube was that an amount of liquid had to be maintained on the suction side of the pump to prevent a possible "dry pump". Thus, the effluent liquid from the

newest packed reactor with sharply increased COD concentration could be directly introduced into the filters. Because the liquid reservoir was not in the system, the amount of liquid volume in the system was not enough for a long term experiment and the third stage experiment only lasted for two weeks.

3.3 Methods Of Sample Analysis

Methods for sample analysis including pH, COD, volatile fatty acids, gas composition, cellulose, hemicellulose, and lignin will be described in the following sections.

3.3.1 pH Measurement

A Fisher Accumet Model 325 expanded scale research pH meter with Corning semi-micro combination electrode was used for pH measurement. Before the pH as a sample was measured, the electrode was first calibrated against a standard buffer solution with pH = 6.98 at 35 ^{o}C (pH meter and buffer solution were placed in the constant temperature room). The pH value was then read to 0.01 unit immediately after the sample was withdrawn from the system. When the electrode was not in use, it was submerged in a pH = 4.02 buffer solution. The standard buffer solution was changed every week.

3.3.2 Chemical Oxygen Demand

The colorimetric method with micro digestion procedures (Hach Co., Loveland, Colordo) was used for measurement of total soluble COD. This method needs only 2.0 ml sample for digestion. The COD vials along with COD reagent, were first purchased from Hach. Later, the frequently used COD reagent was made by the author according to the recipe provided by Hach as shown bellow:

H_2SO_4	$K_2Cr_2O_7$	$AgSO_4$	$HgSO_4$
2.5 ml	0.0245 g	0.03 g	0.03 g

The chemical quantities listed above are for one COD vial only. A batch of reagent for 200 vials was made at one time. The procedures for COD reagent preparation are as follow:

1. Disolve 6.0 grams of silver sulfate in 500 ml of concentrated sulfuric acid.
2. Weight exactly 4.90 grams of anhydrous potassium dichromate and mix into H_2SO_4 solution prepared in step (1) until completely dissolved.
3. Transfer about 0.03 grams of mercuric sulfate into each cleaned COD vial.
4. Use repipetor to pipet exactly 2.50 ml of solution prepared from step (2) into each COD vial prepared from step (3).
5. Store COD vials with reagent in the refrigerator.

A Bausch & Lomb SP-20 spectrophotometer was first used for the

measurement of percent transmittance by directly inserting the digested COD vial (made by HACH) into SP-20. However, it was found that COD vials did not have uniform circumference and that the inside diameter for different vials was not identical. Therefore, COD measured during the first stage period were not precise and were not useful, these data were not used for analysis but were only used as a reference. The COD measurement technique were improved by using precision test tubes (by Bausch& Lomb Co.) and replacing the SP-20 by a SP-70 spectrophotometer. Good calibration curves of COD vs percent transmittance were obtained, as shown in Figure 3-6, by using the improved method. This new method was then used for stage two and stage three experiments.

Methods of sample preparation for COD measurement and the procedures for micro-digestion, and spectrophotometric measurement are described in the following:

1. Filter 2.0 ml of fresh liquid sample through 0.45 μ m filter paper.
2. Accurately pipet 1.0 ml of filtrate into 10 ml volumetric flask and add distilled water to the mark.
3. Gently shake the volumetric flask to completely mix the diluted filtrate.
4. Turn on the COD reactor (Hach, Model 16500) to preheat to 150 degree C. Remove the cap from a COD vial. Holding the vial at a 45^{o} angle, carefully pipet exactly 2.0 ml of diluted filtrate sample into the vial.
5. Replace the cap and tighten cautiously. Hold at the cap and invert the vial several times to mix the sample with the COD

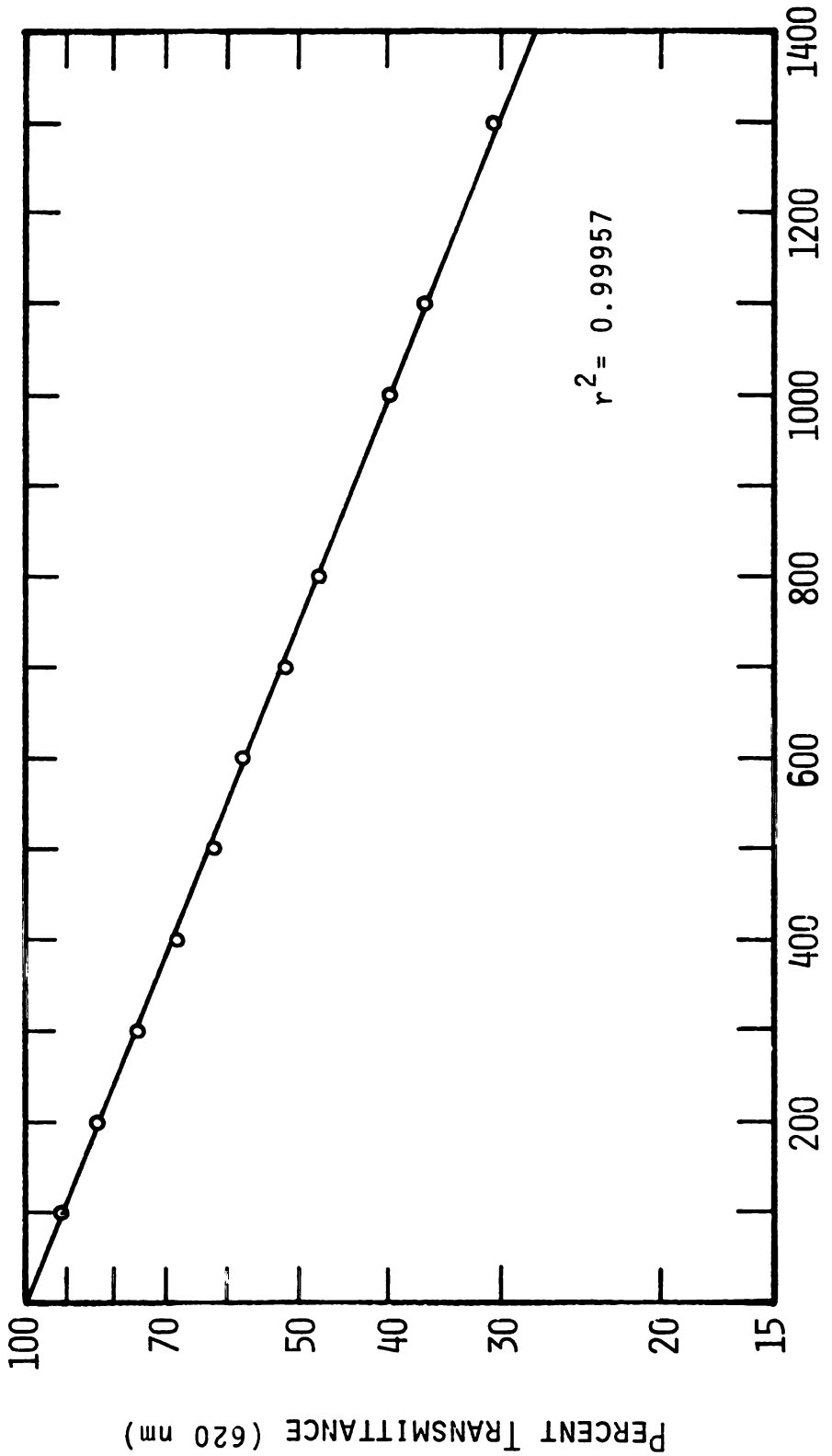


Figure 3-6 Calibration Curve for Colorimetric COD Test

reagent.

6. Place the vial in the preheated COD reactor. Digest the vials for three hours.
7. Remove vials from the COD reactor and allow to cool to room temperature.
8. Turn on the spectrophotometer and set the wavelength to 620 nm and let it warm up for 10 minutes.
9. Carefully transfer digested blank sample from COD vial to special spectrophotometer test tube and adjust zero.
10. Carefully transfer digested sample to special tube and insert the tube into the adapter and replace the cover.
11. Read percent transmittance.
12. Read COD concentration from prepared standard COD curve.

Standard COD solutions were made from potassium acid phthalate (KHP). Fourteen different concentrations (100 to 1400 mg/l) of standard COD solution were carefully made according to the relationship that one milligram of KHP requires 1.175 mg of oxygen for complete oxidation. A standard COD curve was prepared for each bach of COD reagent made; a typical COD standard curve is shown in Figure 3-6.

3.3.3 Individual Volatile Fatty Acids

Low carbon number volatile fatty acids including acetic, propionic, butyric, and valeric acids as well as iso-butryic and iso-valeric acids were measured using a Varian 3700 gas chromatograph equipped with a flame ionization detector. The output was monitored with a Varian CDS-111 data system and a Model 9716 recorder.

Several columns with various packing materials are available for volatile acids analysis. A 193 cm., 0.2 cm. I.D. coiled glass column packed with 10 % SP-1200/1% H_3PO_4 on 80/100 mesh acid washed Chromosorb W (Supelco Inc., Bellefonte, PA) was selected because it was tested to give the fastest and most complete separation over other columns (Ottenstein and Bartley, 1971_a; Ottenstein and Bartley, 1971_b; Robbins et al, 1979). Before use, the column was conditioned overnight at a temperature of 185°C with nitrogen carrier gas flow rate at 40 ml/min. and at a pressure of 60 psig. During operation, the column temperature was maintained isothermally at 125 °C. Hydrogen and air flow rates were adjusted to 30 ml/min and 300 ml/min at pressures of 40 psig, and 60 psig, respectively. The temperature of the flame ionization detector was 250 °C and the temperature for the glass lined injection port was 200 °C. After a number of injections, the glass liner was replaced and cleaned to prevent excess accumulation of nonvolatiles which may cause loss of sample and tailing. The glass column ends were plugged with H_3PO_4 treated glass wool.

The CDS-111 data system was able to automatically analyze the chromatograms. The external standard method was used to quantitatively analyze the individual acid concentrations. A program to control automatic calculation of three different units (mg/l as individual acid, mg/l as acetic acid, and mg/l as equivalent COD) was written and stored in the CDS-111. The equivalent COD conversion factors for each volatile acid was calculated and were given in the Appendix C. These conversion factors were stored in the external standard program. A commercial free fatty acid standard (WSFA-2, by Supelco Co.) was used to calibrate the external standard. The calibration curves for stan-

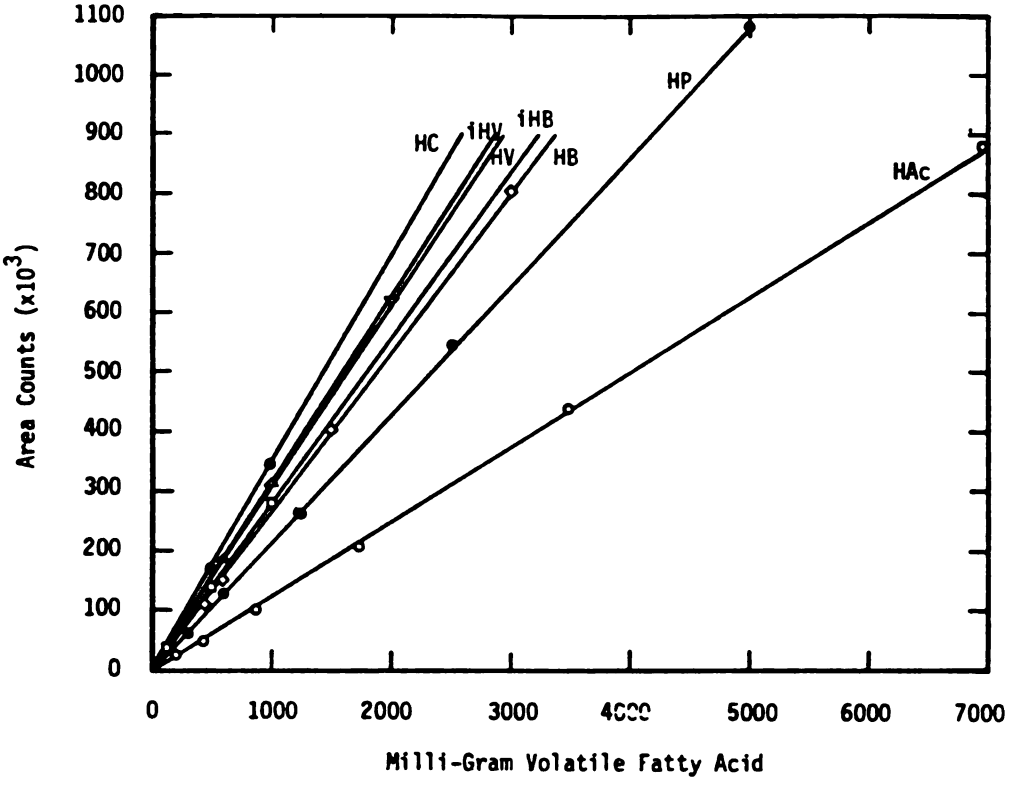
dard volatile fatty acids are shown in Figure 3-7. The measured data for Figure 3-7 are attached in Appendix D-3-1.

Sample preparation was done by using H_3PO_4 acidification and filtration of sample through 0.45 micrometer Millipore filters. A 10 microliter syringe (Hamilton #701) was used for injection of 1.0 microliter samples. Nearly complete base line separation of the volatile acids standard solution and experimental samples were obtained as shown in Figure 3-8 and Figure 3-9. Figure 3-9 is a 77% reduction of the origional plotting. The "10^{-10} x 8" appears in Figure 3-9 represents that the FID detector signal amplification was 10^{-10} amps/mv and the attenuation for the COD-111 reactor was set at "8" position.

3.3.4 Gas Composition

Methane and carbon dioxide were analyzed by using the same gas chromatograph using a thermal conductivity detector instead of the flame ionization detector. The column used was 12 feet long, made of $^1/_8$ inch O.D. stainless steel and packed with 80/100 mesh Porapak Q (Water Associates, Inc., Milford, Mass.). The operating temperatures were: column oven, 65°C; thermal conductivity detector, 150°C; injection port, 150°C. Helium was used as the carrier gas at a flow rate of 30 ml/min and a pressure of 40 psig.

Gas samples were withdrawn from the gas line close to the head of the reactor by using a 0.25 ml pressure lok syringe (Precision Sampling Co., Baton Rouge, Louisiana), and a 0.2 ml sample was injected immediately into the gas chromatograph. Good base line separation was obtained, the major gas detected in the gas samples were nitrogen,



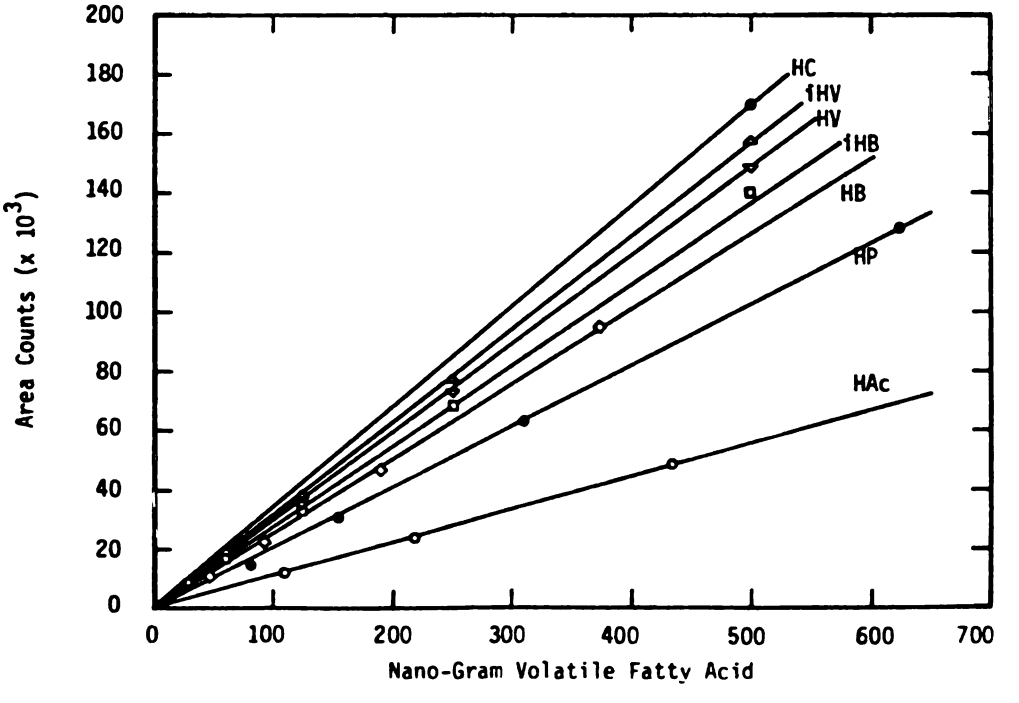


Figure 3-7 Volatile fatty acids standard curves

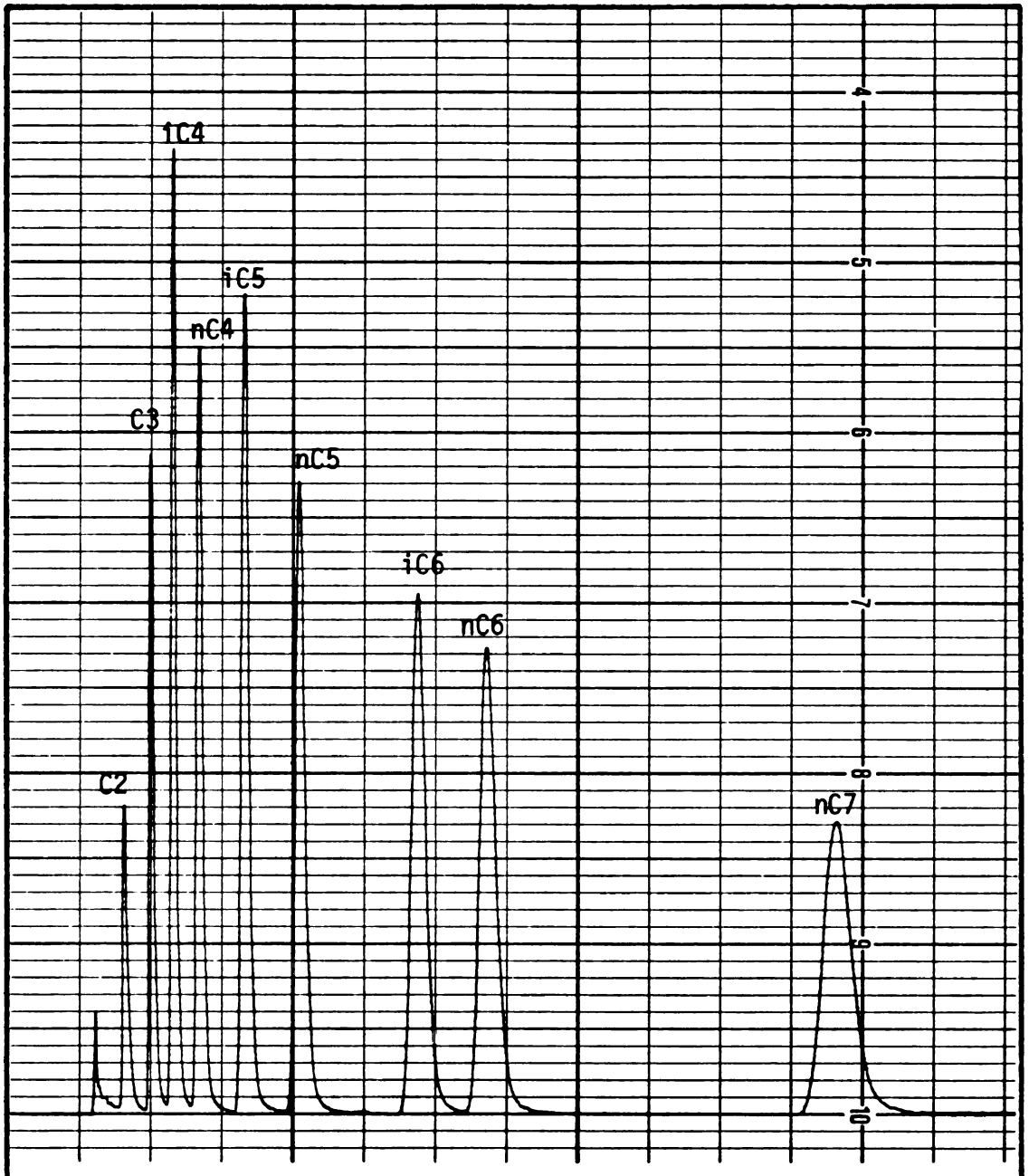


Figure 3-8 Chromatograms of Fatty Acids Standard. 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W AW, 6 ft. x 2mm ID glass colum. Temperature: 125°C, Flow Rate: 40 ml/min., nitrogen, FID detector, 16 x 10^{-10} AFS, Recorder chard speed: 1 cm/min., Sample size: 1.0 μl

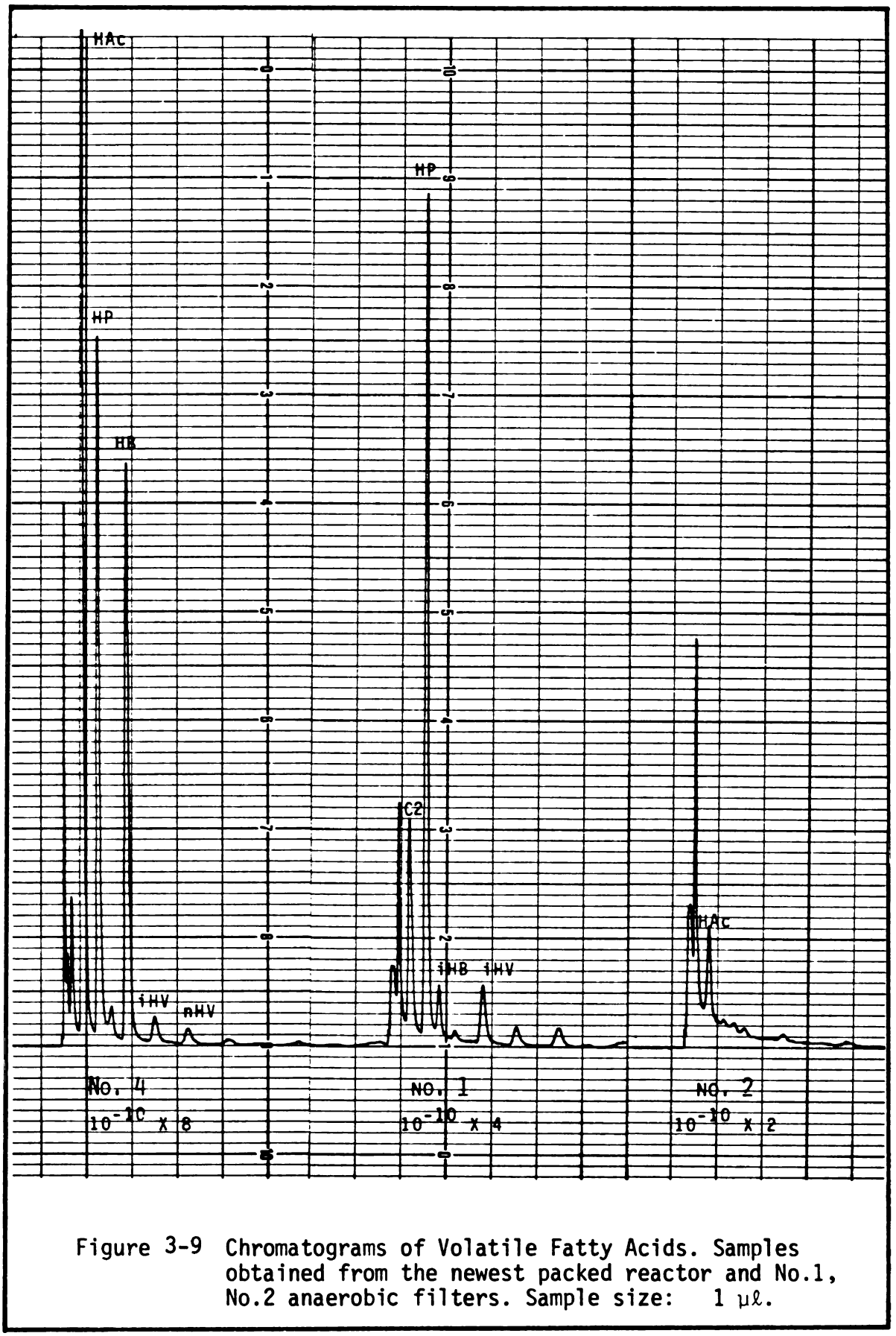


Figure 3-9 Chromatograms of Volatile Fatty Acids. Samples obtained from the newest packed reactor and No.1, No.2 anaerobic filters. Sample size: 1 μℓ.

methane, and carbondioxide, as shown in Figure 3-10.

Standardization of CH_4 and CO_2 were done by injecting various volumes of pure CH_4 and CO_2 gas (Table 3-1 and Figure 3-11). The peak area response for CO_2 was shown higher than that for CH_4. This effect has been corrected by a factor of 1.4 when CH_4 and CO_2 volumes were calculated. The amount of nitrogen detected in the gas samples was assumed to be contributed by the air that was originally existed in the reactor. The percent of nitrogen was normalized when the individual gas volume was calculated

Table 3-1 Response Of Thermal Conductivity Detector To CH_4 And CO_2

Sample Size (ml)	Area Counts		Ratio
	CO_2	CH_4	CO_2/CH_4
0.1	3,039,951	2,217,564	1.371
0.2	6,254,523	4,491,906	1.392
0.3	9,353,356	6,643,089	1.408
0.4	12,425,480	8,754,998	1.419
0.5	15,248,994	10,684,775	1.427
0.6	18,144,232	12,672,222	1.432
0.7	21,004,246	14,533,520	1.445
0.8	23,683,641	16,348,107	1.449
0.9	26,229,318	18,134,616	1.446
1.0	28,861,971	19,866,973	1.453

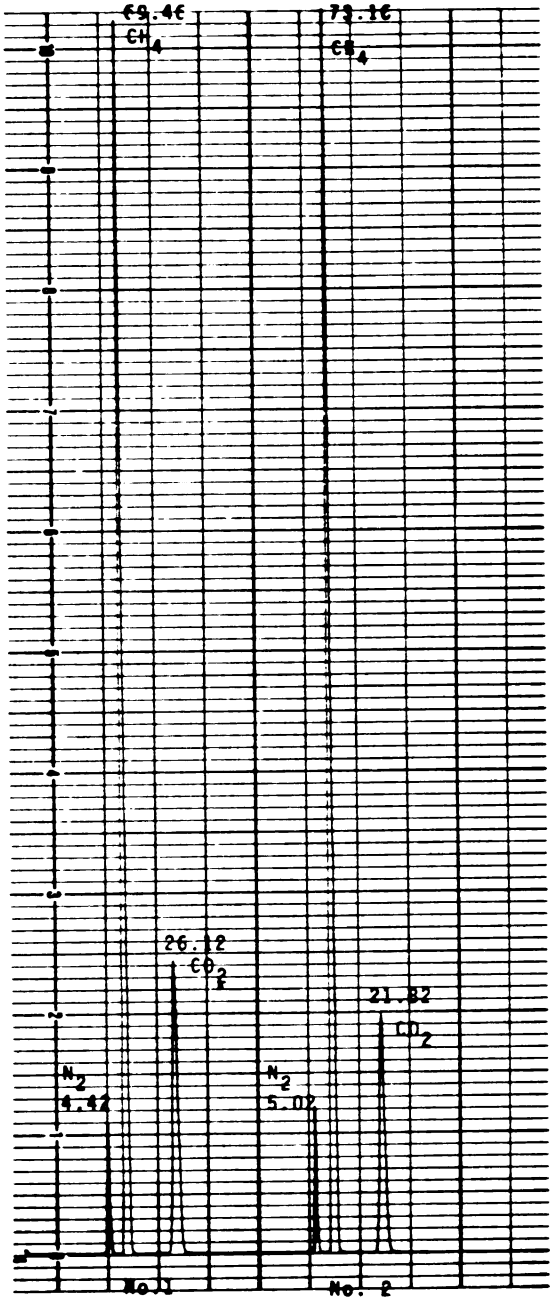


Figure 3-10 Chromatograms of Gases From No.1 And No.2 Anaerobic Filters.
80/100 mesh Porapak Q on 6 ft. x 1/8" SS,
Col. Temp.: 65°C, Flow Rate: 30 ml/min. helium,
Det: thermal conductivity, 128x on range 0.5 mv,
Sample Size: 0.2 ml.

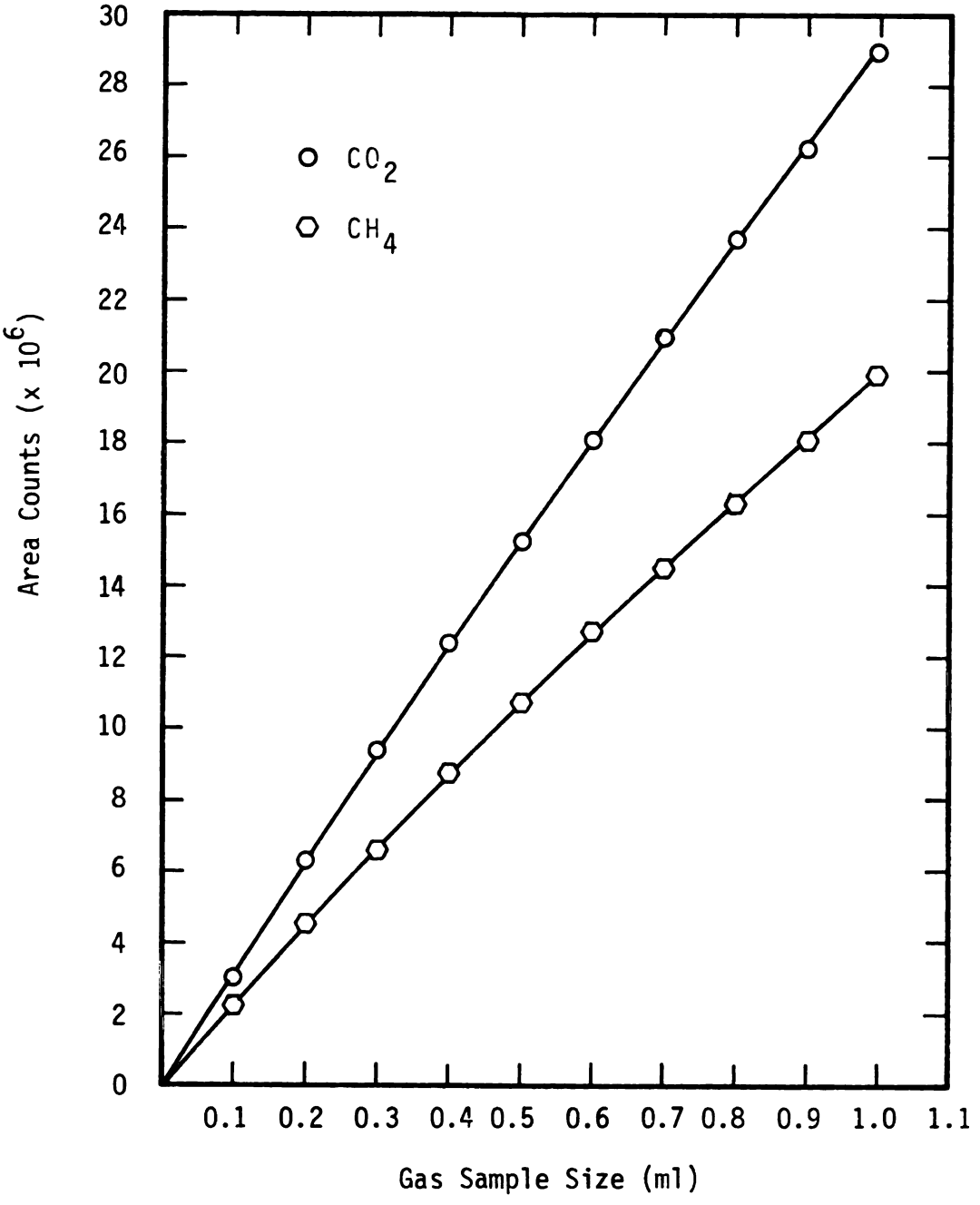


Figure 3-11 Thermal Conductivity Detector Response To Methane And Carbon Dioxide.

3.3.5 Cellulose, Hemicellulose And Lignin

The fermented and fresh straw were analyzed for cellulose, hemicellulose, and lignin according to the methods described by Goering and van Soest (1970). This section will describe the procedures for this method.

The sample was dried and ground in a 2 mm mesh cyclone sample mill (U. D. Co., Boulder, Colorado) to a powdered form to facilitate uniform sampling.

3.3.5.1 Determination Of Cell Wall Constituents (Neutral Detergent Fiber)

This is a fairly rapid method for analyzing the total fiber in vegetable feedstuffs and cellulosic material with low protein content. It divides the dry matter of substrate very near the point that separates the soluble constituents and nutritively available from those that are incompletely available or dependent on a microbial fermentation.

3.3.5.1.1 Procedures

The procedures for neutral detergent fiber (NDF) analysis were as follows:

1. Weigh by difference approximately a 0.5 grams ground sample and place in Berzelius beaker.
2. Add about 75 ml cold (room temperature) neutral-detergent solution into Berzelius beaker.

3. Place the 600 ml Berzelius beaker on the condenser with the heating elements on "high". Heat to boiling in 5 to 10 minutes. Reduce heat as boiling begins, to avoid foaming. Adjust boiling to an even level and reflux for 60 minutes.
4. Place Gooch glass crucibles in 103 degree C oven for 30 minutes. Remove into a dessicator, and after 30 minutes, weigh.
5. Place weighed crucibles on filtering apparatus. Heat crucibles first by pouring boiling water through. Swirl beaker to suspend solids and fill crucible.
6. Turn on the vacuum and use low vacuum at first, increasing vacuum only as more force is needed. Rinse sample into crucible with a minimum of boiling water. Allow sample to drain.
7. Rinse sample with boiling water to clear.
8. Wash with acetone. Drain slowly. Repeat two times. Dry on full vacuum.
9. Dry crucibles over night in forced air oven at 103 degree C. Cool and weigh.
10. Calculate as % oven dry residues recovered.

3.3.5.1.2 Calculation

$$\% \text{ NDF} = 100 \times \frac{(\text{crucible wt.} + \text{cell wall}) - \text{crucible wt.}}{\text{weight of sample}} \qquad (3\text{-}1)$$

3.3.5.2 Determination Of Acid Detergent Fiber

The acid detergent fiber procedures provides a rapid method for ligno-cellulose determination. The difference between the cell wall constituents (NDF) and acid detergent fiber is a rough estimate for hemicellulose.

3.3.5.2.1 Procedures

The procedures used for ADF analysis are as following:

1. Weigh by difference approximately 1.0 gram of ground sample into a Berzelius beaker.
2. Add 100 ml cold (room temperature) acid detergent solution to the beaker. Carefully place the beaker onto a heating element. Put coling flasks on top of the beakers and start a flow of cold water.
3. Heat solution to a rapid boil, then reduce to a slow even boil. Reflux for 60 minutes.
4. Weigh predried Gooch crucible (with asbestos). Place crucibles on the filtration apparatus.
5. Check sample frequently during digestion. If large numbers of sample particles splash on the sides of the beaker, remove the beaker and rinse down the sides with a little acid detergent solution.
6. Begin boiling distilled water for rinsing purposes.
7. After the digestion time, remove the beaker and wash the sample into a corresponding pre-weighted crucible.

8. Allow sample to drain. Rinse the sample in the crucibles with hot water until the filtrate is colorless. Rinse sides of the crucible.
9. Break the mat of fiber sample on the bottom of the crucible with a glass stirring rod and allow sample to dry by vacuum.
10. Repeat wash with acetone until it removes no more color, breaking up all lumps so that the solvent comes into contact with all particles of fiber.
11. Put the crucibles in a 103 degree C oven overnight.
12. Transfer the crucibles to a dessicator and allow 30 minutes cooling time before weighing.
13. Record the weights of crucible and remaining ADF.

3.3.5.2.2 Calculation

$$\% \text{ ADF} = 100 \times \frac{(\text{crucible wt.} + \text{fiber wt.}) - \text{crucible wt.}}{\text{weight of sample}} \qquad (3\text{-}2)$$

$$\% \text{ Hemicellulose} = \% \text{ NDF} - \% \text{ ADF} \qquad (3\text{-}3)$$

3.3.5.3 Determination Of Acid Detergent Lignin

The acid-detergent lignin procedure uses the acid-detergent fiber procedure as a preparatory step. The principle of the procedure is that the acid detergent fiber residue is primarily lignocellulose of which the cellulose is dissolved by a 72% H_2SO_4 solution. The remaining residue consists of lignin and acid insoluble ash.

3.3.5.3.1 Procedures

1. The first step is to prepare acid-detergent fiber as described in section 3.3.5.2.1
2. Place crucible with acid-detergent fiber in pyrex pan. Have one end of the pan 2 cm. higher so that acid will drain away from the crucible.
3. Cover fiber remaining from ADF step with 72% sulfuric acid (10-15 ml).
4. Leave for 3 hours at room temperature. Pour back acid through the sample once per hour.
5. During the last hour of digestion, add new acid to keep the acid level at 2/3 full.
6. Ten minutes before the end of digestion, begin to heat distilled water for rinsing purposees.
7. At the end of 3 hours, place the crucibles on the filtering apparatus and suction off the acid, rinsing immediately with hot water.
8. Continue to rinse with hot water, also rinsing the bottom edge of the crucible free from any residure.
9. Turn off the vacuum. Fill 1/2 full with methyl orange solution. With a glass stirring rod break up the mat and stir with methyl orange. If the solution turns pink, rinse two more times.
10. Rinse two times with hot water and three times with acetone and dry under vaccum.
11. Dry crucible overnight in 103 degree C. Cool and Weigh.

12. Place the crucible in a muffle oven and ignite at 480 degree C for two hours. Cool and weigh.
13. Calculate lignin as % oven dry weight lost on ignition.

3.3.5.3.2 Calculation

$$\% \text{ Cellulose} = \% \text{ ADF} - \frac{\text{wt. from (11)} - \text{crucible wt.}}{\text{sample weight}} \times 100 \quad (3\text{-}4)$$

$$\% \text{ Lignin} = 100 \times \frac{\text{wt. from (11)} - \text{wt. from (12)}}{\text{sample weight}} \quad (3\text{-}5)$$

3.3.5.4 Reagents For Fiber Analysis

Reagents required for the fiber analysis are given as follows:

(A) Neutral Detergent Solution:

Add 30 grams sodium lauryl sulfate, 18.61 grams disodium dihydrogen ethylenediaminetetracetate dihydrate, 6.81 grams sodium borate decahydrate reagent, and 10 ml 2-ethoxy-ethanol (ethylene glycol), per liter of distilled water and agitate to dissolve. Check pH to be within range of 6.9 to 7.0 and adjust as necessary.

(b) Acetone, reagent grade.

(C) Acid Detergent Solution (H-Brobide Solution)

Add the amount of concentrated sulfuric acid indicaed in Table 3-2 to distilled water. Then add the appropriate amount of H-Bromide (hexadecyltrimethyl ammonium bromide). Stir until dissolved and make up to volume.

Table 3-2 H-Bromide Solution

Total Volume (liter)	H_2SO_4 (ml)	H-Bromide (gram)
1	27.75	20

CHAPTER FOUR

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental results obtained from this study will be presented as five major subjects: (1) the performance of the high solids packed reactors; (2) the role of the liquid equilization reservoir; (3) the performance of the anaerobic filters; (4) the extent of substrate degradation; and (5) the response of the anaerobic filters to transient substrate loading.

4.1 Performance of the High Solids Packed Reactors

The performance of the high solids packed reactors will be reported in terms of three parameters: (1) total soluble COD production, (2) volatile fatty acid production, and (3) gas production from the packed reactors. Discussion of items (1) and (2) will use the data obtained from Stage II experiment, while gas production data obtained from Stage I will be used to discuss item (3). The operational parameters for Stage II were as follows:

1. Number of packed reactor involved in the system (see Figure 3-7): 6
2. Substrate input interval : 3 days
3. Substrate solids retention time : 18 days
4. Average liquid flow rate : 26 ml/hour
5. Average hydraulic retention time per reactor: 17.3 hours

The operating parameters for Stage I were the same as those for Stage II, except for the following: (1) eight reactors were involved in the system; (2) substrate input interval was 5 days; and (3) straw retention time was 40 days.

4.1.1 Total Soluble COD Production

The entire course of Stage II was fairly stable. The term "stable" means that the pump flow rate was fairly constant and the effluent COD concentration from the anaerobic filters was maintained at stable and low levels. Total soluble COD of the effluent from packed reactors was measured at a frequency from two hours for the newest reactor to two days for old reactors over a period of 30 days in Stage II. Experimental results of effluent total soluble COD for packed reactors are shown in Figure 4-1 and in Appendx D-4-1. Part of the earlier experimental results were not included due to a minor mechanical failure. As shown in Figure 4-1, three curves representing the change of total soluble COD concentration versus time for three packed reactors all demonstrated the same characteristics. The production of COD by the packed reactors was most active within 36 hours after the new substrate was introduced. The concentration reached the highest value between 12 to 14 hours, and then rapidly decreased until approximately hour 72. Beyond hour 72, the COD concentration decreased slowly at a rate of about 30 mg/l/day. The initial low soluble COD concentrations as exhibited in the Figure 4-1 for each packed reactor were due to dilution with water which was added in the new reactor. The least-squares polynomial curve fitting technique was applied to

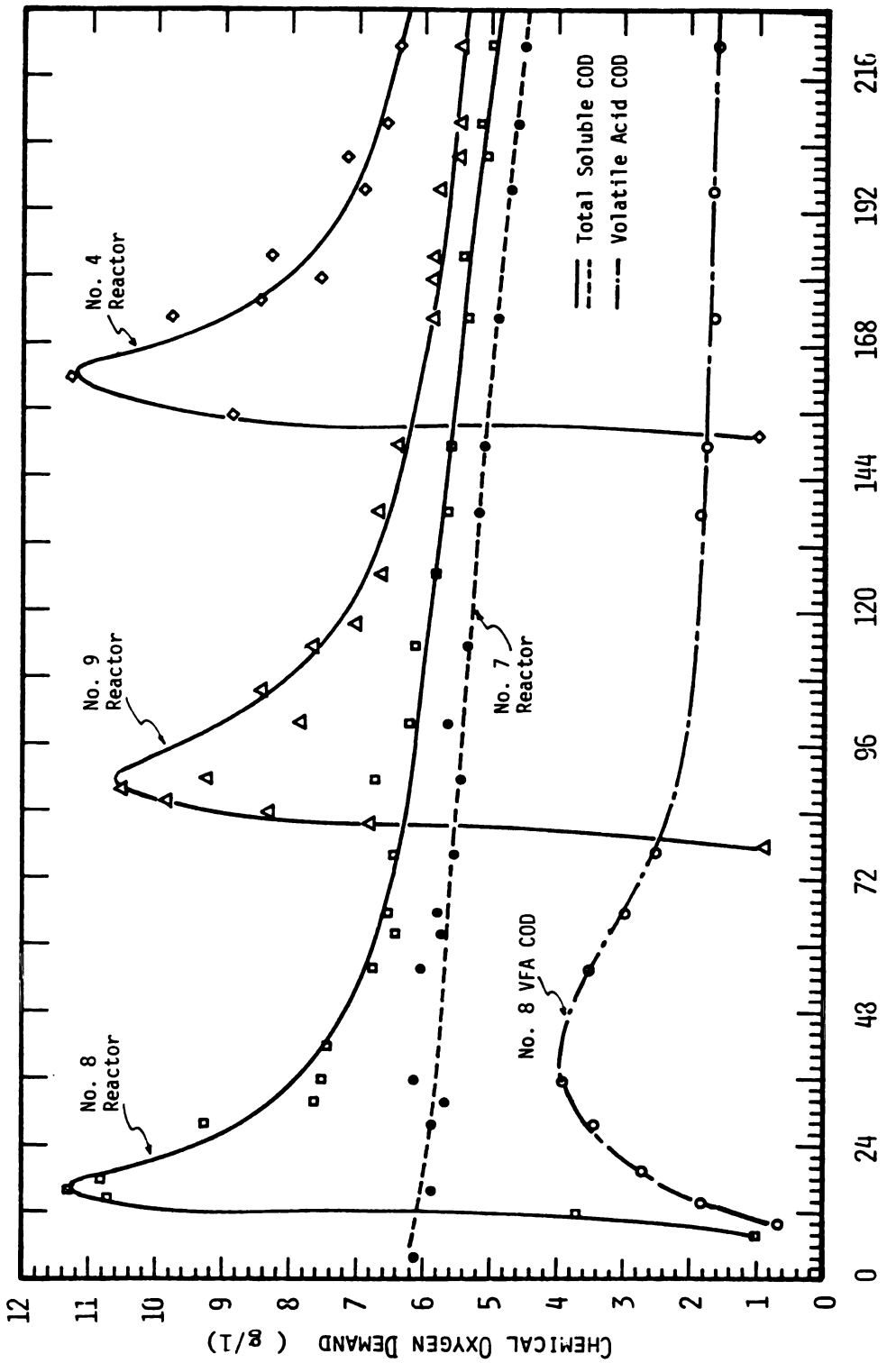


FIGURE 4-1 TOTAL SOLUBLE COD AND VOLATILE ACID COD OF EFFLUENT LIQUID FROM PACKED REACTORS

approximate the soluble COD concentration for the points after the peak concentration obtained from four packed reactors. A FORTRAN program CUVFIT was written to handle the matrix manipulation and curve fitting procedures. This computer program was executed both on the CDC CYBER/750 mainframe computer and a DEC PDP-11/23 minicomputer. Program CUVFIT can be found in Appendix E-1. Figure 4-2 shows the experimental data points from four packed reactors as well as the approximation curve calculated from the 7-degree, least-squares polynomial equation generated from CUVFIT as shown below:

$$y = 1.4301 \times 10^{4} - 3.7852 \times 10^{2} X + 7.2724 X^{2}$$
$$- 7.5897 \times 10^{-2} X^{3} + 4.5712 \times 10^{-4} X^{4} - 1.5929 \times 10^{-6} X^{5}$$
$$+ 2.9847 \times 10^{-9} X^{6} - 2.3287 \times 10^{-12} X^{7}$$

in which y = soluble COD, (mg/l).

X = time, (hr).

If the experimental data are plotted on a log-log scale, as shown in Figure 4-3, it can be seen that COD concentration in the packed reactor is decreasing logarithmically with respect to time after the peak occurs.

The characteristic of the sharply increasing COD concentration during the first 12 hours resulted from rapid leaching of soluble and colloidal substances associated with the wheat straw. The subsequent slow COD production was contributed by microbial degradation of the straw. This leaching from the straw was verified by pumping distilled

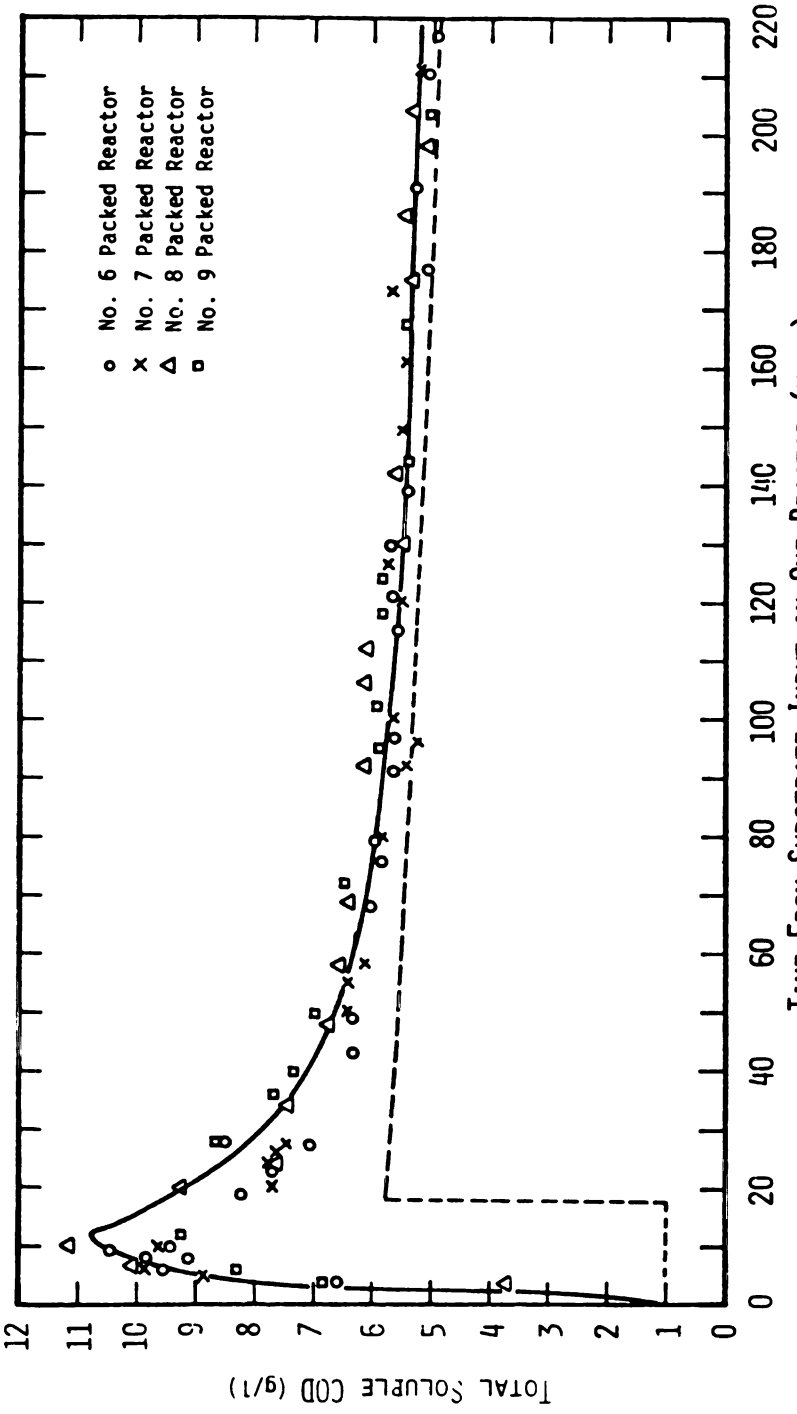


Figure 4-2 Total soluble COD of effluent liquid from packed reactors.

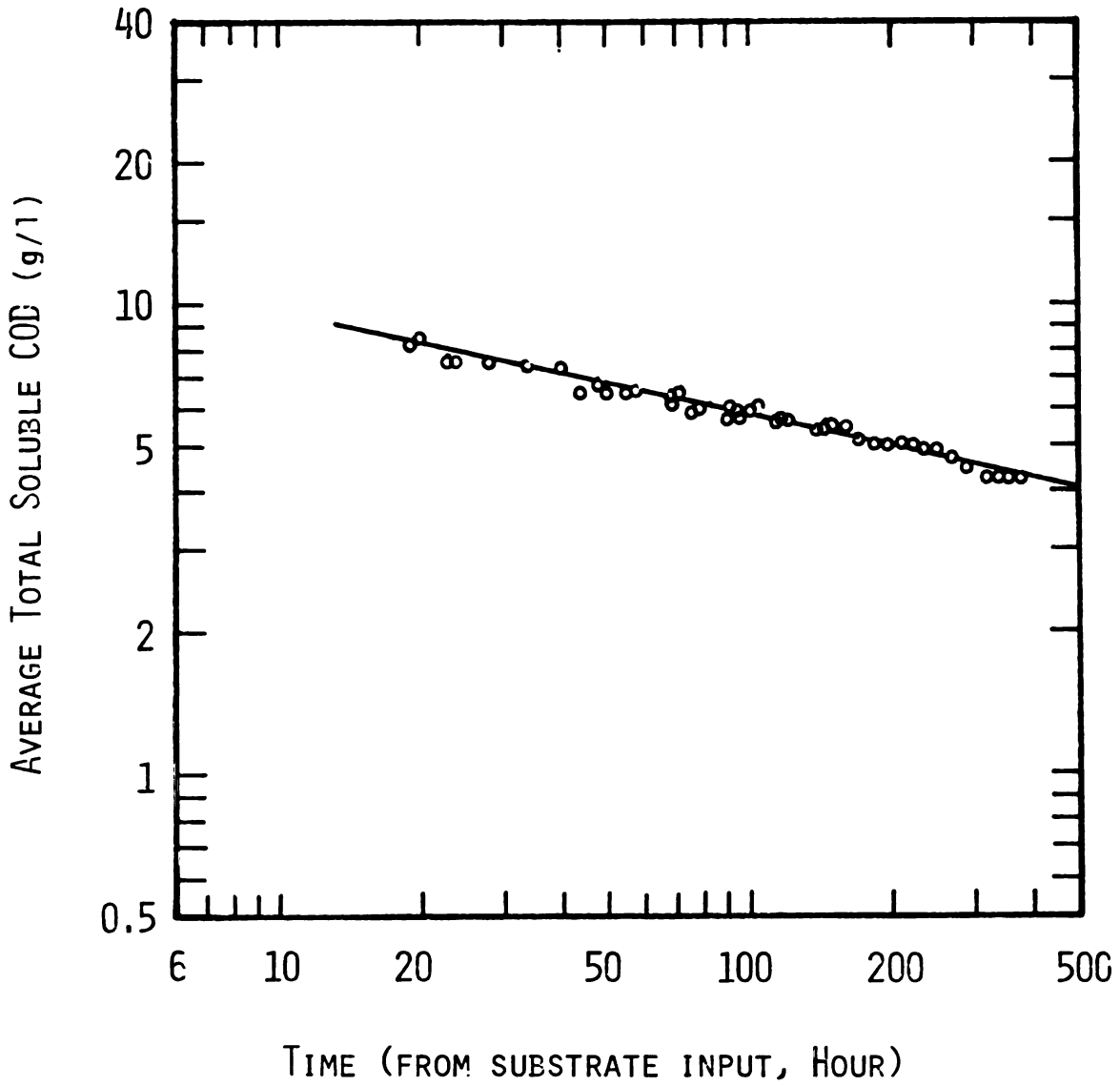


Figure 4-3 Average total soluble COD of effluent from packed reactors after peak.

water into a single packed reactor which was filled with 60 grams of fresh straw at room temperature. The effluent soluble COD was measured for a period of 50 hours, and the results are plotted in Figure 4-4. The pump flow rate during this test was 0.86 l/day (36.0 ml/hr) and the hydraulic retention time in the single reactor was 12.5 hours. The same curve shape as in Figure 4-1 was observed in Figure 4-4 indicating that the sharp increase of COD concentration in the early hours was not contributed by microbial degradation because the influent distilled water did not contain enough bacteria to degrade the cellulosic substrate in such a short period of time.

The mass of soluble COD produced per unit weight of straw for the flow-through leaching test and for a packed reactor can be obtained by integrating the area under the curve of Figure 4-4 and the area between the two curves in Figure 4-2, using the mathematical procedures shown in Appendix B. The amount of soluble COD produced was 60.4 mg COD/gm straw for the flow-through leaching test and 93.1 mg COD/gm straw for the packed reactor.

Another straw leaching test was performed by placing 50 grams of fresh straw into 1500 ml distilled water. Total soluble COD was measured until the maximum concentration (2,880 mg/l) was reached (Figure 4-5). The mass of COD produced per gram of straw of the batch straw leaching test was 86.4 mg COD/gram straw.

Comparing the COD production by leaching and in the packed reactor, it is found that the leachable COD in the first 66 hours should fall between a maximum of 93% (86.4/93.1) and a minimum of 65% (60.4/93.1). Because the flow-through leaching test has the similar hydraulic conditions as that of the packed reactors, it is reasonable

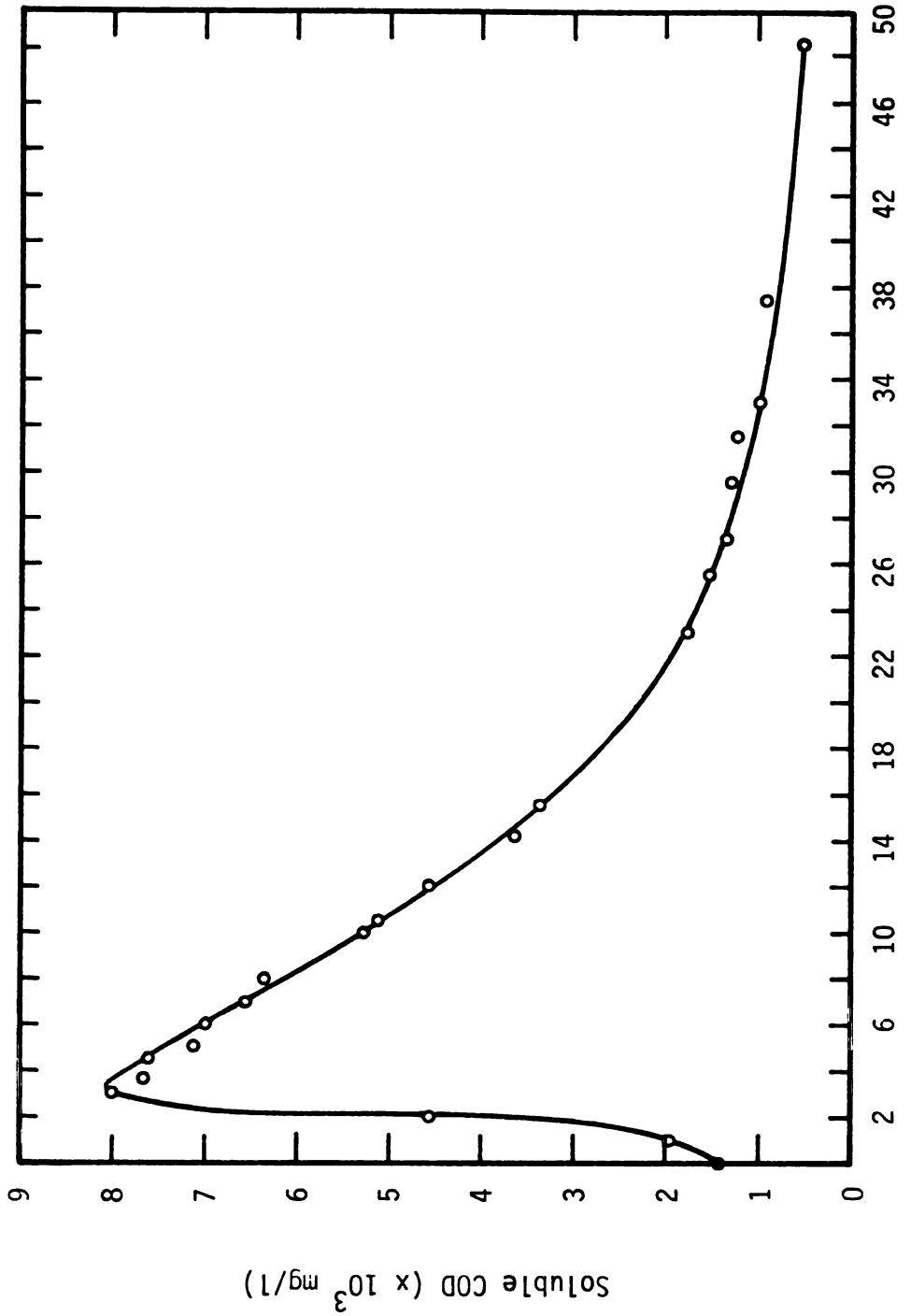


Figure 4-4 Straw leaching test in a packed reactor.

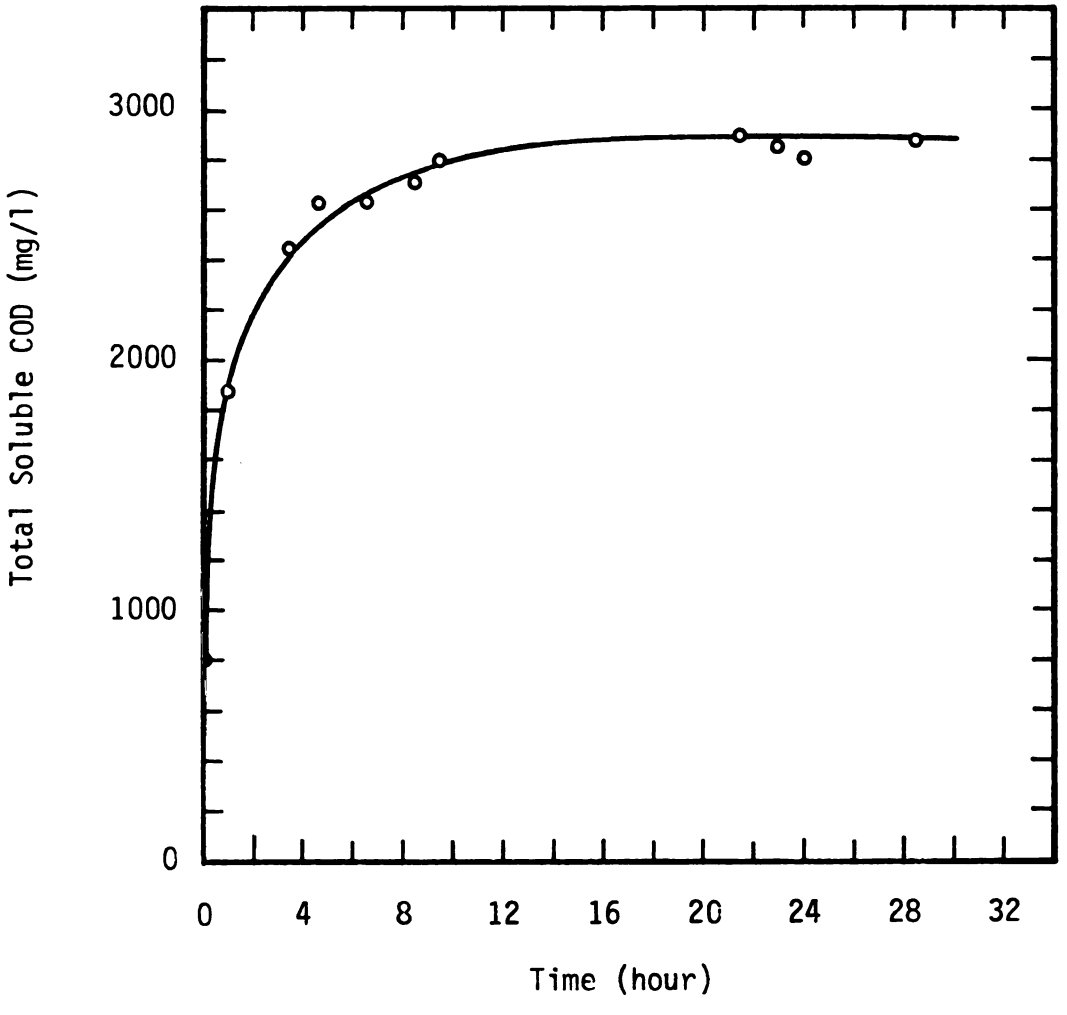


Figure 4-5 Straw Leaching Test in a Bach Reactor.

to assume that 65% of the total soluble COD produced by a packed reactor in the first 66 hours was contributed by leaching. The maximum value of 93% was obtained from a batch reactor in which the straw was completely submerged in the liquid. While in the packed reactor, the solid substrate was not totally submerged in the up-flowing liquid due to the build up of gas spaces within the reactor.

To investigate the amount of soluble COD produced in a single packed reactor, the effluent total soluble COD concentration from one packed reactor at a specific time was reduced by the COD in the effluent of the immediately preceeding packed reactor at one hydraulic retention time (18 hours) earlier. Figure 4-6 shows the COD production computed from the difference between the solid curve and the dashed curve in Figure 4-2.

The effluent COD concentration is a function of several parameters such as: (1) liquid flow rate (or hydraulic retention time), (2) the amount of straw introduced into the reactor, (3) the degree of substrate pretreatment, (4) substrate solids retention time in the system, and (5) proper environment for normal microbial activity. Therefore, the effluent COD characteristics as shown in Figure 4-2 are valid only for the case when the operating conditions are as described in Section 4-1. The selection of three days straw input interval in Stage II and five days for Stage I were only suitable for the above operating conditions.

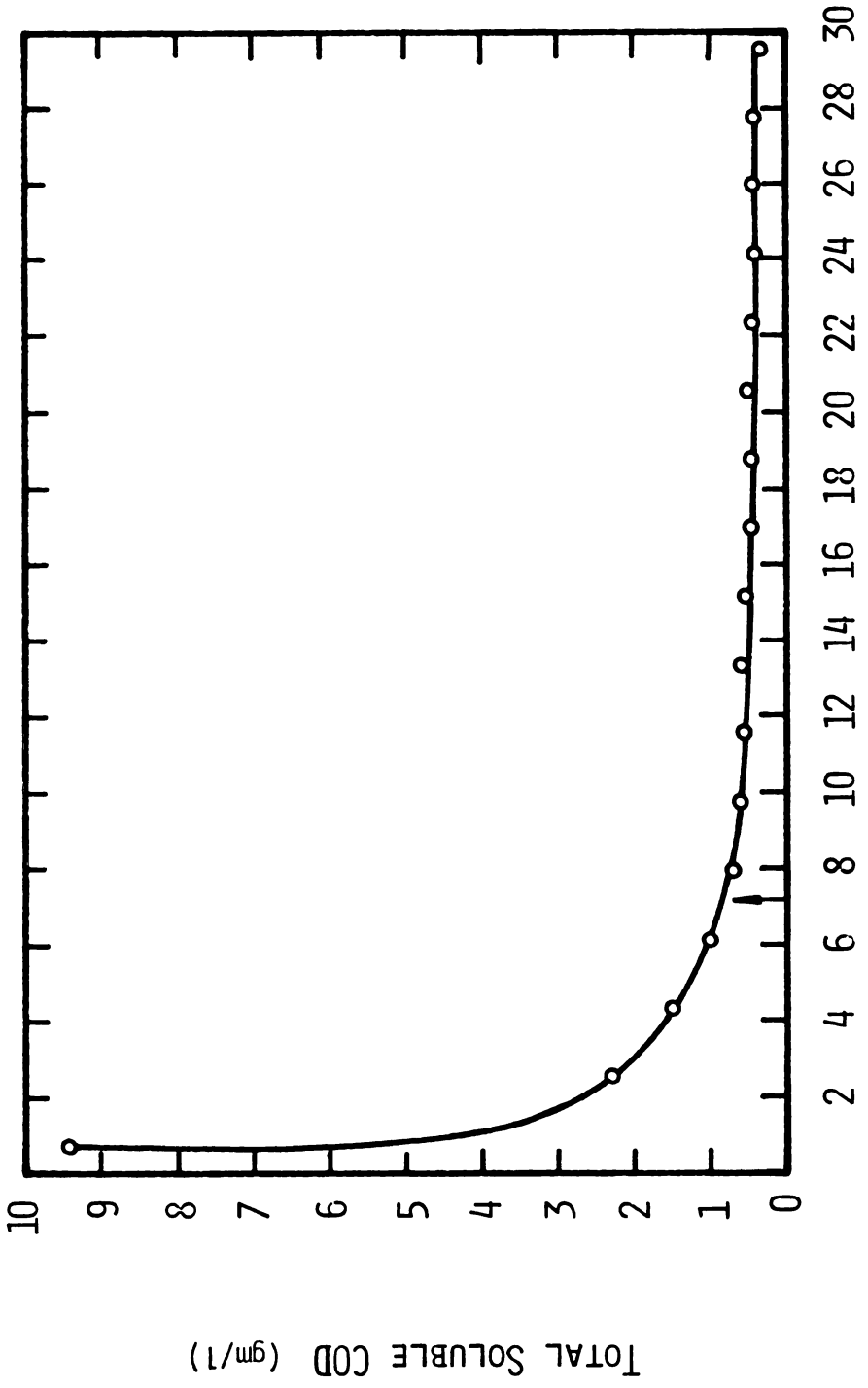


Figure 4-6 Total soluble COD production from packed reactor.

4.1.2 Volatile Fatty Acids Production

The effluent volatile fatty acid concentration was measured continuously at intervals of 6 to 24 hours for two consecutive packed reactors in Stage II over a period of 10 days. Figure 4-7 shows the concentrations of the three major volatile acids found in the samples. The percentage of the the total VFA COD contributed by three major acids can be computed by integrating the curves (Simpson's Rule was used) in Figure 4-7 and multiplying the areas by the flows to obtain the numbers shown in Table 4-1.

Table 4-1 Individual Volatile Fatty Acid Concentration in the Packed Reactor

	Acetic	Propionic	Butyric	Total
Mass	747	418	198	1363
Percent	55	31	14	

It can be seen that acetic acid was the predominant acid being produced in the packed reactors, accounting for more than half of the total VFA COD, followed by propionic acid and butyric acid. Although other acids of higher carbon number were also detected, their concentrations were very low and consquently were not shown in Figure 4-7 but are included in Appendix D-4-2.

As Figure 4-7 shows, the peak concentrations of the three major volatile acids in the packed reactor did not occur at the same time. The butyric acid concentration reached its highest value of 340 mg/l

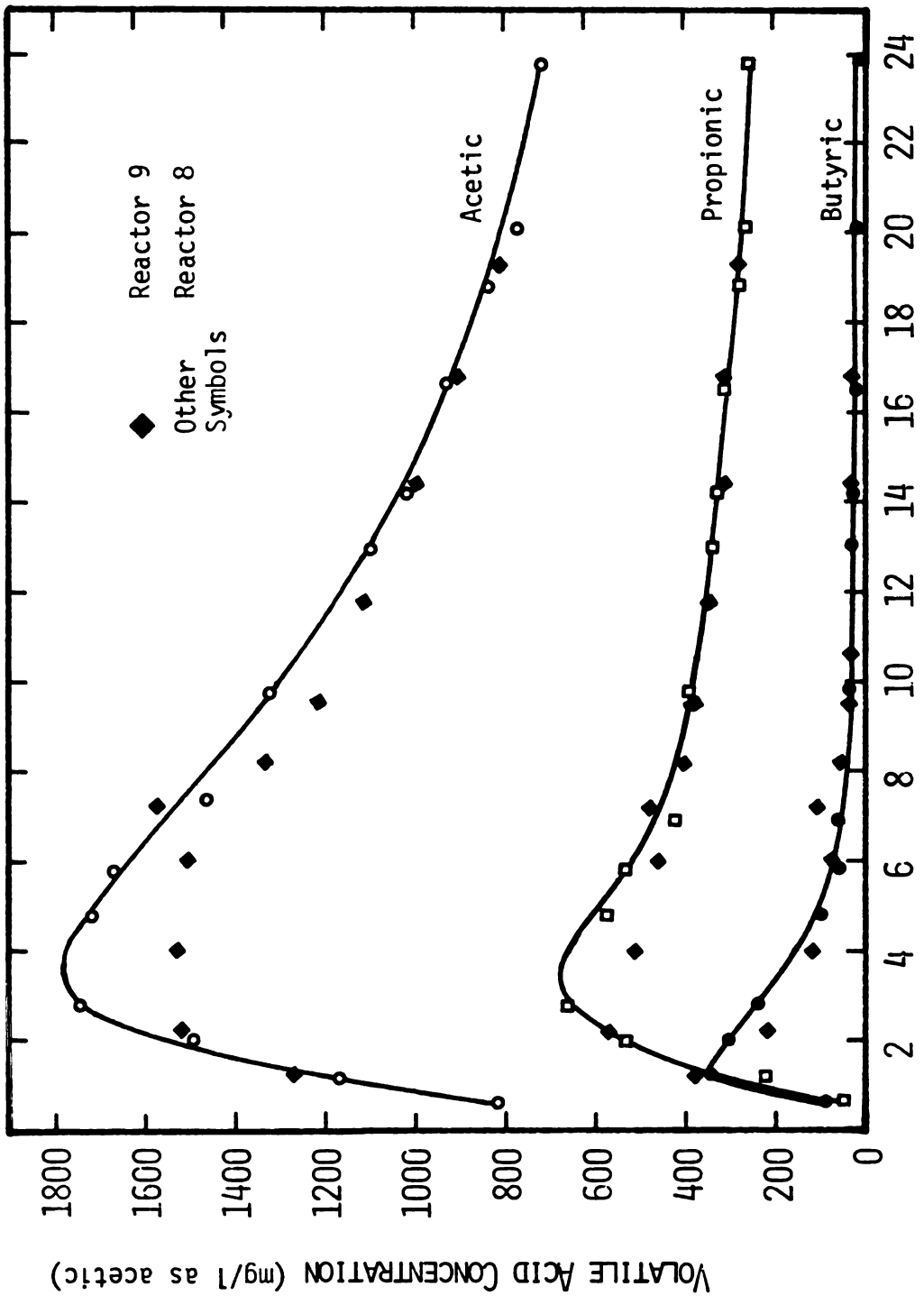


Figure 4-7 Individual volatile acid concentration in one packed reactor

(as acetic acid) at about 12 hours after new substrate was introduced while propionic acid and acetic acids peaked about 18 hours later at 660 mg/l and 1800 mg/l as acetic acid, respectively. About 60 hours after substrate input, the butyric acid concentration had decreased to less than 50 mg/l (as acetic acid) and then gradually declined to as low as 6 mg/l. This suggests that the butyric acid produced at the earlier time has been washed out or been converted partly to acetic and propionic acids. Also the concentration of iso-butyric acid (Appendix D-4-2) at 6.5 hours was higher than the concentrations of propionic and butyric acid. By 20 hours the iso-butyric acid concentration had dropped to less than 20 mg/l. This suggests that the straw surface contains small amounts of substances, possibly a certain kind of amino acid, for instance, valine, which is readily converted to iso-butyric acid (Barker, 1961).

The total of the volatile fatty acids, expressed as COD, is presented in Appendix D-4-2 and plotted in Figure 4-1. Comparing the VFA COD curve in Figure 4-1 with the total soluble COD curve shows that VFA were produced at a slower rate than total soluble COD. The VFA COD reaches a peak concentration approximately 24 hours after the peak for total soluble COD. These characteristics are further evidence that the initial soluble COD production in the packed reactor was not contributed by microbial activity but rather by leaching.

4.1.3 Gas Production From The Packed Reactor

The main function of the packed reactors was the production of soluble COD from solid substrate, not the production of methane gas. However, a small quantity of methane was produced daily from each packed reactor. Figure 4-8 shows the composition of methane and carbon dioxide of several packed reactors, and Figure 4-9 shows the cumulative gas production from a single packed reactor in Stage I. As shown in Figure 4-9, more carbon dioxide than methane was produced from the packed reactor. Figure 4-8 shows that the methane content gradually increased from a low level, about 15%, at the beginning when the packed reactor was installed. By Day 8, the methane content had increased to a value higher than the percentage of carbon dioxide where it remained until Day 31. At Day 31 the packed reactor had moved to the position as the second oldest reactor in the system, and at Day 36, it was the oldest reactor in the system. Examining Figures 4-8 and 4-9 reveals that the methane content is decreasing and the total gas production rate is increasing after Day 31. These phenomena can be explained by two observations. First the pH value in the influent liquid, which is the effluent of Filter No.2, was higher than the pH inside the last two packed reactors; the drop of pH resulted in more carbon dioxide escaping from the liquid phase. Secondly, a larger bacterial population may have developed in the last two packed reactors than in the other reactors due to the carrying over of microorganisms from Filter No.2; higher microbial population in the two older reactor resulted in the higher gas production rate. The change of pH versus time of a single packed reactor from the first day of installation until the last day

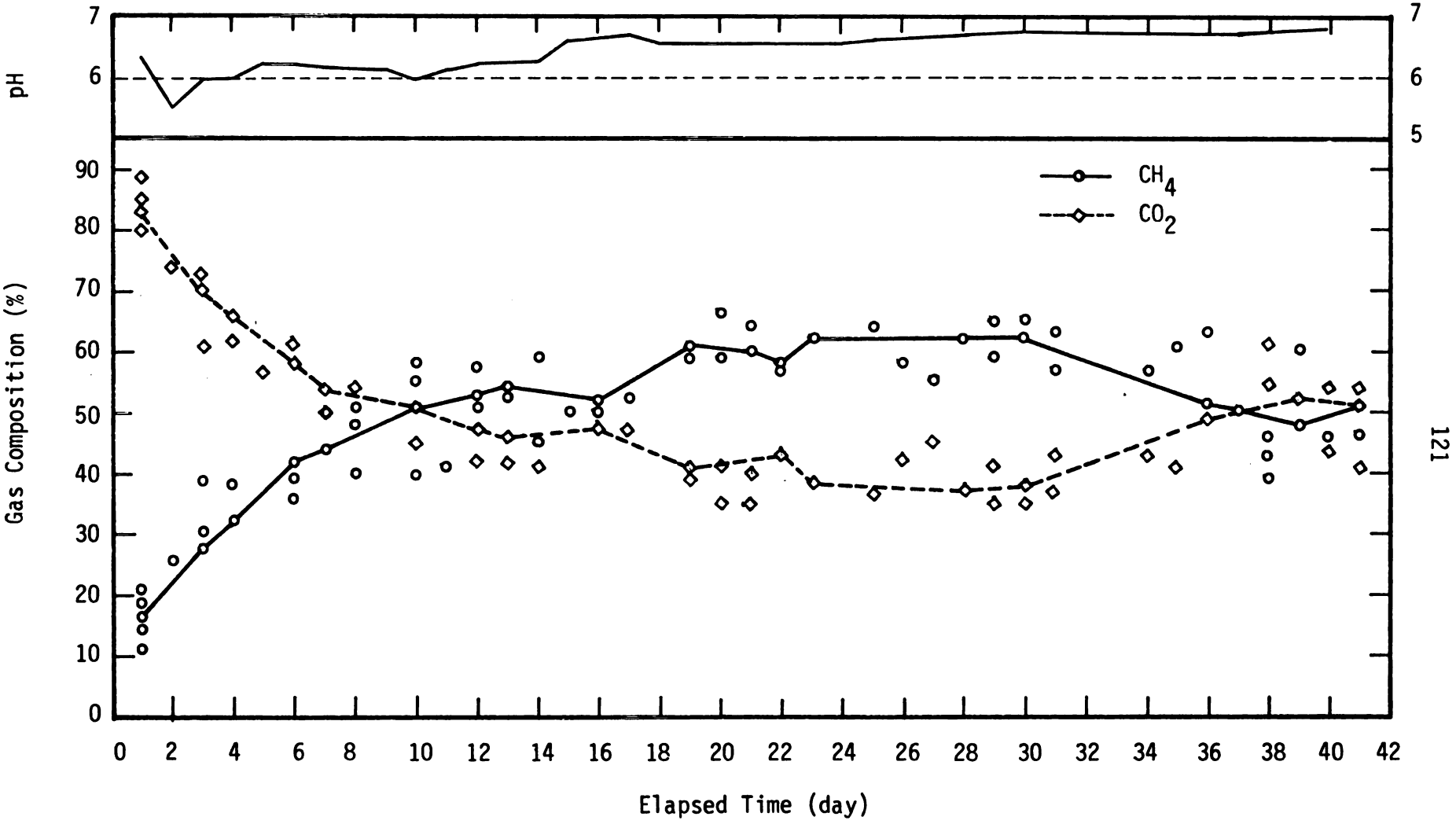


Figure 4-8 Gas Composition in Stage I Packed Reactors

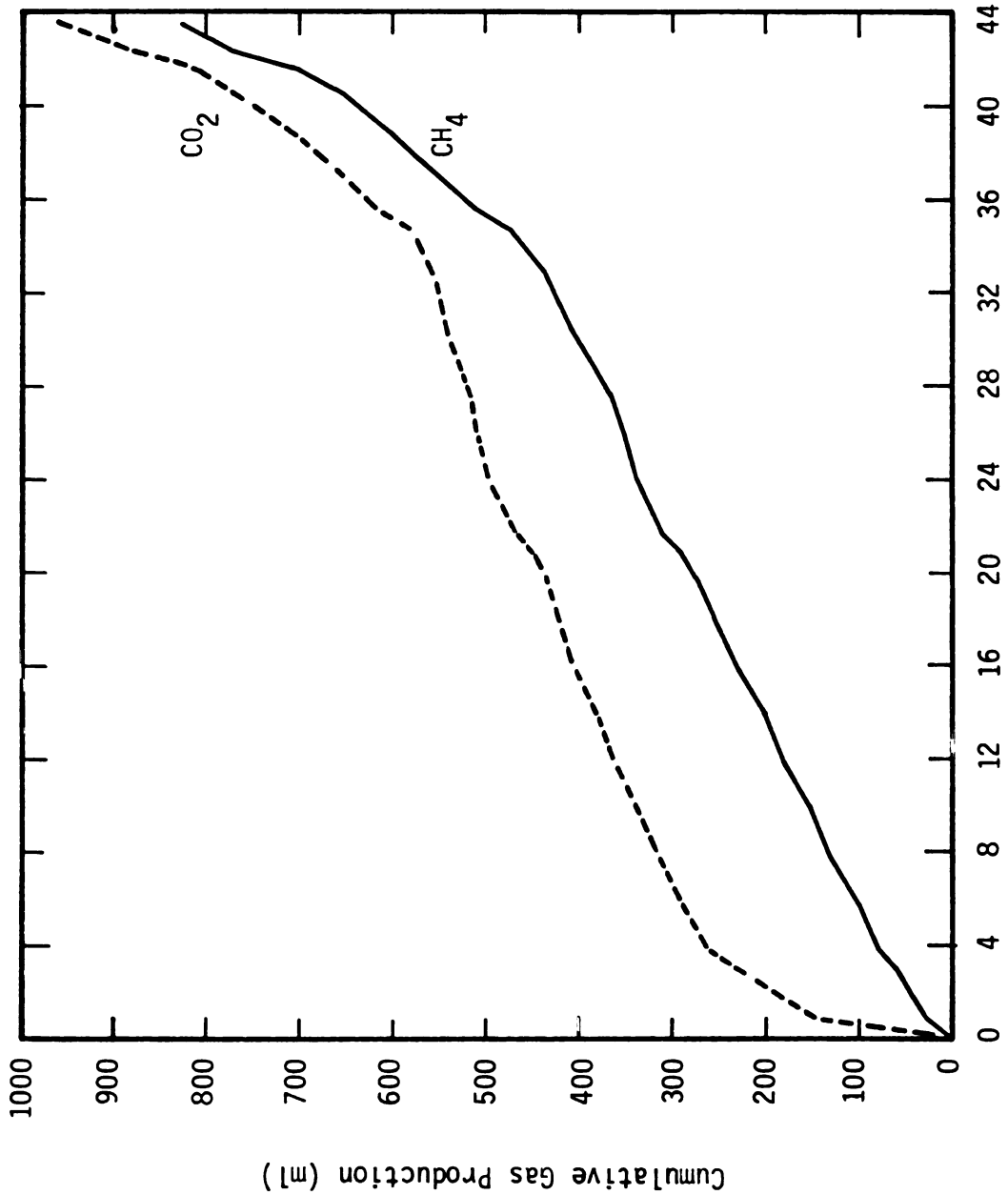


Figure 4-9 Cumulative Gas Production From a Single Packed Reactor.

when it was removed was also plotted in Figure 4-8. The pH of a new packed reactor was as low as 5.5 and then gradually increased to about 6.8 on the last day. The initial low pH value was due to the high concentration of volatile fatty acids produced from carbohydrate fermentation.

Hydrogen was also detected in first day after a new packed reactor was installed. This finding agreed with the theoretical concepts discussed in Section 2.1.1 that hydrogen would be produced during the first stage (substrate hydrolysis and organic acids formation) of anaerobic fermentation. Methane produced in this period mostly came from the reduction of carbon dioxide by using hydrogen (see section 2.1.4.2).

The daily production of methane and carbon dioxide was calculated based on the normalized percentages for CH_4 and CO_2 only. Since the nitrogen gas was assumed to result from the air originally present in the reactor. The total amount of methane producted from the packed reactors was about 15 % of the total methane produced from the entire system. Table 4-2 shows the volume of methane produced from anaerobic filters and packed reactors in two long term periods in Stage I.

Table 4-2 Comparison of Methane Production in Packed Reactors and in The Entire System (Stage I)

Dates 1982 (1)	Days (2)	Methane Production, (ml) Packed Reactors (3)	Filter 1+2 (4)	Total System (5)	Percentage Packed Reactors (6)	Filter 1+2 (7)
1/21 - 3/10	48	10676	58900	69576	15.3	84.7
3/29 - 5/15	47	8269	46684	54917	15.1	84.9

The above values were calaulated based on the data obtained from the extented experimental period. Therefore it is reasonable to assume that the packed reactors can produce about 15 % of the total methane that is produced from the entire system. This information will be used for data analysis in the latter section. However, the information given above is not suitable for use to estimate the daily methane production from a specific packed reactor; for instance, if the daily methane production from the filters is 1000 ml it is not always true that the packed reactors will produce 150 ml of methane in the same day.

4.2 Role of the Liquid Equalization Reservoir

The average volume of liquid stored in the liquid reservoir during Stage II was about 1.0 liter for a detention time of about 1.6 days. Every 3 to 6 days, a measured amount of distilled water (50 - 100 ml) was added to the reservoir to maintain approximately the same liquid volume. As mentioned before, pH control chemicals such as NH_4HPO_3,

and NaOH were also added to the liquid reservoir when pH adjustment was necessary. Liquid samples taken from the reservoir were analyzed for total soluble COD and volatile fatty acid concentrations.

The change of individual volatile acid concentrations in the reservoir with respect to time are presented in Figure 4-10, and the total soluble COD and total VFA COD versus time are plotted in Figure 4-11. By comparing Figure 4-7 with Figure 4-11, it was found that the variation of VFA COD inside the liquid reservoir was not as significant as in the packed reactors. Although Figure 4-1 and Figure 4-11 are not plotted with the same scale and therefore are not directly comparable, it still can be seen that the total soluble COD has been equalized by the reservoir to a large extent. The difference between the highest and the lowest COD concentration, was about 1300 mg/l or about 19 % of the average soluble COD concentration. The maximum difference of total soluble COD concentration for the influent and the effluent of a packed reactor was greater than 5000 mg/l as can be seen from Figure 4-1.

It is apparent that the liquid reservoir played an important role as an equalization basin that reduced the variability of COD before it was brought into the anaerobic filters, preventing possible damage to the anaerobic filters due to shock loading.

The hydraulic retention time in the liquid reservoir during Stage II was approximately 1.67 days which is not long enough for acid utilizing methane bacteria to grow. Although methane gas was produced in the liquid reservoir, the amount of CH_4 produced, 450 ml in 40 days period, was not significant when compared with the gas produced from the anaerobic filters. Another important function of the liquid reservoir was that it stored enough liquid volume for the necessary

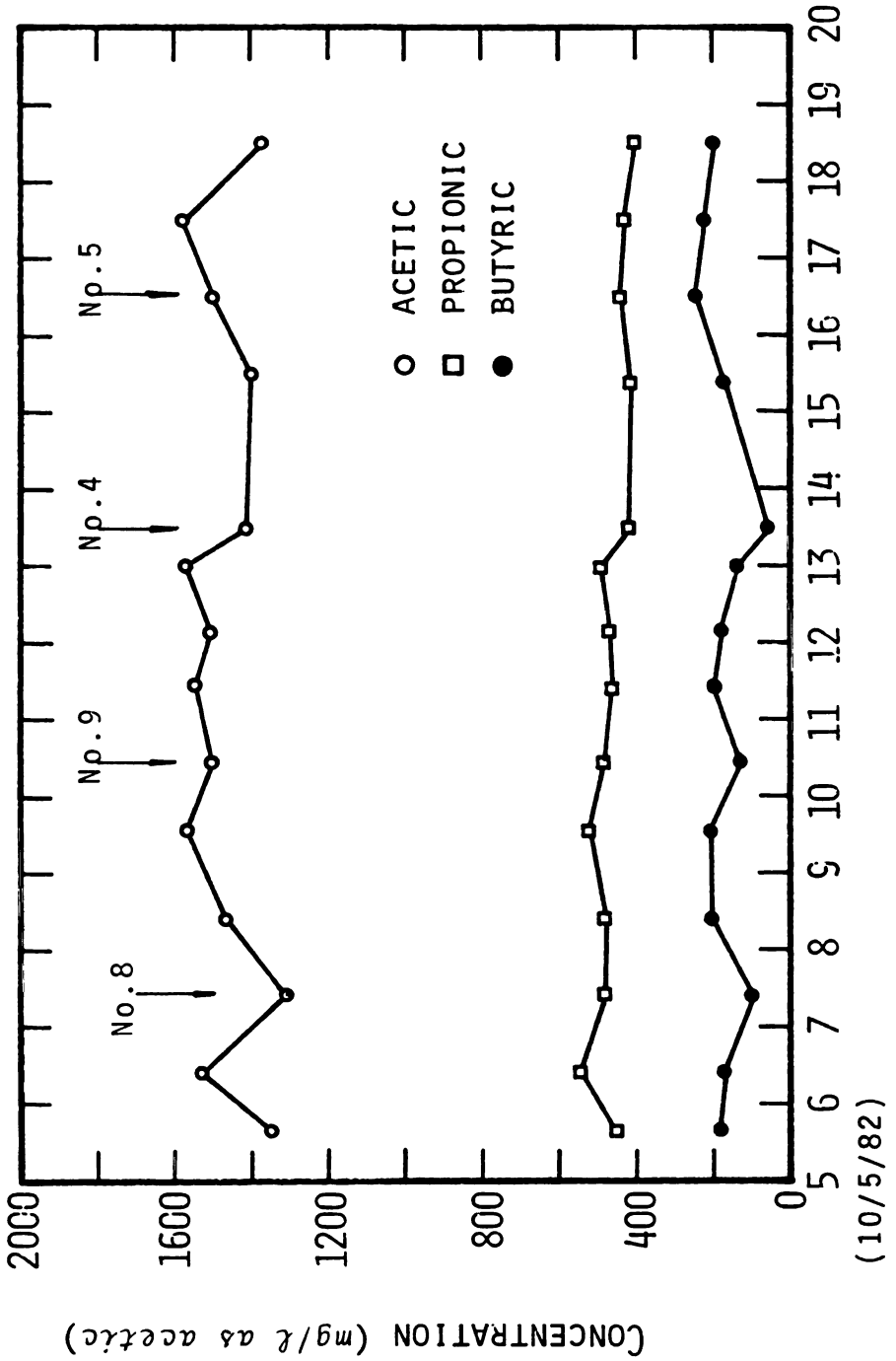


FIGURE 4-10 INDIVIDUAL VOLATILE FATTY ACID CONCENTRATION IN LIQUID RESERVOIR.

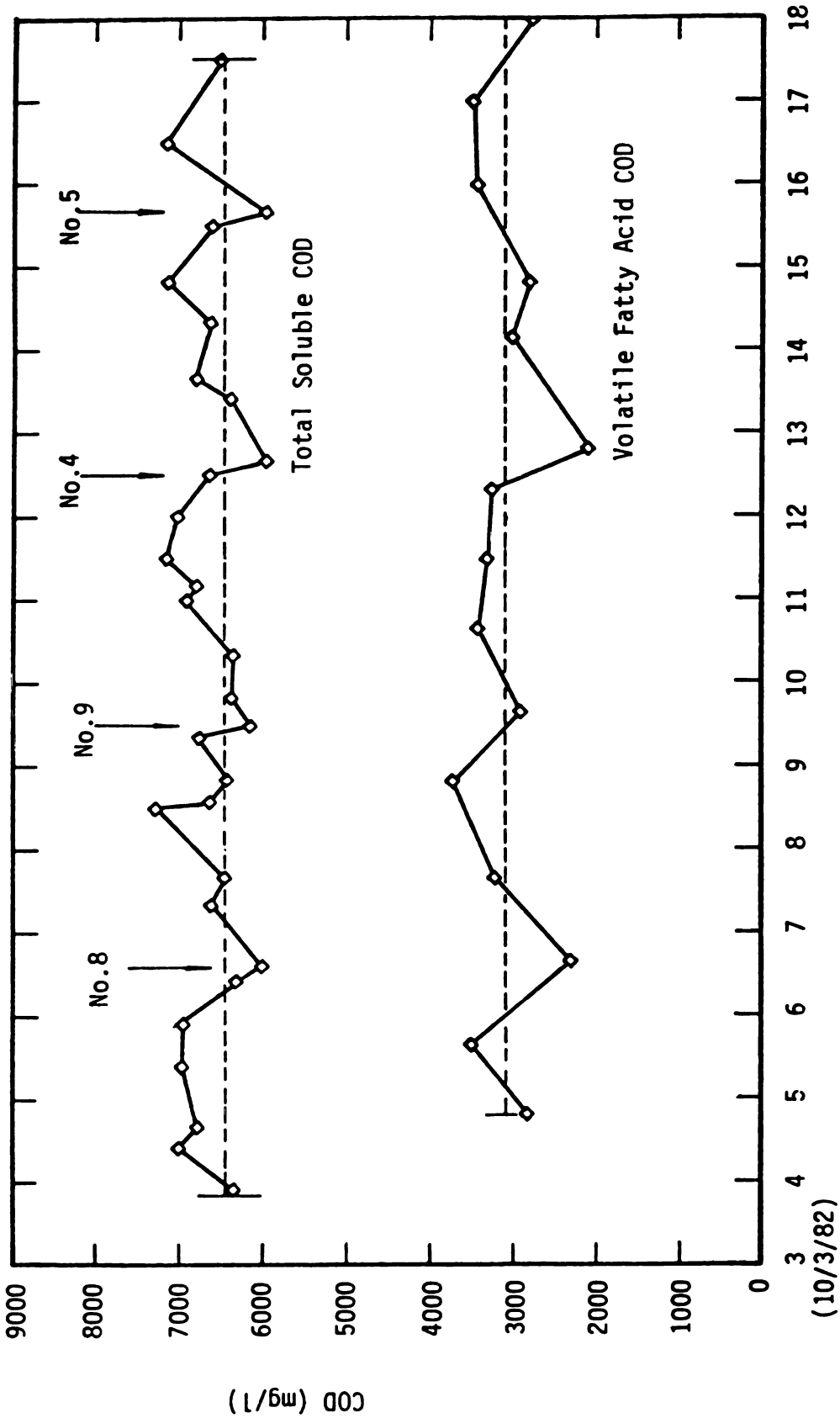


Figure 4-11 Effluent total soluble COD and volatile fatty acid COD of liquid reservoir.

sampling. The arrow marks in Figure 4-11 indicate the times when a new packed reactor was installed in the system. The drop in COD concentration when a new reactor was added is due to dilution from the make up water added at the same time.

Valeric and iso-valeric acids were found in the liquid reservoir in concentrations ranging from 15 to 30 mg/l as acetic. The presence of these acids which are rarely found in the packed reactors suggests that further degradation of organic substrate was taking place in the liquid reservoir. The change of pH in the liquid reservoir was also plotted in Figure 4-12. It should be noted that buffering chemicals were used occasionally to adjust pH in the liquid reservoir.

4.3 The Performance of the Anaerobic Filter

As mentioned in Chapter One, the anaerobic filters used in this study were up-flow reactors in to which straw was packed and served as the filter medium. Because the majority of the microorganisms were attached on the surface of the medium or trapped within the interstitial void spaces, a long biological solids retention time was able to be maintained.

Due to the special substrate input method, the packed reactors received substrate intermittently and produced effluent with highly variable COD concenetration. Although the variability was reduced by the liquid reservoir, the effluent from the liquid reservoir exhibited a cyclic COD concentration fluctuation, as can be seen from Figure 4-11. Therefore, the influent soluble COD concentration of the anaerobic filters was never at a constant state but was changing periodically

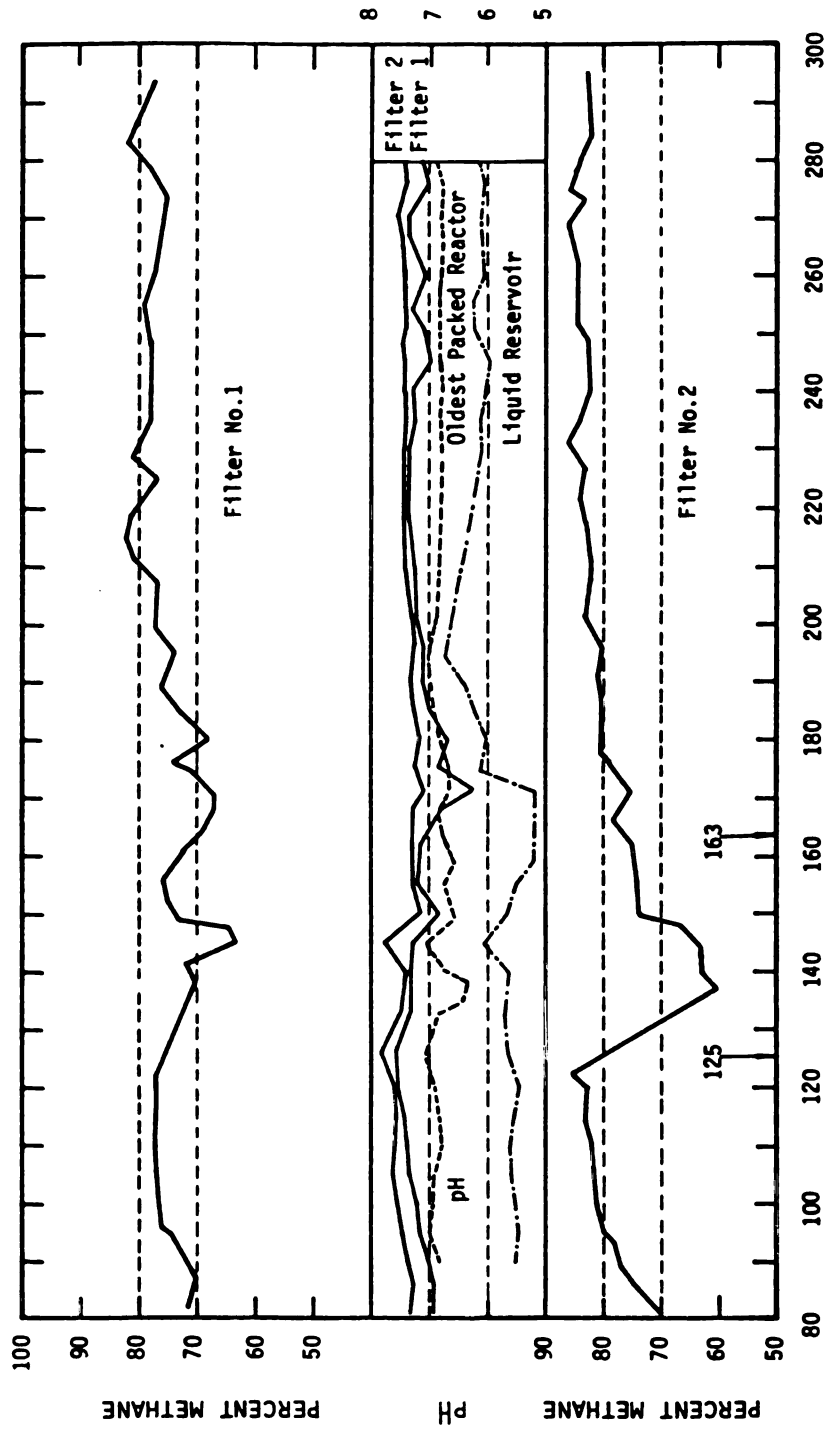


Figure 4-12 Methane gas composition of Filters No.1 and No.2. pH change of anaerobic filters, liquid reservoir, and the oldest packed reactor in Stage I.

in a fairly regular up and down pattern.

The performance of the anaerobic filters will be reported in terms of two parameters: (1) gas production, and (2) COD removal efficiency.

4.3.1 Gas Production From the Anaerobic Filters

Two anaerobic filters were the major reactors designed for methane production. Gas production was measured according to the methods described in Section 3.1, and the data to be presented in this section represent daily averages. Results obtained from Stage I and Stage II will be reported separately.

4.3.1.1 Stage I Gas Production

Figure 4-12 shows the gas composition for methane versus time in Stage I. The pH values for the anaerobic filters, the liquid reservoir, and the oldest packed reactor are also shown. It can be seen from the figure that the gas composition varied to a certain extent, especially during the early period of the experiment due to a pump failure on Day 125. The system limped along until Day 163 when a new pump was installed. Over the next 40 days the gas composition stabilized and then held relatively constant for the rest of the experiment.

Three observations from Figure 4-12 reflect the response to the filters to variable liquid flow rates and short-term inoperation:

1. The methane content decreased continuously from Day 125 to Day 145. In this period the origional pump head was leaking and it delivered only a very small flow, as well as being turned off several times. The results of the very small flow rate

and short-term inoperation was the reduced supply of organic material that caused a low COD loading and long hydraulic retention time, resulting in low concentrations of volatile fatty acids, high pH values, decreased methane composition, and high carbon dioxide composition. The high CO_2 content measured in the gas samples in this period was possibly contributed in part by the unknown amount of air leaking into the system through the failing pump.

2. Between Day 145 and Day 163, the old pump head was sealed with vacuum grease and reconnected to the system. However, it was unable to maintain a constant flow rate, so that the gas composition continued to fluctuate.
3. At Day 163, the old pump was replaced by a new one. It was later found that the flow rate of the new pump was too fast, although the pump head was adjusted to the same position as the old one. The obvious response of the filters to the continous high flow rate was the reduction of pH caused by the high influent VFA concentration. Also the methane composition, which had initially increased from 53 % to 62 % after installing the pump, dropped to 56 %. These fluctuations resulted from the high COD loading due to the high liquid flow rate. The pump flow rate was then reduced and finally maintained at approximatly 0.628 liter per day (S.D. = 0.074 l/day) for the rest of Stage I. After the filters acclimated to the new flow rate, the methane composition steadily increased from Day 180 stabilizing at about 78 to 85 % until the end of Stage I.

The cumulative methane, and total gas production (CH_4 + CO_2) from the two anaerobic filters for the 300 days of Stage I experiment are shown in Figure 4-13. It may be observed that each of the curves in Figure 4-13 exhibits periods of steady gas production with inflections corresponding to the times when the pump flow rate changed or the pump stopped.

Examination of Figures 4-12, and 4-13 reveals that the most steady period of operation was from Day 220 (3/29/82) to Day 260 (5/8/82) in which both the gas production rate and methane composition were maintained at a fairly constant level. As will be discussed later, the volatile fatty acid concentration in the filter effluent was very low during this period of stable operation. The average daily methane production from the two filters in this period was 1035 ml/day (S.D. = 230 ml/day) and the average methane production from Filter No.1 in the same period was 761 ml/day (S.D. = 158 ml/day). Thus, 75% of the total methane was produced in the first filter.

4.3.1.2 Stage II Gas Production

At the beginning of Stage II, the brine solution was sucked into Filter No.1 by accident. Accordingly, a spare filter, which was previously made by seeding active digester sludge into a removed old packed reactor, was used to replace the original filter. Because a certain level of methanogenic activity had already developed in the spare filter, it only took two weeks of acclimation before sampling for Stage II could be resumed.

Unlike Stage I, the gas composition in this stage was fairly con-

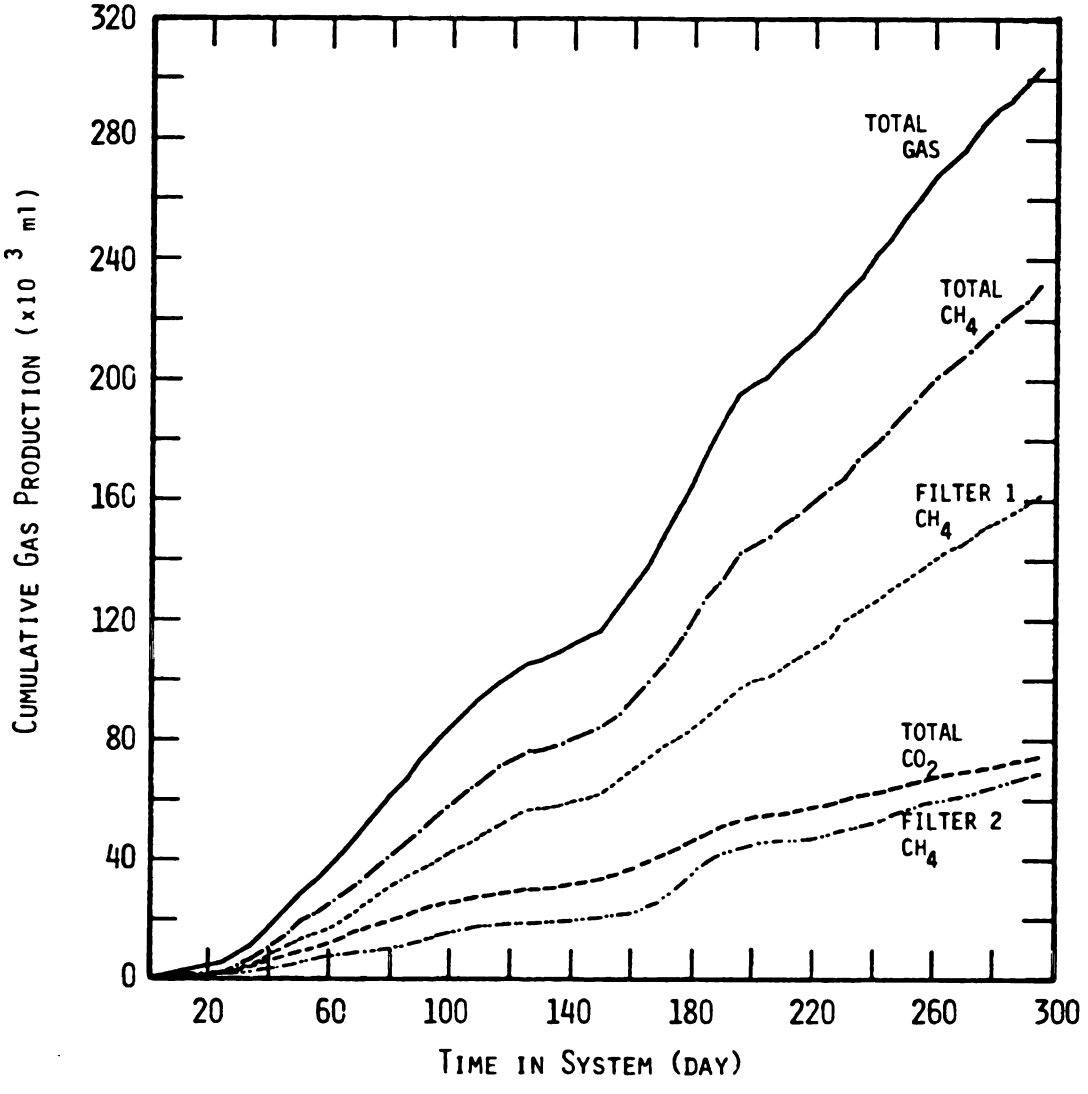


FIGURE 4-13 TOTAL GAS PRODUCTION FROM FILTER NO.1 AND FILTER NO.2

stant as shown in Table 4-3:

Table 4-3 Composition of Gas Produced in Stage II Anaerobic Filters

	Hydrogen (%)	Methane (%)	Carbon Dioxide (%)
Filter No.1	none	70.8 - 73.7	26.3 - 29.2
Average		72.9	27.1
S.D.		1.1	1.1
Filter No.2	none	74.4 - 80.3	20.0 - 25.6
Average		78.8	21.3
S.D.		2.2	2.2

* S.D. = Standard Deviation

Some nitrogen gas was also observed in each sample. This nitrogen was attributed to air existing in the gas collection cylinders and gas lines at stard up, and possibly due also to the diffusion of air through the tygon tubing. The amount of air that might have diffused into the system was neglected and the gas composition was normalized to exclude nitrogen on the assumption that all the gas actually produced was either methane or carbon dioxide.

The cumulative methane and carbon dioxide production from the anaerobic filters is shown in Figure 4-14. Inspecting the cumulative methane production curve of Filter No.1 reveals that the first filter produced methane at a fairly constant rate in the period from day 10 to day 20. The average daily methane production in Filter 1 in this 11 day period was 925 ml/day (S. D. = 116 ml/day), and the total methane production rate from both filters in this period was 1052 ml/day

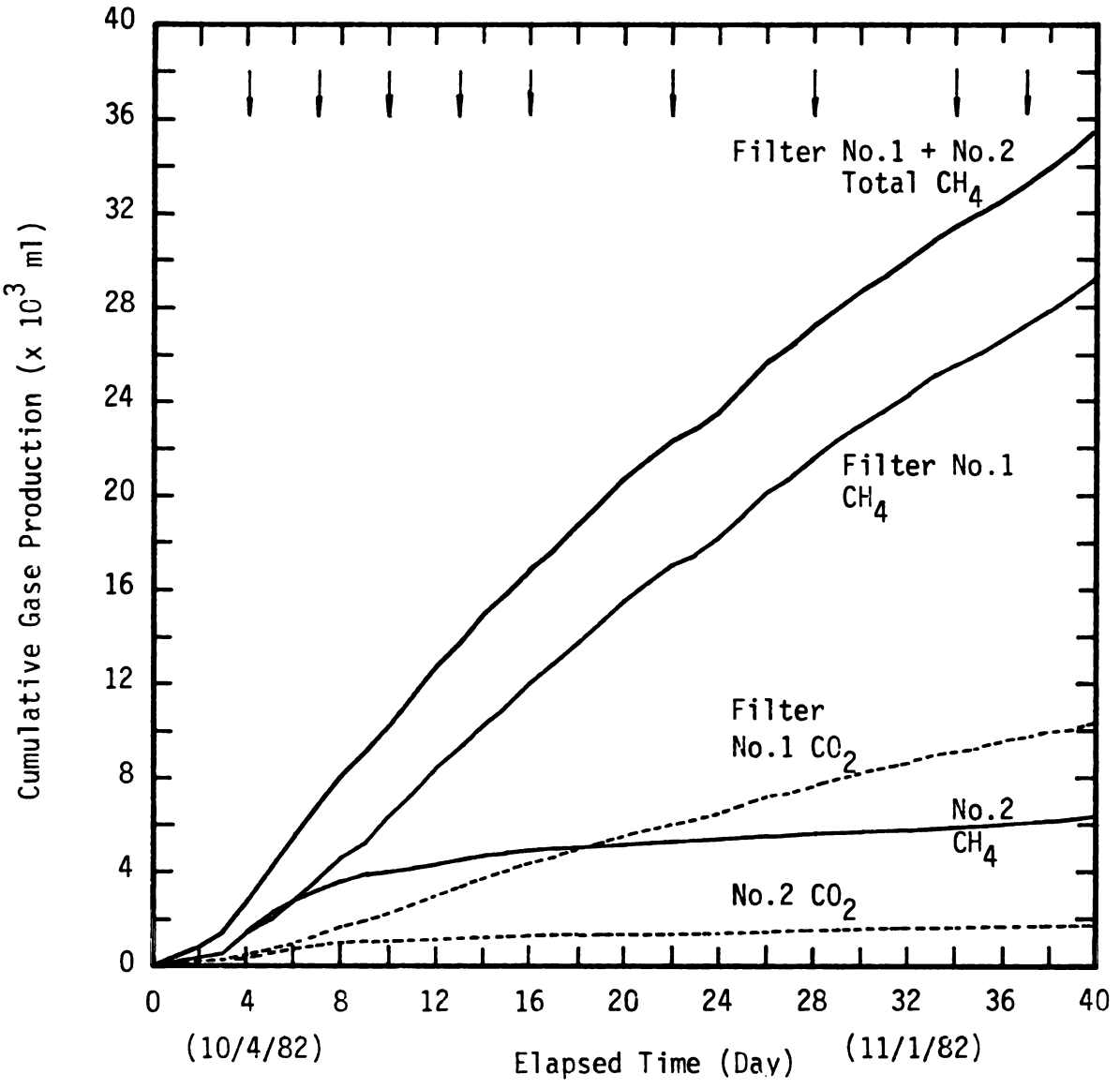


Figure 4-14 Cumulative Methane and Carbon Dioxide Production From Anaerobic Filters. (Stage II)

(S.D. = 149). Thus, 88% of the total methane was produced in the first filter. After day 20, methane production decreased slowly until day 34. The declining gas production was due to extending substrate input interval from 3 days to 6 days. The arrow marks shown in the top of Figure 4-14 represent the dates that fresh straw was introduced. There was no special objective for the extended substrate input. Data obtained from day 10 to day 20 were used for analysis.

4.3.1.3 Specific Methane Production

The specific methane production from the anaerobic filters will be expressed in terms of two units: (1) ml/day/unit reactor volume, (2) ml CH_4/gram substrate input.

In Stage I, the average daily methane production rate in the period between Day 220 and Day 260 was 761 ml/day for Filter No.1 and 1035 ml/day for the combination of two filters. As described in Chapter 3, the volume of the straw holding area in the anaerobic filter was 435 ml. Therefore the total methane production per unit volume of filter was 1.19 liter CH_4/liter reactor volume per day, and the methane production from Filter No.1 was 1.75 liter CH_4 per day per liter reactor volume. The total methane produced from the entire system during the period from Day 220 to Day 260 (3/29/82 to 5/8/82) can be computed as follows:

Methane gas produced from two anaerobic filters = 42,432 ml

Methane produced by the two filters is about 84.8 % of that produced from the entire system.

Therefore methane production from the system = 42,432/0.848

= 50,038 ml

The total substrate input into the system during this period was 480 grams. Thus, the total methane production per gram of substrate input was: 104.3 ml CH_4 per gram substrate input.

In Stage II, the average daily total methane production from both filters was 1052 ml/day, and the average methane production from Filter No.1 was 925 ml/day. As described in the above paragraph, the methane production in Stage II for two filters and for Filter No.1 were 1.20 l CH_4/liter reactor volume per day and 2.12 l CH_4/liter reactor volume per day, respectively. As Figure 4-14 shows, the total cumulative methane production from 10/4 to 11/10 in Stage II from two anaerobic filters was 34,935 ml. Using the same method as shown above for Stage I, the total methane production from the whole system is: $34{,}935/_{0.848}$ = 41,197 ml. During this period, 540 grams of straw was introduced into the system, therefore, the methane production per unit weight of substrate input was 41,197/540 = 76.3 ml CH_4/gram substrate. The ratio of volume methane produced per gram substrate input for Stage I to Stage II is: 104.3/76.3 = 1.37.

It should be noted that the expression of methane production in terms of per unit reactor volume is not an absolute unit. It should not be interpreted for the estimation of methane production for other types of filters. The amount of methane produced is affected by several factors including, (1) the type of substrate, (2) COD loading, (3) hydraulic retention time, and (4) the type of filter medium. However, this expression provides insight into filter performance at certain known operating conditions. At known COD loading, the specific methane

production per unit reactor volume provides the information of filter efficiency. Comparing the performance of the anaerobic filters in terms of methane production per gram of substrate input, it is apparent that the filters conducted in Stage I exhibited higher methane production rate than that obtained during Stage II. This phenomenon may be explained by the fact that the substrate solid retention time in Stage I was 40 days compared with 18 days in Stage II. This additional time allowed increased straw degradation resulting in higher COD loading to the filters.

4.3.2 COD Removal Efficiency

COD removal efficiency was evaluated as the ratio of the amount of COD removed by the anaerobic filters to the influent COD concentration. Because the influent and effluent COD concentration of the anaerobic filters varied somewhat, average values during the "stable" operation period were used for COD removal efficiency calculations.

4.3.2.1 Total Soluble COD Removal

As previously mentioned, the total soluble COD measurements in Stage I were unsatisfactory, so only the data obtained from Stage II will be presented for the total soluble COD removal efficiency. Figure 4-15 shows the influent and effluent total soluble COD concentrations for the anaerobic filters of Stage II. Since the liquid reservoir, Filter No.1 and Filter No.2 were connected in series, the effluent from the liquid reservoir was the influent for Filter No.1. The average influent soluble COD concentration for Filter No.1 was 6636 mg/l and

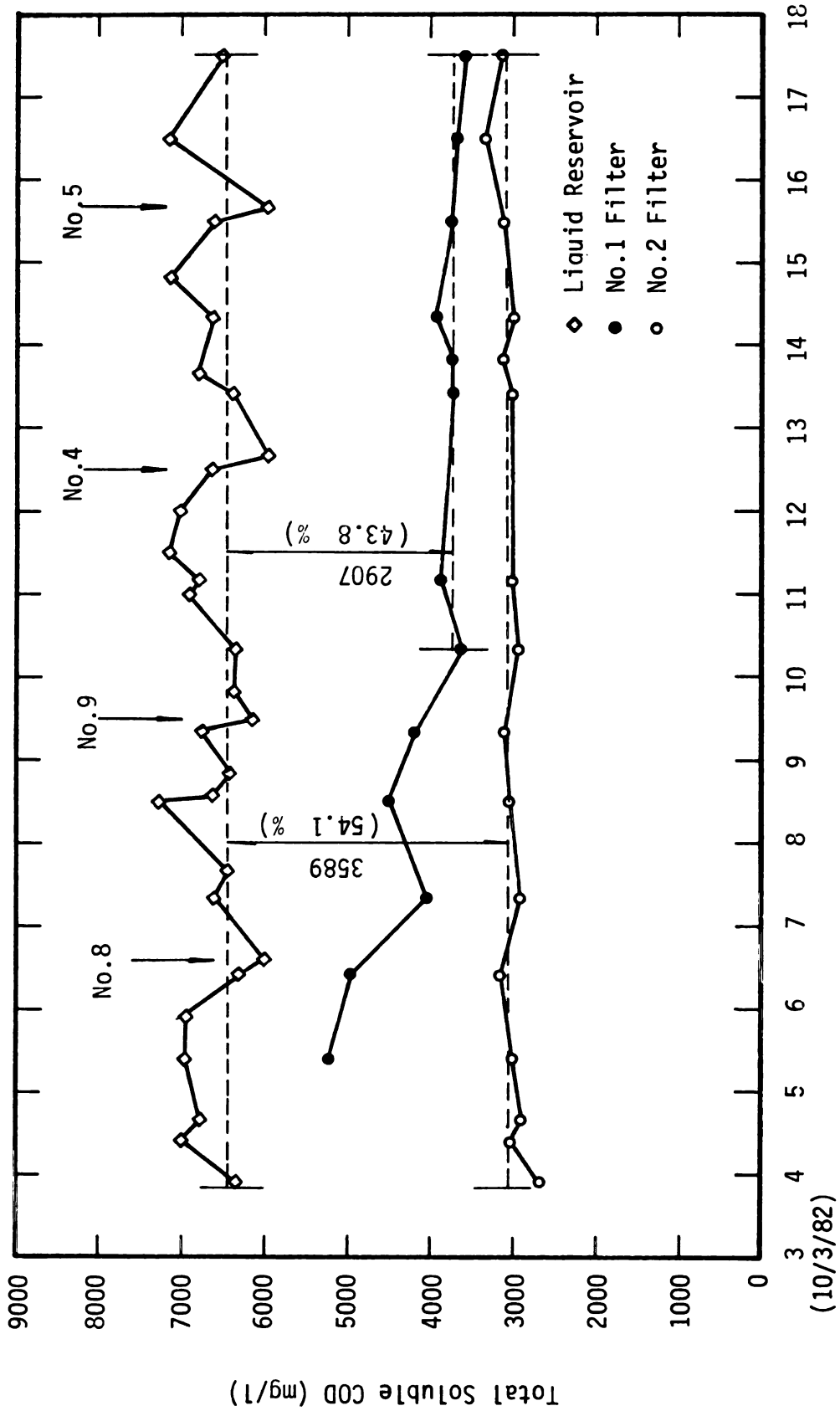


Figure 4-15 Total soluble COD of effluent from the liquid reservoir and the anaerobic filters. (Stage II)

the average effluent COD concentration for Filters No.1 and No.2 were 3729 mg/l and 3047 mg/l, respectively. It is noted that the average effluent COD concentration of Filter No.1 was computed from the data after 10/11/82 when this filter had stabilized. Therefore, the average COD removal efficiency for Filter No.1 can be calculated as follows:

$$E_1\ (\%) = \frac{6636 - 3729}{6636} \times 100 = 43.8\% \qquad (4\text{-}1)$$

If the two filters are considered as a unit, then the total COD removal efficiency become:

$$E_t\ (\%) = \frac{6636 - 3047}{6636} \times 100 = 54.1\% \qquad (4\text{-}2)$$

The average COD loading of Filter No.1 was:

$$6636\ (\text{mg/l}) \times 26\ (\text{ml/hr}) \times \frac{1}{1000}\ (\text{l/ml}) \times 24\ (\text{hr/day}) \times \frac{1}{435\ (\text{ml})}$$

$$= 9.52\ \frac{\text{g COD/day}}{\text{liter reactor volume}} \qquad (4\text{-}3)$$

or

$$\frac{9.52 \times 2.205 \times 10^{-3}}{0.03531} = 594\ \frac{\text{lb COD/day}}{10^3\ \text{ft}^3} \qquad (4\text{-}4)$$

The total average COD loading for two filters together was:

$$\frac{6636 \times 0.026 \times 24}{430 \times 2} = 4.76\ \frac{\text{g COD/day}}{\text{liter}} \qquad (4\text{-}5)$$

or

$$\frac{4.76 \times 2.205 \times 10^{-3}}{0.03531} = 297\ \frac{\text{lb COD/day}}{10^3\ \text{ft}^3} \qquad (4\text{-}6)$$

* 1 gram = 2.205×10^{-3} lb.

1 liter= 0.03531 ft^3

In view of the results obtained from the above calculations, the overall removal efficiency of 54.1 % does not seem significantly large. The reason for the high effluent COD is that non-biodegradable COD is leached from the straw and accumulates due to recirculation, stabilizing at a value determined by the rate at which liquid is removed due to sampling and replacement of packed reactors. As shown in the next section, degradation of volatile fatty acids was nearly complete indicating that the filters were not overload. It should be emphasized that the anaerobic filters were designed to produce methane from the organic substances contained in the liquid phase and not necessarily to produce a high quality effluent since the effluent is not being discharged from the system.

4.3.2.2 Volatile Fatty Acid COD Removal

Data obtained both from Stage II and Stage I will be presented for VFA COD removal efficiency.

4.3.2.2.1 Stage II VFA COD Removal

The change of VFA COD concentration for the influent and effluent of the anaerobic filters is presented in Figure 4-16 (data in Appendix D-4-4). It can be seen that the curve of effluent VFA COD for Filter No.2 still shows a declining trend, which indicates that the microbiological population in the Filter No.2 was still developing during that

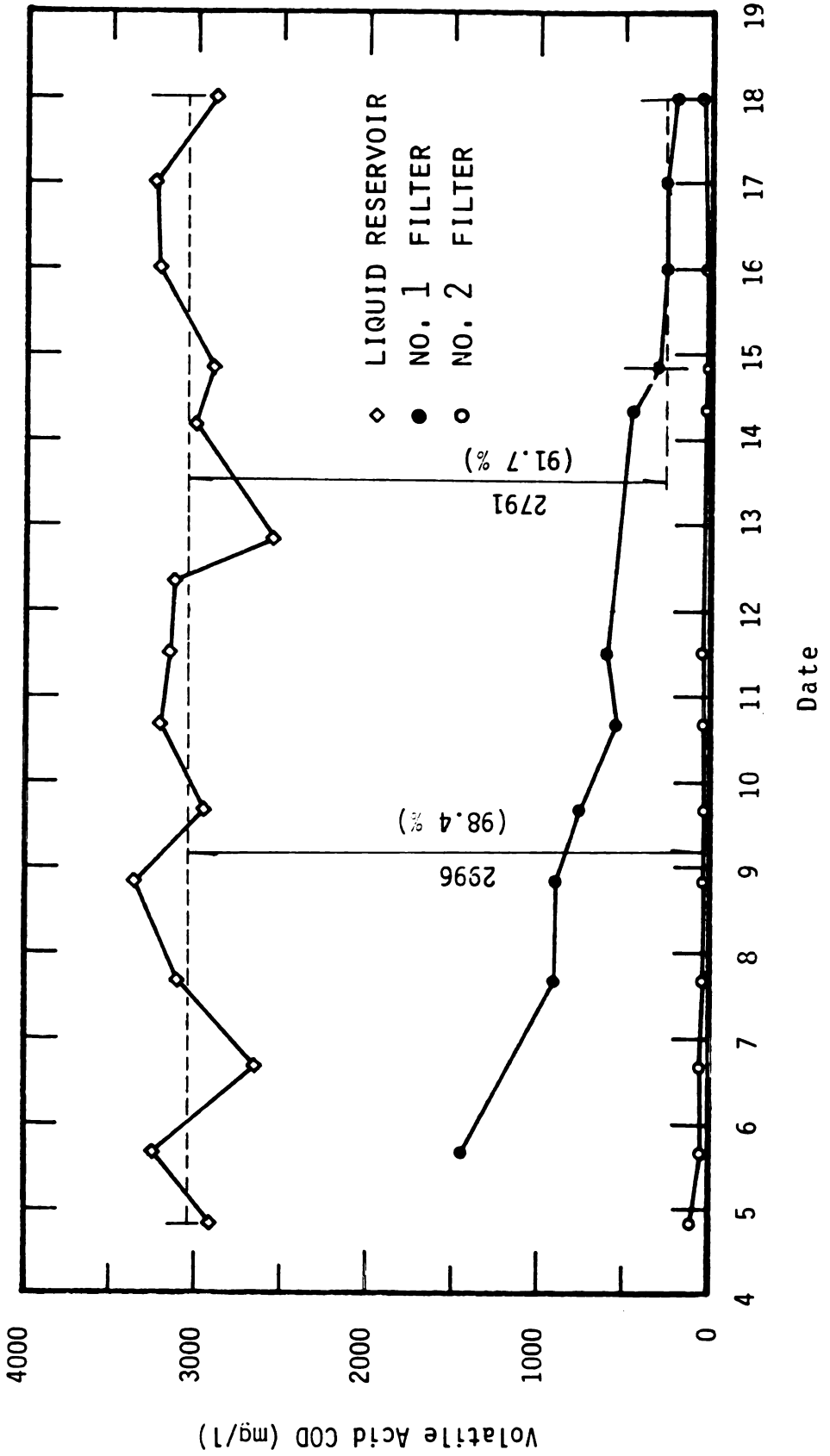


Figure 4-16 Effluent volatile fatty acid COD of the liquid reservoir and anaerobic filters. (Stage II)

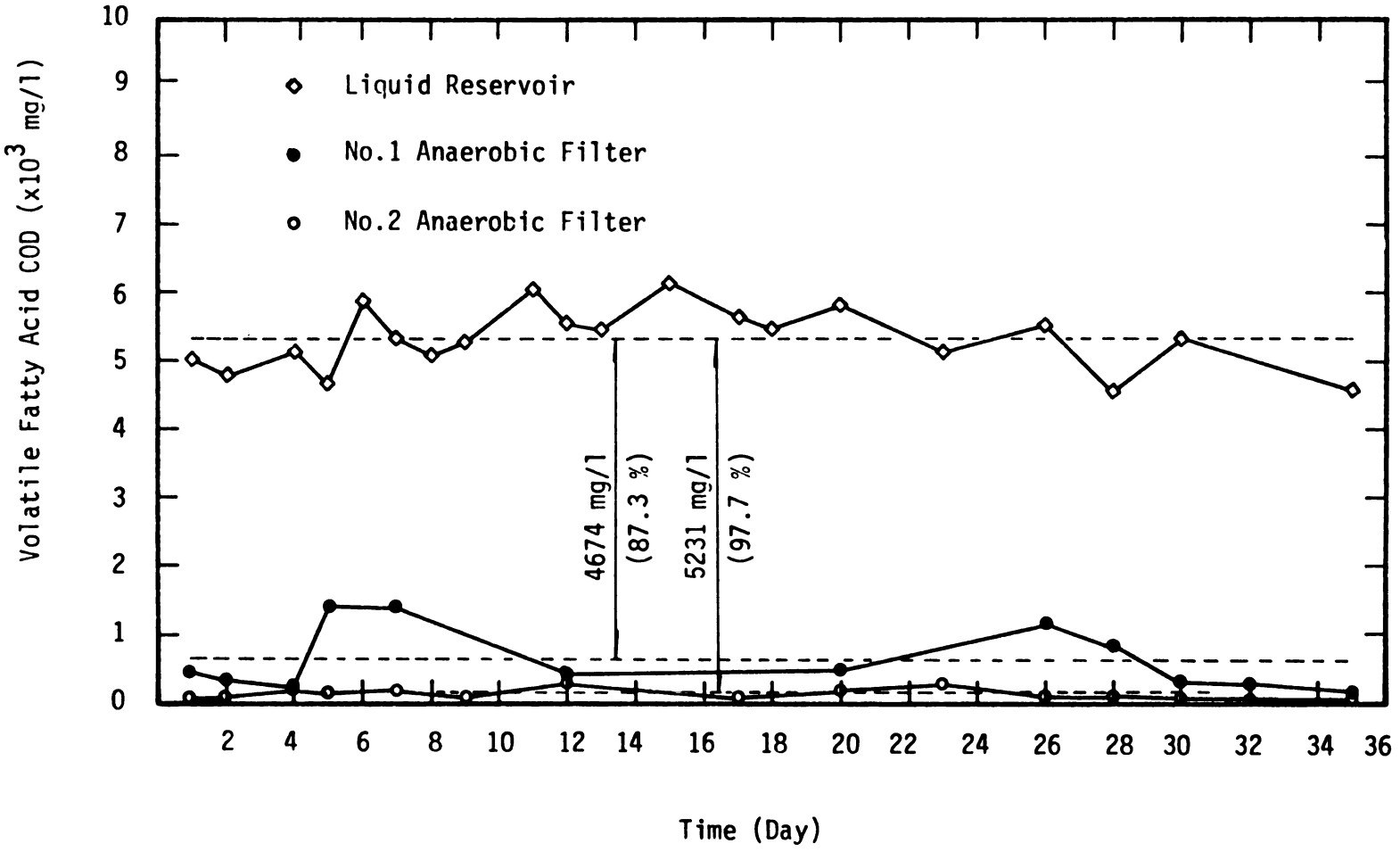
period. In other words, the COD removal efficiency of Filter No.1 was increasing and eventually would reach a level that would make the Filter No.2 unnecessary.

As shown in Figure 4-16, the average influent VFA COD concentration of Filter No.1 was 3044 mg/l and the average concentration of effluent from Filter No.2 was 48 mg/l. Using the same method for the calculation of total soluble COD removal efficiency, described earlier, the VFA COD removal efficiency for the two filters was 98.4 %. For the purpose of comparing the performance of the two filters, the last four days of Filter No.1 were used to compute the average of 253 mg/l. Therefore the VFA COD removal efficiency for Filter No.1 was 91.7 %. The VFA COD loading for Filter No.1 was 4.37 g COD/day/liter or 273 lb COD/day/10^3 ft^3. And the total VFA COD loading for two filters together was 2.19 g COD/day/liter or 136 lb COD/day/10^3 ft^3. It is noted that the VFA COD produced in the filters was not included in the above calculation.

4.3.2.2.2 Stage I VFA COD Removal

As has been pointed out in Chapter 3, the original pump used in the early period of Stage I failed and it was replaced by a new pump. The experimental results to be presented in this section were obtained from the 35 days of "stable" data from 4/7/82 to 5/10/82 (Day 229 to Day 263) after the new pump was used.

From Figure 4-17 and Appendix D-4-5 it was found that the average VFA COD concentration of the influent liquid for Filter No.1 was 5356 mg/l and the effluent VFA COD concentrations were 682 mg/l and 125 mg/l



Firugr 4-17 Volatile Fatty Acid COD of Effluent From Liquid Reservoir And Anaerobic Filters.(Stage I)

for Filters No.1 and No.2 respectively. Therefore, the average VFA COD removal efficiency for Filter No.1 was 87.3 %, and the total removal efficiency for two filters was 97.7 %. Furthermore, the COD loading, in terms of VFA COD, for Filter No.1 in this stage was 7.94 g VFA COD/liter/day or 496 lb COD/day/10^3 ft^3, and the total VFA COD loading for two filters was 3.97 g VFA COD/liter/day or 278 lb COD/day/10^3 ft^3.

4.3.2.3 Summary of The COD Removal Efficiency

The above calculations for the COD removal efficiency may be summarized as in the tables shown below. Table 4-4 shows the influent and effluent COD concentrations of the anaerobic filters; Table 4-5 summarizes the COD removal efficiency for Stage II; and Table 4-6 shows the COD removal efficiency for the Stage I.

Table 4-4 Influent and Effluent COD Concentrations for The Anaerobic Filters (Stage II)

	Influent Filter No.1	Effluent	COD Removed Filter No.2
(1) Total Soluble COD, mg/l	6636	3047	3589
(2) VFA COD	3044	48	2996
Difference (1) & (2)	3592	2999	593

Table 4-5 COD Removal Efficiency in the Anaerobic Filters (Stage II)

	COD Loading (lb COD/Day/1000 ft^3)		COD Removal Efficiency (%)	
	Soluble COD	VFA COD	Soluble COD	VFA COD
Filter No.1	594 (9.52)*	273 (4.37)*	43.8	91.7
No.1 + No.2 Filter	297 (4.76)*	136 (2.19)*	54.1	98.4

* unit in g COD/day/liter

Table 4-6 Volatile Fatty Acid COD Removal Efficiency in Stage I Anaerobic Filters

	COD Loading $\frac{\text{lb COD/day}}{10^3\ ft^3}$	Removal Efficiency (%)
Filter No.1	496 (7.94)*	87.3
No.1 + No. 2 filters	278 (3.97)*	97.7

* unit = g COD/day/liter

As mentioned earlier, the influent liquid to the anaerobic filters contained non-biodegradable and biodegradable substances which included particulate COD and soluble COD, and the soluble COD included VFA COD and non-VFA COD. Examination of Tables 4-4 and 4-5 shows that 3589 mg/l of soluble COD was removed by the anaerobic filters while only

2996 mg/l of VFA COD was removed. This indicated that 593 mg/l of soluble COD, about 16.5 % of the degradable soluble COD, was fermented to volatile acids and further converted to methane and carbon dioxide inside the filters. In other words, about 16.5 % of non-VFA COD in the influent stream was converted to VFA COD in the anaerobic filters, and this portion of the COD was not included in the calculation of VFA COD removal efficiency. If this 593 mg/l is added to the influent VFA COD then the removal efficiency becomes:

$$\frac{(593 + 3044) - 48}{593 + 3044} \times 100 = 98.7\ \% \qquad (4\text{-}7)$$

for both filters in Stage II. It is concluded that at a total soluble COD loading of 4.63 g COD/liter per day (289.12 lb. COD/day/10^3 ft^3), for two anaerobic filters, 98.7 % of volatile fatty acids were converted to CH_4, CO_2 and microbial cell solids.

The fact that a volatile acid COD removal efficiency of greater than 90 % was obtained suggests that efficient methane production may be accomplished at even higher COD loading rates once sufficient microbial mass is established in the anaerobic filters. Also by comparing the removal efficiency and the operating parameters of this study, such as type of substrate, COD loading, hydraulic retention time, with the results obtained from previous studies (Table 2-15), it is found that the anaerobic filters conducted in this study performed better than those of many previous studies.

It also concluded from Table 4-5 that 81 % of the soluble COD removal was accomplished by Filter No.1. These finding agreed with the results reported by several other investigators (Young and McCarty,

1967; Lovan & Foree, 1971) that the major portion of the COD is removed in the lower section of an anaerobic filter.

4.3.3 Individual Volatile Fatty Acids in Anaerobic Filters

A rough picture of the fate of volatile fatty acid degradation in the anaerobic filters can be observed by determining the individual acid concentrations of the effluent stream. Tables 4-7 and 4-8 give the concentrations of individual acids that were measured after 10/6/1982. The data in these two tables are plotted in Figure 4-18.

Table 4-7 Individual Volatile Fatty Acid Concentrations in Filter No.1 Effluent. (Stage II)

		Volatile Acid Concentration (mg/l as HAc)					
Date	Time	HAc	HP	iHB	HB	iHV	HV
10/6	1000	433	367	10	57	17	18
10/7	1000	399	550	40	126	_	_
10/8	1000	131	230	9	93	--	--
10/9	1400	189	280	17	2	22	14
10/10	1030	150	270	13	--	16	--
10/11	1000	133	185	8	--	12	4
10/12	0400	145	189	9	--	14	6
10/14	2200	167	122	6	--	11	4
10/15	1000	106	85	1	1	5	2
10/16	1200	76	84	2	--	4	2
10/18	1300	77	51	2	--	--	--

* Stage II, Year 1982.

Table 4-8 Individual Volatile Fatty Acid Concentrations in Filter No.2 Effluent. (Stage II)

		Volatile Acid Concentration (mg/l as HAc)					
Date	Time	HAc	HP	iHB	HB	iHV	HV
10/5	1600	103	1.5	--	--	--	--
10/6	1000	58	0.6	--	--	--	--
10/7	1000	55	2.5	--	--	--	--
10/8	1000	32	--	--	--	--	--
10/9	1400	27	--	--	--	--	--
10/10	1030	22	--	--	--	--	--
10/11	1000	30	1.0	--	--	--	--
10/12	0400	35	--	--	--	--	--
10/14	2200	6	--	--	--	--	--
10/15	1000	8	--	--	--	--	--
10/16	1200	5	--	--	--	--	--
10/17	1200	43	28	--	--	--	--
10/18	1300	47	--	--	--	--	--

* Stage II, Year 1982.

As has mentioned in Section 4.3.1.2, the original Filter No.1 was replaced by a spare filter. The new filter was acclimated for two weeks before VFA COD measurements were resumed. However, the new Filter did not achieve stable operation until the final days of Stage II. This can be observed from the continuously decreasing concentration of volatile fatty acids in the effluent of Filter No.1. The VFA COD escaping from Flter No.1 was removed by Filter No.2. Therefore, the over-all VFA COD removal capability of the anaerobic filters was not affected.

As shown in Figure 4-18, the effluent acetic acid concentration in Filter No.1 decreased sharply from above 400 mg/l to about 150 mg/l and then decreased at a slower rate. Figure 4-18 also shows that butyric acid was not effectively removed before 10/8 and the propionic acid

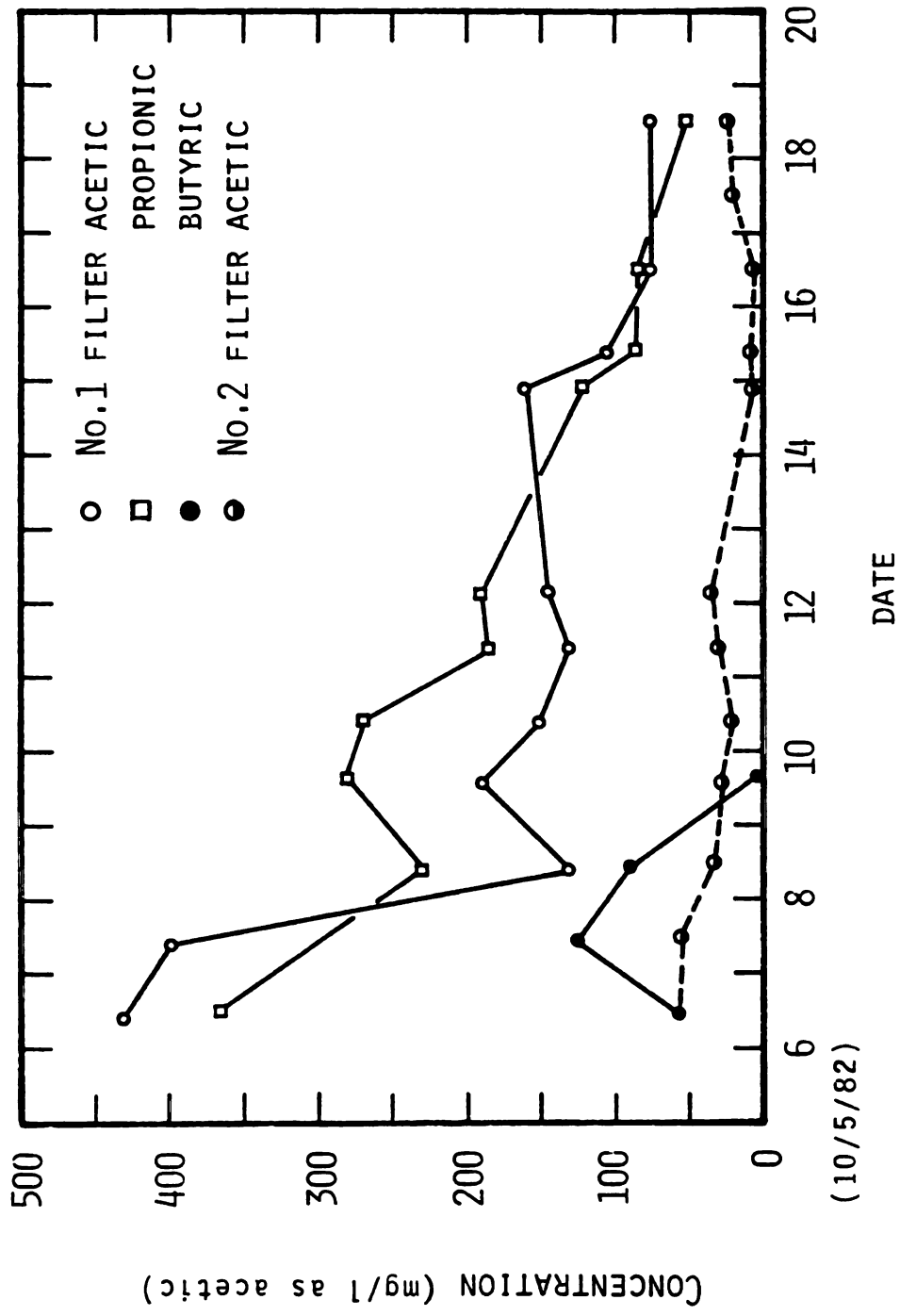


FIGURE 4-18 INDIVIDUAL VOLATILE FATTY ACID CONCENTRATION IN FILTER NO.1 AND FILTER NO.2. (Stage II)

concentration was higher than that of acetic acid before 10/13 in Filter No.1. These phenomena suggest that a longer time is required for H_2-producing acetogenic bacteria to grow to a sufficient microbial population to completely degrade the propionic and butyric acids to acetic acid than for degradation of acetic acid.

4.3.4 Estimation of Particulate COD Degradation in Anaerobic Filters

Biodegradable organic substances that contribute to methane production include particulate COD and soluble COD. Although liquid samples were not analyzed for particulate COD, the portion of particulate COD that was degraded in the anaerobic filters may be estimated by theoretical calculations.

As previously described in Section 4.3.1.2, the average daily methane production from both anaerobic filters in Stage II was 1052 ml/day. The theoretical maximum methane production per unit COD destroyed is 0.35 l/g COD at standard conditions, equivalent to 0.396 l CH_4/g COD at 36°C (see Appendix C calculation). If assumed that 98 % of the COD destroyed was converted to methane with the remaining COD used for cell growth, then the amount of the total biodegradable COD destroyed by the anaerobic filters was:

$$1.052\ (^{l}/_{day}) \times \frac{1}{0.396}\ (^{g}/_{l}) \times \frac{1}{0.98} \times \frac{1}{0.628}\ (^{day}/_{l})$$

$$= 4.32\ (^{gm}/_{l}) = 4320\ (^{mg}/_{l}) \qquad (4\text{-}8)$$

From Table 4-7, the total soluble COD removed by the anaerobic filters was 3589 $^{mg}/_{l}$. Therefore, the theoretical particulate COD removed by

the filters was:

$$4,320 - 3,589 = 731 \ (^{mg}/_{l}) \qquad (4\text{-}9)$$

The above computational results are only an estimation and they are only valid providing the following conditions are valid: (1) the average liquid flow rate was equal to 0.628 $^{l}/_{day}$ (S.D. = 0.074 l/day), (2) no methane produced diffused out or leaked out from the gas collection system, (3) the percent of methane composition measurements were accurate, and (4) 98 % of the total COD destroyed were converted to methane. Of the factors which affect this calculation, the consistency of the pump flow probably has the grestest impact. The standard deviation of the average pump flow rate was 0.074 l/day which was 11.8 % of the average flow rate. Therefore, the variation of flow rate could substantially affect the computational result.

4.4 Extent of Wheat Straw Degradation

The substrate used in the packed reactor was un-pretreated, chopped wheat straw. The extent to which this substrate was degraded has been studied and will be presented in terms of three parameters: (1) the substrate weight loss after fementation, (2) the percent of cellulose and hemi-cellulose degradation, and (3) by calculation from methane production.

4.4.1 Percent Substration Degradation Based on Weight Loss

After a packed reactor was removed from the system at the end of its full retention period, the fermented straw inside the reactor was carefully transfered to a 185 mm dia., 765 ml evaporation dish. The fermented straw was placed in a 103^{o}C oven for two days to dry. It was then cooled and weighed. The weighed sample was ground and stored for analysis of cellulose, hemi-cellulose, and lignin at a later date.

The moisture content of the fresh straw was 5.67% (S.D. = 0.11). The straw weight loss after fermentation was obtained by subtracting the fermented weight from the dry fresh straw weight. Table 4-9 shows the weight loss of fermented straw from 3/6/82 to 5/28/82 (Day 197 to Day 280) in Stage I. Table 4-10 shows the percent of straw weight loss in Stage II.

Table 4-9 Straw Weight Loss After Fermentation. (Stage I)

Date (1)	Days (2)	Reactor (3)	Weight Loss (g) (4)	Percent Weight Loss (%) (5)
3/06 - 4/18	43	5	14.79	26.13
3/11 - 4/28	43	6	16.02	28.30
3/18 - 4/28	41	7	17.08	30.18
3/23 - 5/03	41	8	15.27	26.98
3/28 - 5/08	41	9	15.65	27.65
4/03 - 5/15	42	10	15.15	26.77
4/08 - 5/20	42	3	16.19	28.60
4/14 - 5/24	40	4	14.12	24.95
4/18 - 5/28	40	5	15.98	28.23
Average			15.58	27.53
S.D.			0.87	1.53

Fresh Substrate Weight = 60.0 x 0.9433 = 56.6 grams
(5) = (4)/56.6 x 100
Year : 1982

Table 4-10 Straw Weight Loss After Fermentation. (Stage II)

Date (1)	Days (2)	Reactor (3)	Weight Loss (g) (4)	Percent Weight Loss (%) (5)
9/28 - 10/16	18	5	10.44	18.45
10/01 - 10/22	21	6	12.51	21.10
				Average = 20.28

* Fresh Substrate Weight = 60.0 x 0.9433 = 56.6 grams
(5) = (4)/56.6 x 100

The weight loss is apparently a function of solids residence time. The weight loss in Stage I with a solids retention time of 40 to 43 days was 1.36 times the weight loss in Stage II when the solids retention time was only 18 to 21 days. From Section 4.3.1.3, the methane production per gram straw input was 104.3 ml/g in Stage I and 76.3 ml/g in Stage II for a ratio of 1.37, essentially the same as the weight loss ratio.

4.4.2 Percent Substrate Degradation Based on Fiber Analysis

The Goering and van Soest (1970) method, as described in Chapter 3, was used to analyze the cellulose, hemi-cellulose, and lignin contents for fresh straw and fermented straw samples. Table 4-11 gives the results of the fiber analysis for Stage I and Stage II. The fresh straw samples were analyzed at the same time as the fermented samples.

Table 4-11 Cellulose, Hemi-cellulose, Lignin Contents of Wheat Straw

Date	Days	Cellulose (%)	Hemi-Cellulose (%)	Lignin (%)
Fresh Straw		43.64	30.25	6.70
		43.64	29.75	6.9
		44.46	31.63	5.69
		43.22	33.03	6.78
Average		43.74	31.17	6.52
S.D.		0.52	1.48	0.56
Stage I				
3/06 - 4/18	43	43.30	29.47	10.90
		41.48	31.61	10.75
3/11 - 4/23	41	41.40	30.01	11.44
		40.20	31.50	11.14
3/18 - 4/28	41	41.47	28.85	11.06
		39.51	30.84	11.35
3/23 - 5/03	42	43.28	30.36	10.74
		41.75	31.79	11.01
4/08 - 5/20	41	43.41	31.20	10.92
		41.79	32.70	10.04
Average		41.75	30.83	10.94
S.D.		1.30	1.17	0.39
Stage II				
10/16 - 11/5	20	46.11	30.55	9.35
		45.83	30.82	9.20
Average		45.97	30.69	9.28

Because lignin is almost nonbiodegradable, it is reasonable to assume that the lignin content in the straw was not changed during the fermentation period. Therefore, based on the 6.52 % lignin content for the fresh straw, the percent of fiber contents in the fermented samples may be adjusted as in Table 4-12.

Table 4-12 Adjustment of Straw Fiber Contents (Stage I)

	Cellulose (%)	Hemi- (%) Cellulose	Lignin (%)	Ash (%)	Other (%)
(1) Fresh Straw	43.74	31.17	6.52	4.90	13.67
(2) Fermented Before Adjust	41.75	30.83	10.94	8.22	8.26
(3) Fermented After Adjust	24.88	18.37	6.52	4.90	4.93
(4) Difference (1) - (3)	18.86	12.80	0.0	0.0	8.75

Assume 50% of the materials under "other" item are degradable, then the total material degraded in Stage I was 36.04% (18.86 + 12.80 + 8.75 x 0.5) of the initial dry weight. The percentage of cellulose and hemi-cellulose degraded were 43.1% (18.86/43.74) and 41.1% (12.8/31.17) respectively.

Using the same approach, fiber contents for the second stage sample can be adjusted as shown in table 4-13.

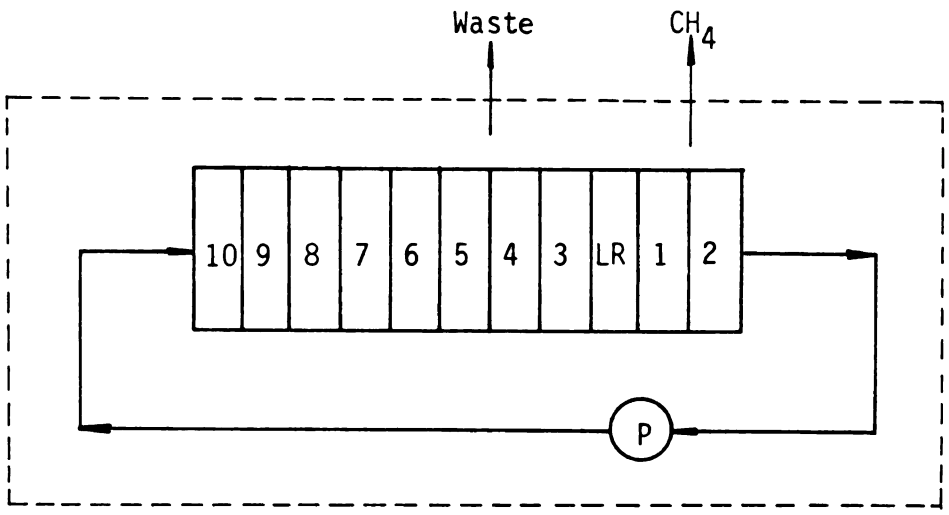
Table 4-13 Adjustment of Straw Fibrous Contents (Stage II)

	Cellulose (%)	Hemi- (%) Cellulose	Lignin (%)	Ash (%)	Other (%)
(1) Fresh Straw	43.74	31.17	6.52	4.90	13.67
(2) Fermented Before Adjust	45.97	30.69	9.28	6.97	7.09
(3) Fermented After Adjust	32.29	21.56	6.52	4.90	4.98
(4) Difference (1) - (3)	11.45	9.61	0.0	0.0	8.69

The amount of material degraded in Stage II is therefore equal to 25.40% (11.45 + 9.61 + 8.69 x 0.5) of the initial dry weight. The percentage of cellulose and hemi-cellulose degraded in this stage were 26.2% and 30.8% respectively. The ratio of percent degradation in Stage I to Stage II is then equal to : $^{36.04}/_{25.40} = 1.42$.

4.4.3 Percent of Substrate Degradation Based on Mass Balance Calculation

Considering the packed reactors, liquid reservoir, and anaerobic filters as one unit, and using the simplified sketch shown below, a mass balance can be used to relate substrate degradation and methane production.



The total amount of biodegradable COD produced from straw degradation in the system is further converted by microorganisms to fermentative product COD and microbial cell solids COD. The fermentative COD includes organic acid COD in the liquid phase and methane COD in the gas phase. Although the type of COD is changed, the over-all COD should be not reduced. Based on this concept, the extent of substrate degradation can be calculated according to the total methane produced from the system.

The experimental results presented in Section 4.3 have shown that no organic acids accumulated in the system. Therefore, most of the organic acids should have been converted to methane and carbon dioxide. It has been pointed out in the previous sections that the majority, about 98 %, of the degraded substrate COD is converted to methane while the rest is utilized by microorganisms for cell growth. Under "stable" operating conditions, it may be assumed that the total organic acid COD in the system remains unchanged. Then, based on mass balance concepts, the product COD must be equal to the summation of methane COD and

microbial cellular COD as the expression below shows:

$$\left[\text{Product COD}\right] = \left[CH_4\ \text{COD}\right] + \left[\text{Cellular COD}\right] \qquad (4\text{-}10)$$

In Stage I, the total amount of straw input into the system in the period from 3/29/82 to 5/8/82 (Day 220 to Day 260) was 453 grams (60 x 8 x 94.33%) and the total methane produced from the system was 50,038 ml (see Section 4.3.1.3). Thus, the equivalent methane COD can be calculated as:

$$2.525\ (^{g\ COD}/_{1}) \times 50.038\ (1) = 126.35\ \text{gm COD} \qquad (4\text{-}11)$$

Assume y percent of the straw is lost due to degradation and leaching. Since 1.0 gram of cellulose can produce 1.186 gram of COD (Appendix C), and the fresh straw contains 74.91 % of cellulose and hemi-cellulose, the equivalent product COD is:

$$453(y) \times 1.186 \times 0.7491 = 402.46(y)\ \text{gm COD} \qquad (4\text{-}12)$$

The amount of VFA COD wasted from the system in this period resulting from liquid sampling was 2.76 g COD. Assume 50% of the total soluble COD was VFA COD. And assume 200 ml of liquid was wasted every time the oldest packed reactor was removed and that the total COD concentration of the oldest reactor was 3000 mg/l. Then, the total amount of COD wasted was:

$$2.76/0.5 + 0.2 \times 8 \times 3.0 = 10.32\ \text{g COD}$$

Since 98 % of the product COD was converted to CH_4, the percent of

straw degraded can be obtained from the relationship:

$$126.35 = (402.46(y) - 10.32) \times 0.98$$

$$y = 34.6\ \% \qquad (4\text{-}13)$$

In Stage II, the amount of methane produced in the period between 10/4/82 and 11/10/82 was 41,197 ml and the total straw introduced into the system during this period was 509.4 gram (60 x 9 x 94.3%). The amount of VFA COD wasted from the system was 7.82 g COD (1.21/0.5 + 0.2 x 9 x 3.0). Again, assuming that K percent of the straw was degraded, and applying the same approach as above, the percent of substrate degradation (K) can then be calculated from the following expression:

$$(509.4(K) \times 1.186 \times 0.7491 - 7.82) \times 0.98 = 2.525 \times 41.197$$

$$K = 25.2\ \% \qquad (4\text{-}14)$$

The ratio of percent substrate degradation in Stage I to Stage II based on this method is equal to: $\frac{34.6}{25.2} = 1.37$, which is the same as the ratio of methane production per gram substrate input in Stage I to Stage II.

4.5 Summary of The Performance of Anaerobic Filtrs And The Extent of Substrate Degradation.

The performance of the anaerobic filters in Stage I and Stage II as well as the extent of substrate degradation in these two stages are summarized in Table 4-14. As shown in Table 4-14, the extent of solid

substrate degradation obtained by mass balance calculation from methane production was 34.6% in Stage I and 25.2% in Stage II. These two values agreed favorably with the values obtained from fiber analysis. The values obtained from the weight loss measurement were both smaller than the data obtained from the other two methods. A possible reason for the difference may be due to liquid phase solids that adhering to the straw surface and then being included when the straw was dried for weighing. The ratio of the percent of substrate degradation in Stage I to Stage II range from 1.34 to 1.37, that are very close to the number of the ratio of methane production per unit weight of straw input.

Table 4-14 Summary of The Performance of Anaerobic Filters and The Extent of Substrate Degradation

	Stage I	Stage II	Ratio
(1) CH_4 Production (ml/day)			
Filter 1	761 (S.D.=158)	925 (S.D.=116)	
Filter 1+2	1035 (S.D.=230)	1052 (S.D.=149)	
(2) Specific CH_4 Production			
(a) l/day/l reactor			
Filter 1	1.75	2.12	
filter 1+2	1.19	1.20	
(b) ml CH_4/g substrate input			
Filter 1+2	104.3	76.3	1.37
(3) Extent of Substrate Degradation (%)			
(a) Weight Loss	27.5	20.3	1.36
(b) Fiber Analysis	36.0	25.4	1.34
(c) Mass Balance	34.6	25.2	1.37

(4) COD Loading, lb COD/day/1000 ft^3				
	TSCOD	VFACOD	TSCOD	VFACOD
Filter 1	--	496	594	273
Filter 1+2	--	278	297	136
(5) COD Removal Efficiency (%)				
Filter 1	--	87.3	43.8	91.7
Filter 1+2	--	97.7	54.1	98.4

TSCOD = Total Soluble COD
VFACOD = Volatile Fatty Acid COD

4.6 Production Rate of Nonbiodegradable COD

If assume that the nonbiodegradable COD is constant throughout the system and that the soluble COD in Filter 2 is nonbiodegradable, the rate of nonbiodegradable COD (NDCOD) production in the system can be calculated according to the following expression:

$$\left[\begin{array}{l}\text{Rate of NDCOD}\\ \text{Production}\end{array}\right] = \left[\frac{\text{Average Vol. of New Water Added}}{\text{Day}}\right] \times \text{NDCOD}$$

In stage II, 243 liquid samples were taken from the system from 10/1/82 to 11/9/82 (39 days) and the average volume of one sample was 4.5 ml. During this period, nine packed reactors were removed and replaced with the fresh straw. Assume 200 ml of liquid was wasted each time the oldest reactor was removed. Then, the total liquid wasted was:

$$4.5 \text{ (ml/sample)} \times 243 \text{ (samples)} + 200 \text{ (ml)} \times 9 = 2894 \text{ ml.} \quad (4\text{-}15)$$

and the mass of NDCOD wasted was: 3000 (mg/l) x 2.894 (l) = 8682 mg COD. Therefore, the average nonbiodegradable COD production rate in Stage II was:

$$8682 \text{ (mg COD)} / 39 \text{ (days)} = 222.6 \text{ (mg COD/day)} \quad (4\text{-}16)$$

The amount of total methane produced from the whole system from 10/4/82 to 11/10/82 (37 days) was 41,197 ml. As has described in Section 4.4.3, 98% of the degraded COD was converted to methane and the

amount of COD wasted from the system was 7.82 grams. The total COD produced in this period was:

$$\frac{2.525 \text{ (g COD/l } CH_4\text{)} \times 4.1197 \text{ (l)}}{0.98} = 113 \text{ g COD} \qquad (4\text{-}17)$$

Because the rate of total COD production was: 113 g/37 (days) = 3063 mg COD/day. Thus, the ratio of the nonbiodegradable COD production rate to the total COD production rate is: 222.6/(222.6 + 3063) = 6.8%.

4.7 Response of Anaerobic Filters to Transient Substrate Loading

In Stages I and II when the liquid euqalization reservoir was connected in the system, it has been shown that the anaerobic filters were capable of accommodating the mild fluctuations of influent substrate concentration and temporary periods of inoperation. In order to determine whether the liquid reservoir was essential for the system or whether it may be excluded from the system to minimize the overall cost, the third stage experiment was conducted without the liquid equalization reservoir in the system. Therefore, the anaerobic filters were receiving the highly variable COD concentration directly from the newest packed reactor. This section will present the response of the anaerobic filters to this kind of transient substrate loading.

The filter response to loading changes as indicated by total soluble COD is illustrated in Figure 4-19 which includes two figures; Figure B represents the change of soluble COD versus time after the liquid reservoir was removed, and Figure A shows the effluent COD con-

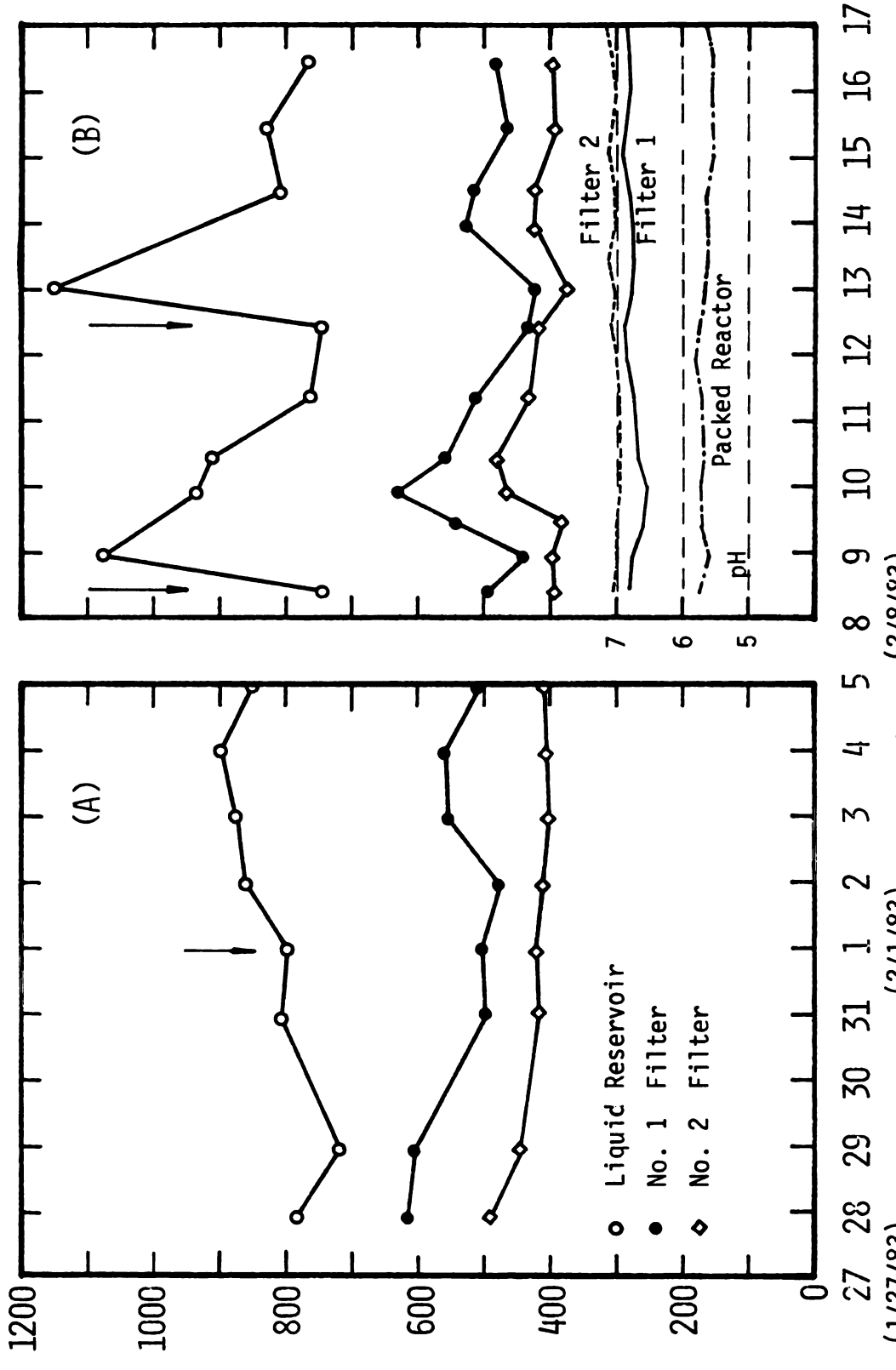


FIGURE 4-19 TOTAL SOLUBLE COD, BEFORE (FIG.A) AND AFTER (FIG.B) DIRECT LIQUID SUBSTRATE INPUT INTO ANAEROBIC FILTERS.

centration when the liquid reservoir was still in the system. Also shown in Figure B are the pH values of the effluent from the two anaerobic filters and the newest packed reactor. As shown in Figure 4-19 B, the effluent COD concentration in the filters increased rapidly about 20 hours after the installation of a new packed reactor. The existing microbial population which had developed inside the anaerobic filters was not able to completely utilize the suddenly increased influent substrate resulting in a higher COD effluent. With the liquid reservoir in place, (Figure 4-19 A) the gradually increasing influent concentration did not create a jump in effluent COD from the anaerobic filters.

The effects of transient loading is also demonstrated by Figure 4-20, which shows that the individual volatile fatty acids in the filter effluents exhibited a sharp rise, especially for the acetic acid.

Even though the effluent VFA COD in Filter No.1 increased to a certain extent, the pH values, as shown in Figure 4-19, did not drop below 6.5. Therefore, the methanogenic bacteria were not damaged, although their activity was affected. Figure 4-19 also shows that the majority of VFA COD was utilized by both filters. The escaping COD peak would recirculate back to the filters for later degradation.

The limited data provided by Stage III indicated that effluent quality of the anaerobic filters was affected by the sudden increase in COD loading but the filters did not fail.

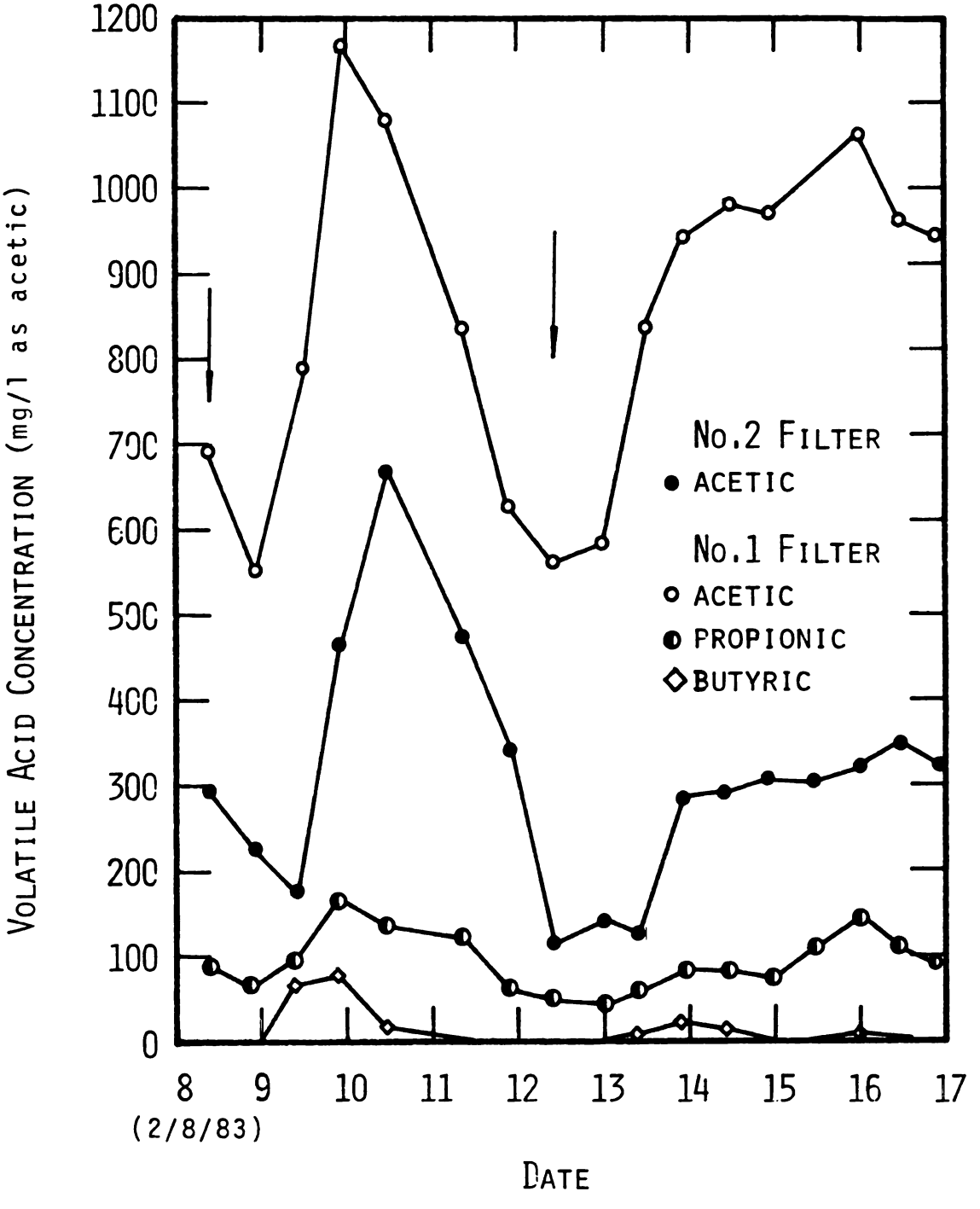


FIGURE 4-20 INDIVIDUAL VOLATILE FATTY ACID CONCENTRATION OF ANAEROBIC FILTER EFFLUENT. (DIRECT LIQUID INPUT)

CHAPTER FIVE

MATHEMATICAL MODEL OF SOLID SUBSTRATE DEGRADATION IN PACKED REACTORS

The characteristic of soluble COD production in packed reactors has been presented in terms of the experimental data in Section 4.1.1. In this chapter, the special nature of COD production will be studied in terms of a mathematical model based on mass balance concepts. The theoretical results generated from the mathematical model will be graphically related to the experimental data.

5.1 Model Development

The following assumptions have been made in order to develop the model:

1. The solid substrate is uniformly distributed throughout the packed reactor (in the straw holding section).
2. The solid substrate has a uniform porosity.
3. The liquid flow is uniform and no short-circuiting occurs in the reactor.
4. Nothing other than the wheat straw contributs to the soluble COD production.
5. No external electron acceptors other than carbon dioxide exist in the reactor.
6. Temperature and pH are constant during the course of fermentation.

Consider a single packed reactor (Figure 5-1) which is divided into N segments, each segment having the same longitudinal length, L, and containing the same amount of liquid volume and solid substrate.

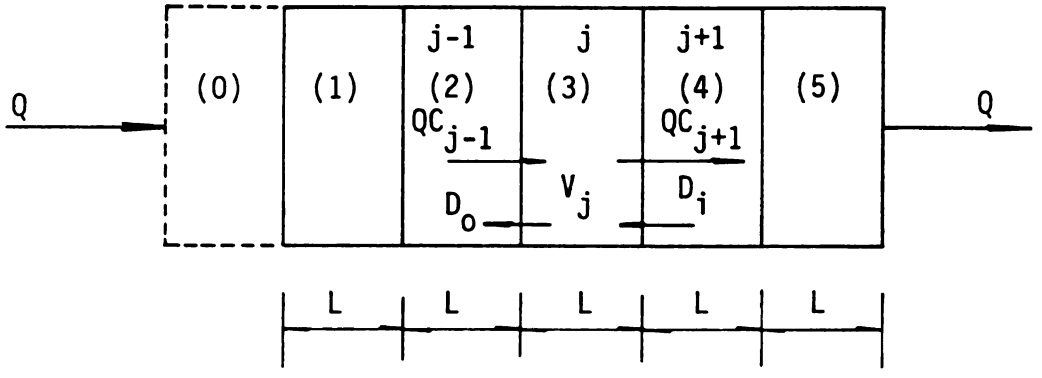


Figure 5-1 Schematic diagram of a packed reactor divided into five segments.

Further assume that the rates of soluble COD production for all pieces of straw in one segment are equal, and that the concentration of soluble COD in each segment is uniformly distributed. Then, a mass balance equation on soluble COD around Segment j can be written as:

$$\left[\begin{array}{l}\text{Rate of change of}\\ \text{COD in Section j}\end{array}\right] = \left[\begin{array}{l}\text{Influent}\\ \text{COD}\end{array}\right] - \left[\begin{array}{l}\text{Effluent}\\ \text{COD}\end{array}\right] + \left[\begin{array}{l}\text{COD}\\ \text{Diffused in}\end{array}\right]$$

$$+ \left[\begin{array}{l}\text{COD}\\ \text{Diffused Ont}\end{array}\right] + \left[\begin{array}{l}\text{Substrate COD}\\ \text{Production}\end{array}\right]$$

$$+ \left[\text{COD Utilized}\right]$$

or written in the mathematical form,

$$V_j\left(\frac{\partial C_j}{\partial t}\right) = QC_{j-1} - QC_j + A_jD_{ij}\left(\frac{\partial C_{ij}}{\partial X}\right) - A_jD_{oj}\left(\frac{\partial C_{oj}}{\partial X}\right) + V_jP_{nj} - R_jV_j \qquad (5\text{-}1)$$

in which:

V_j = volume in segment j, (cm^3)

A_j = cross sectional area in segment j, (cm^2)

Q = liquid flow rate, (ml/hr)

C_j = soluble COD concentration in segment j, (mg/l)

t = time, (hr.)

D_{ij} = dispersion coefficient, into segment j, (cm^2/hr)

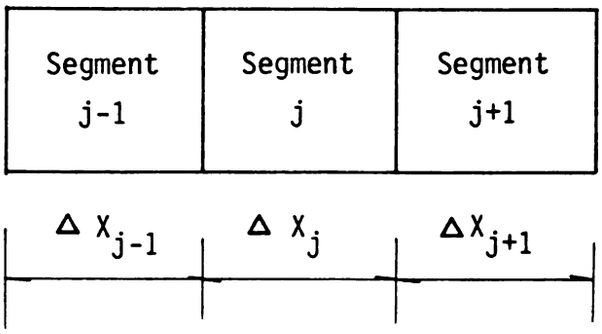
D_{oj} = dispersion coefficient, out from segment j, (cm^2/hr)

X = length of one segment, (cm)

P_{nj} = rate of soluble COD production from solid substrate in segment j, (mg/l/hr), n =1, 2

R_j = rate of COD utilization in segment j, (mg/l/hr)

The dispersion differentiaton terms, $A_jD_{ij}\left(\frac{\partial C_{ij}}{\partial X}\right)$ and $A_jD_{oj}\left(\frac{\partial C_{oj}}{\partial X}\right)$, in Equation 5-1 can be simplified by applying the finite difference technique:



let:

$$\frac{\partial C_{oj}}{\partial X} = \frac{C_{j+1} - C_j}{\frac{1}{2}(X_j + X_{j+1})} = \frac{C_{j+1} - C_j}{L} \qquad (5\text{-}2)$$

$$\frac{\partial C_{ij}}{\partial X} = \frac{C_j - C_{j-1}}{\frac{1}{2}(X_j + X_{j-1})} = \frac{C_j - C_{j-1}}{L} \qquad (5\text{-}3)$$

Substitute Equation 5-2 and Equation 5-3 into Equation 5-1, to obtain:

$$\frac{\partial C_j}{\partial t} = \frac{1}{V_j}\left[QC_{j-1} - QC_j + A_j D_{ij} \frac{(C_{j+1} - C_j)}{L} - A_j D_{oj} \frac{(C_j - C_{j-1})}{L} \right] + \left[P_{nj} - R_j \right] \qquad (5\text{-}4)$$

Rearrange the terms in Equation 5-4:

$$\frac{\partial C_j}{\partial t} = C_j \left[\frac{1}{V_j} \left(-Q - \frac{A_j D_{ij}}{L} - \frac{A_j D_{oj}}{L}\right)\right]$$

$$+ C_{j-1} \left[\frac{1}{V_j} \left(Q + \frac{A_j D_{oj}}{L} \right) \right] + C_{j+1} \left[\frac{1}{V_j} \times \frac{A_j D_{ij}}{L} \right]$$

$$+ \left[P_{nj} - R_j \right] \qquad (5\text{-}5)$$

Now define;

$$B_{1j} = \frac{1}{V_j} \left(-Q - \frac{A_j D_{ij}}{L} - \frac{A_j D_{oj}}{L}\right) \qquad (5\text{-}6)$$

$$B_{2j} = \frac{1}{V_j} \left(Q + \frac{A_j D_{oj}}{L} \right) \qquad (5\text{-}7)$$

$$B_{3j} = \frac{1}{V_j} \times \frac{A_j D_{ij}}{L} \qquad (5\text{-}8)$$

Substitute Equations 5-6, 5-7, 5-8 into Equation 5-5, to obtain:

$$\frac{\partial C_j}{\partial t} = B_{1j} C_j + B_{2j} C_{j-1} + B_{3j} C_j + P_{nj} - R_j \qquad (5\text{-}9)$$

The COD production term, P_{nj}, in Equatin 5-9 has two forms:

(1) From the "zero" time when the packed reactor was connected into the system to time "hour 6.0", the COD production term is:

$$P_{1j} = S_t f_j + S_j K_{1j} \log(t) \qquad (5\text{-}10)$$

(2) After hour 6, the COD production term is:

$$P_{2j} = S_j K_{2j} t^{-\alpha} \qquad (5\text{-}11)$$

Where f_j is the soluble COD concentration per unit time per unit weight of straw when water is just added to the reactor due to instentaneous leaching. While K_{1j}, K_{2j}, and are COD production kinetic constants. Equations 5-10 and 5-11 were obtained according to the characteristic curves of the experimental COD measurements shown in Figure 4-1. Therefore, Equation 5-9 actually contains two series of system differential equations.

Since:

$$A_j = A_1 = A_2 = \cdots = A$$
$$V_j = V_1 = V_2 = \cdots = V_t/N = V$$
$$S_j = S_1 = S_2 = \cdots = S_t/N = S$$

where:

A = cross-sectional area

V_t = total volume of the straw holding section

S_t = total straw weight.

And assume:

$$R_j = R_1 = R_2 = \cdots = R$$

$$f_j = f_1 = f_2 = \cdots = f$$

$$D_{ij} = D_{i1} = D_{i2} = \cdots = D_i$$

$$D_{oj} = D_{o1} = D_{o2} = \cdots = D_o$$

$$K_{1j} = K_{11} = K_{12} = \cdots = K_1$$

$$K_{2j} = K_{21} = K_{22} = \cdots = K_2.$$

Then, Equations 5-6 to 5-8 can be rewritten as:

$$B_1 = \frac{1}{V}\left(-Q - \frac{AD_i}{L} - \frac{AD_o}{L}\right) \tag{5-12}$$

$$B_2 = \frac{1}{V}\left(Q + \frac{AD_o}{L}\right) \tag{5-13}$$

$$B_3 = \frac{1}{V}\left(\frac{AD_i}{L}\right) \tag{5-14}$$

Therefore, Equation 5-9 can be rewritten as:

$$\frac{\partial C_j}{\partial t} = B_1 C_j + B_2 C_{j-1} + B_3 C_{j+1} + P_n + R \tag{5-15}$$

$$n = 1, 2$$

in which:

$$P_1 = S f + S K_1 \log(t) \tag{5-16}$$

$$P_2 = S K_2 t^{-\alpha} \tag{5-17}$$

As can be seen from Figure 4-1, the COD production rate was not a constant value. Therefore, Equation 5-15 cannot be simplified by just making $\frac{\partial C}{\partial t} = 0$. Instead, it has to be solved for the COD concentration for any given time t. Examining Equation 5-15, it is found that the equation contains three unknown variables. Hence, two boundary conditions have to be provided in order to satisfy the equation.

If a packed reactor is divided into five segments as shown in Figure 5-1, the first segment, shown with a dashed line and designated Segment 0, is a dummy segment which provides the lower boundary condition for Segment 1; and the upper boundary condition of Segment 5, is given by making $C_5 = C_{5+1}$. In other words, assume that the influent COD concentration is the concentration in the dummy segment and the effluent COD concentration is the same as the COD concentration inside Segment 5. Thus, there are six system partial differential equations describing the six segments. These equations are readily solved numerically by using a computer. The Runge-Kutta method was applied to solve the equations and a FORTRAN program MODEL was written to handle the calculations and to create the data files for computer plotting. The program MODEL is included in Appendix E-2.

The three known variables, B_1, B_2, and B_3, can be computed from Equations 5-12 to 5-14 given the basic hydraulic and reactor dimension data. The COD utilization term, R, can be estimated from the amount of methane produced by the packed reactor. The average methane produced from a packed reactor in one solids retention time was about 1.98 % of the total methane produced from two anaerobic filters in the same period of time. From Figure 4-14, the total methane produced from the two filters from 10/10/82 to 10/27/82 was about 17,000 ml. Therefore, the

daily COD consumption in a packed reactor can be calculated by:

$$\frac{2.525\ (g\ COD/_{l}\ )\ x\ 17.0\ (l)\ x\ 0.0198}{18\ (day)}$$

$$= 0.0472\ (gm\ COD/day) = 1.967\ (mg\ COD/hr) \qquad (5\text{-}18)$$

The volume of the straw holding section in a packed reactor is equal to 435 ml. Therefore, COD utilization per unit volume in the packed reactor is:

$$R = \frac{1.976}{0.435} = 4.52 \frac{mg/l}{hr}$$

$$R_j = \frac{R}{N} = \frac{4.52}{N} \qquad (5\text{-}19)$$

where N = No. of segments in a packed reactor.

Before Equation 5-15 can be solved, four constants: f, K_1, K_2, α, for Equations 5-16 and 5-17 have to be provided. The value of f can be obtained from Figure 5-2 which is the semi-log plot of the measured effluent soluble COD concentration versus time for a packed reactor before the COD concentration reached the peak point. If we let $F = S_t f$, the interception point of the straight line and the Y-axis in Figure 5-2 gives the value of F which is approximately equal to 1,000. Because S_t = 60 grams, then f = F/60 =16.7. The exponentional constant, α, in Equation 5-17 can be obtained from the slope of the straight line in Figure 4-3. The value of α is approximately - 0.23. The other two constants, K_1 and K_2, were determined by trial and error. The best values of K_1 and K_2 as well as the final values of f and

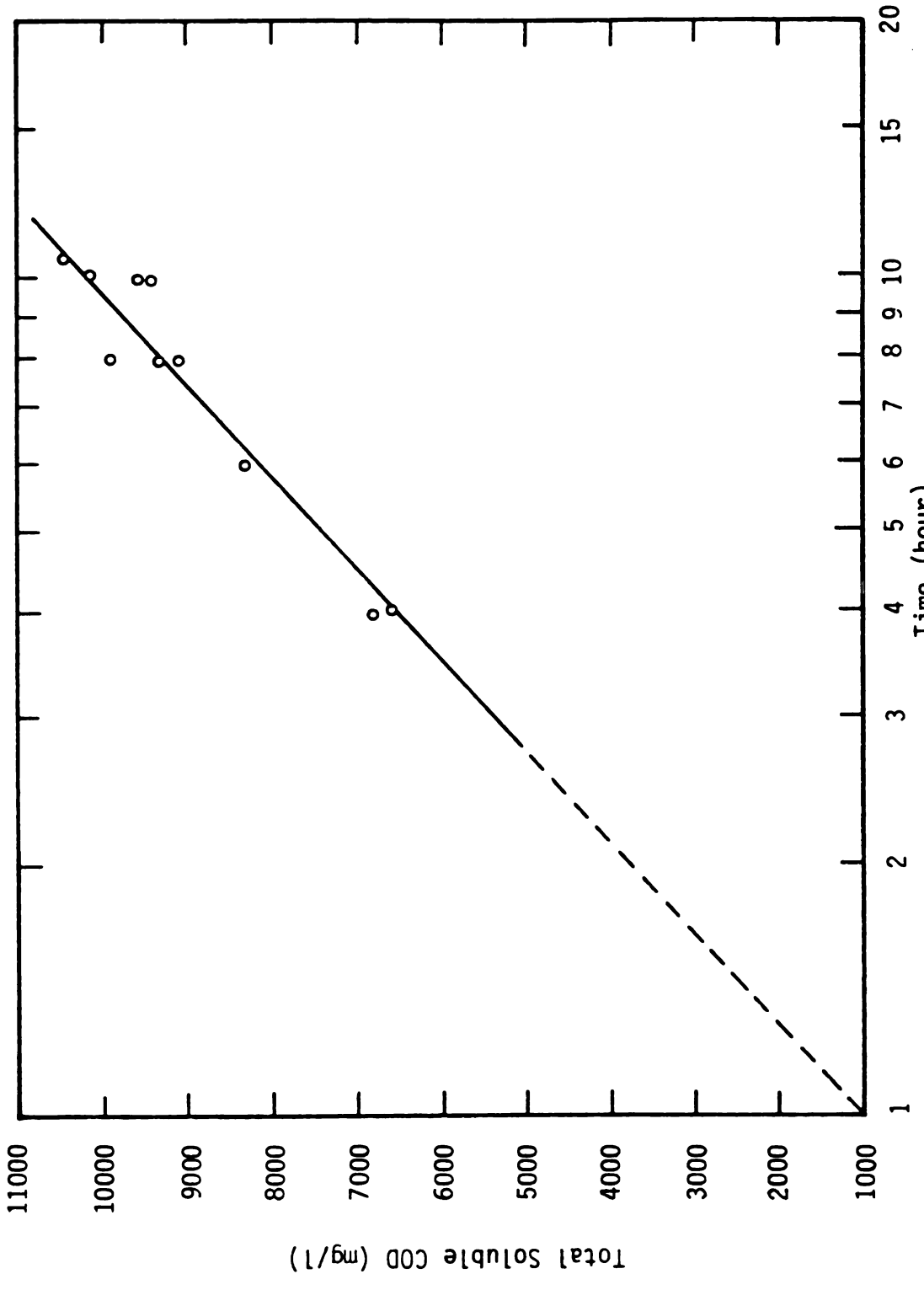


Figure 5-2 Experimental Effluent Soluble COD Concentration From Newest Packed Reactor (Stage II)

α are those that result in the best fit of the experimental data by Equation 5-15. The dispersion coefficients, D_o and D_i, were assumed identical and their values were selected from the literature (Motta, 1976) as 0.00482 CM^2/hr.

The program MODEL was executed several times on a DEC PDP-11/23 minicomputer until the best fit curve was obtained and the best values of K_1 and K_2 were determined. Program MODEL was designed to be interactive so that the variables of Equation 5-15 could be changed conveniently from the terminal screen without exiting and restarting the program.

Good results were obtained from the model, since the soluble COD concentration calculated from MODEL agreed with the experimental data very well. Figure 5-3 demonstrates the computational results from MODEL compared with the real experimental COD data to evaluate how well the results computed from the model agreed with the measured data.

The final parameters for Equations 5-16 and 5-17 were found to be:

$f = 16.7$ (mg/l/hr·gm)

$\alpha = -0.238$

$K_1 = 9.86$ (mg/l/hr·gm)

$K_2 = 7.98$ (mg/l/hr·gm)

$R = 4.52$ (mg/l/hr)

Other input data for MODEL can be found from Appendix E-2.

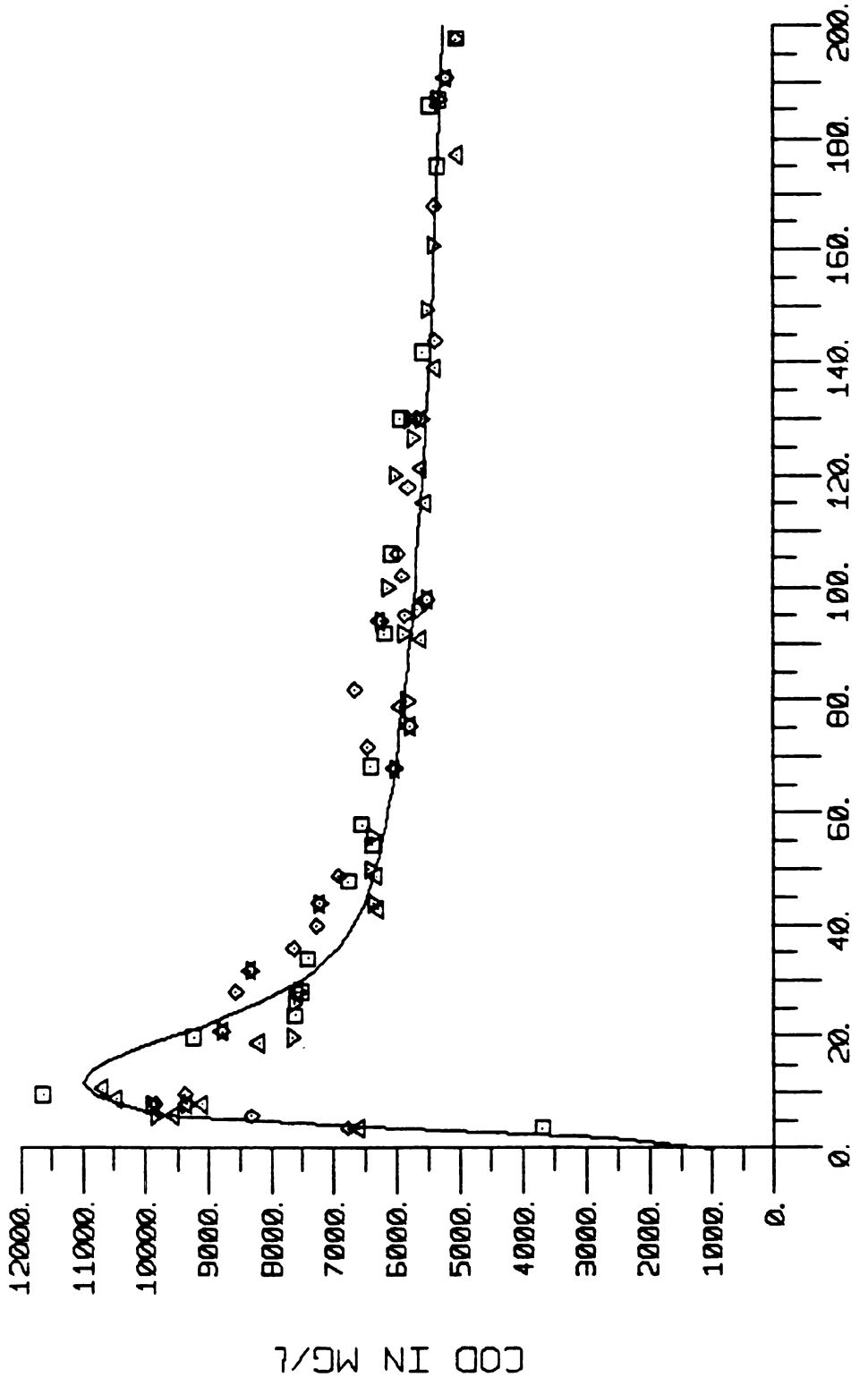


Figure 5-3 Effluent soluble COD concentration of packed reactors obtained from MODEL and from experimental measurements.

5.2 Limitations and Discussion of The Application of MODEL

Most mathematical models, including the one presented in this chapter, are established under certain assumptions. Therefore, their applications are subjected to some limitations attributed by their fundamental conditions. The assumptions described in the preceding section limit the application of this model as well. Among those conditions, the two factors most affecting the computional results when MODEL is used are: (1) the liquid flow characteristics, in that the flow rate was assumed strictly steady, was identical everywhere inside the straw holding area, and no short circuiting existed in the reactor; and (2) the straw substrate input conditions, such as substrate solids retention time and substrate solids concentration. The two COD production kinetic parameters, K_1 and K_2, were determined, and are only valid for a substrate input interval of three days, a straw weight of 60 grams, and a packed reactor divided into five segments.

In order to test the suitablity of the values of K_1 and K_2 in MODEL when the packed reactor is divided into a different number (N) of segments than five, the same values of K_1 and K_2 as obtained from N = 5 were used to run MODEL. The number N was varied from 2 to 6. Figure 5-4 shows the theoretical soluble COD concentrations for five different conditions, the lowest curve (N = 2) represents the effluent soluble COD concentration when the reactor was divided into two segments, and the highest curve, six segments. It can be seen that using the same values of K_1 and K_2 resulting in similar curves for different segment numbers. Therefore, it can be concluded that the two COD production parameters obtained from this study were suitable for various segment

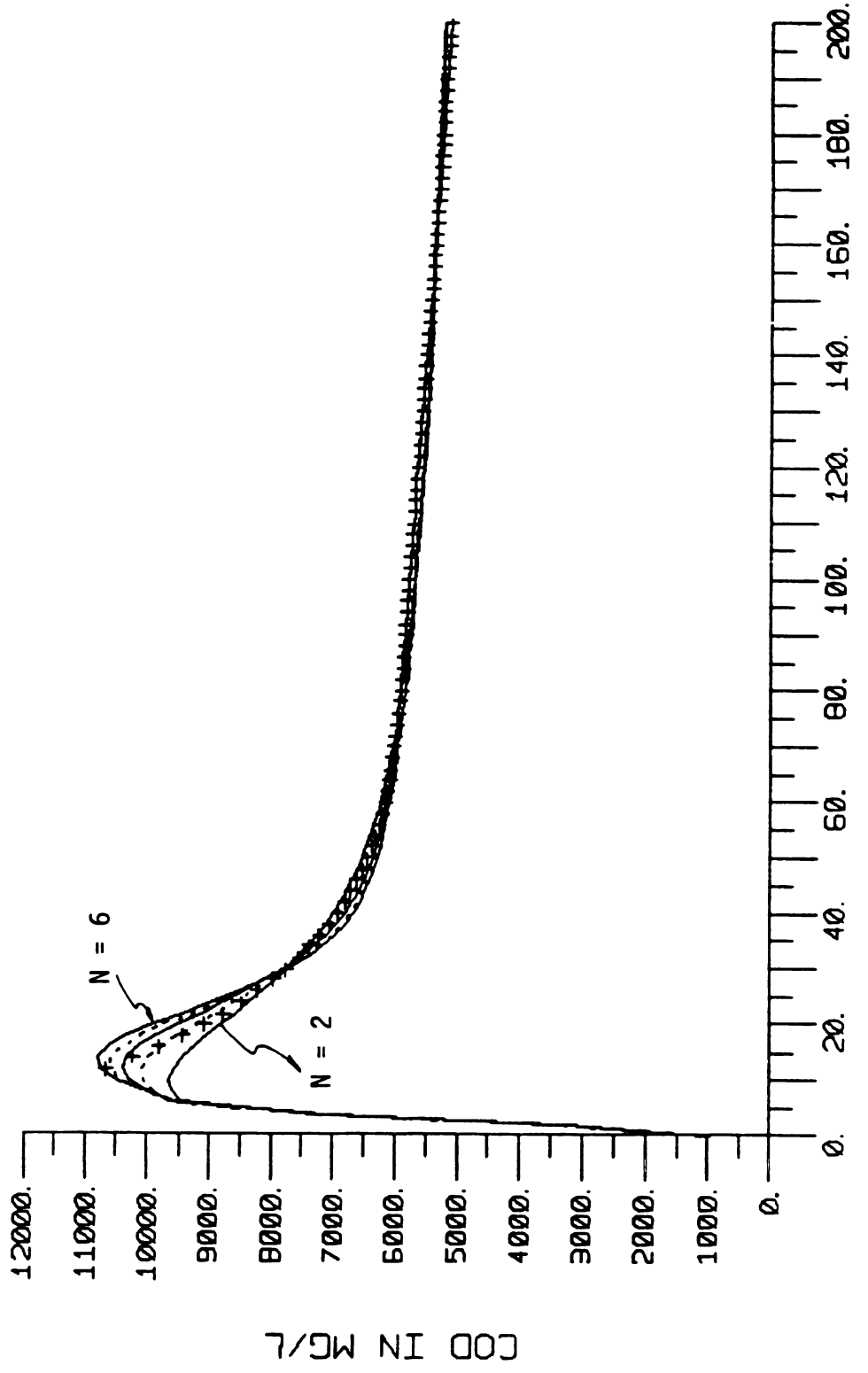


Figure 5-4 Estimated soluble COD concentrations of packed reactors by MODEL at various segment numbers.

nembers (N > 1) in MODEL.

The model presented in this chapter is still a prototype. Once the relationships between different hydraulic retention times and substrate solids retention times with the values of K_1, K_2, and α are developed in the future, MODEL will be able to predict the effluent COD concentration of a high solids packed reactor at any hydraulic retention time and solids retention time without running wet chemical measurements, and can be applied to associate with other automatic process control devices, such as pH control.

CHAPTER SIX

ENGINEERING APPLICATION

The experimental results of the proposed process have shown the technical feasibility of producing methane from un-pretreated wheat straw. This chapter will discuss the application of this process to a full scale system.

6.1 Configuration and Liquid Flow Pattern of the Packed Reactors

For full scale operation, there are three possible basic flow configurations for the packed reactors: (1) counter-current series; reactors connect in series with the liquid flow opposite to the solid substrate movement as studied in this project, (2) co-current series; reactors connect in series but with the liquid flow in the same direction as the solid substrate movement, and (3) parallel; packed reactors connected in parallel as shown in Figure 6-1.

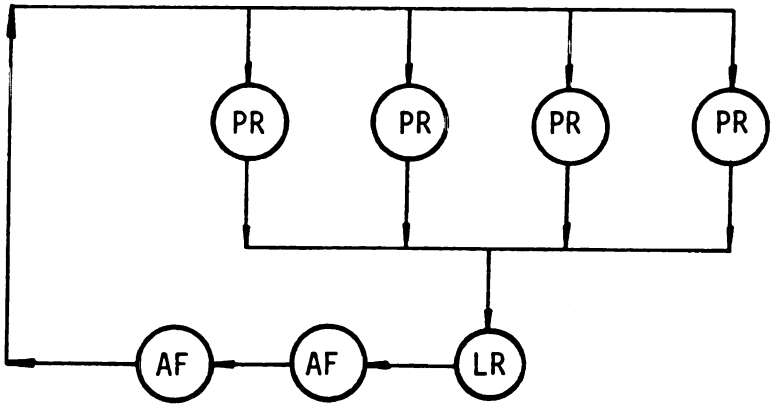


Figure 6-1 Schematic diagram of parallel connected reactor system

6.2 Comparsion of The Three Types of Reactor Systems

Among the three reactor systems metioned above, the last two types have not been experimentally studied. The likely nature of their performance will be disscused and compared based on the knowledge obtained from the studies of the first reactor configuration. Comparsion of the operation for the three reactor systems will be made in terms of the following parameters: (1) the need for a liquid reservoir, (2) the extent of solid substrate hydrolysis, (3) the extent of leaching, and (4) operational simplicity.

6.2.1 The Need for A Liquid Reservoir

The first configuration of counter-current, series connected reactors always has the liquid phase passing through the newest packed reactor last so that the high COD and low pH liquid can be removed shortly after it is produced. As previously mentioned, a liquid reservoir can be used to equalize the highly variable COD before it is introduced into the anaerobic filter. The second configuration, on the contrary, has the newest packed reactor located at the begining of the liquid flow stream. Thus the high COD liquid flows from the newest reactor through a series of packed reactors that will provide additional liquid volume to dilute the high COD fluid due to dispersion. Therefore, the liquid reservoir can more easily be eliminated from the co-current reactor system.

The third system, packed reactors connected in parallel, may require a liquid reservoir downstream of the packed reactors to equalize the peak of high COD liquid when a new packed reactor is added to

the system and to simplify the operation procedure.

6.2.2 Extent of Solid Substrate Hydrolysis in The Packed Reactors

The extent of solid substrate hydrolysis is affected by several factors such as pH, microbial population, and reaction time. The pH in a single packed reactor for the counter-current system increased gradually from 5.5 when new to 6.8 when removed as shown in Figure 4-7. In the co-current system, the pH values in all the packed reactors are expected to be lower than 6.0 because the newest reactors are located at the upstream end. The high COD and VFA's produced in the newest packed reactor would be augmented as the liquid flows through the other reactors. Therefore, the pH would remainn low, probably not higher than 6.0.

In the parallel system, the initial pH value in a new packed reactor would fall between 5.5 and 6.0; then it would quickly rise to a higher value because the influent to each reactor would always be high pH liquid from the anaerobic filter. Since the best pH range for substrate hydrolysis is between 6.2 and 7.4, the parallel reactor system would have the most favorable pH range for substrate hydrolysis.

Effluent from the anaerobic filter is likely to contain active bacteria which can be partly retained in the packed reactors. The parallel connected reactor system has every packed reactor directly connected to the anaerobic filter. Therefore, packed reactors in this type of system would contain more bacteria and hence provide a higher degree of hydrolysis than the two types of series connected reactor systems.

If the number and the size of packed reactors were the same for the three types of reactor systems, the total solids retentation time in each system would be the same for all three cases.

As a summary of the above discussion, packed reactors connected in parallel should provide the highest degree of substrate hydrolysis followed by the counter-current series system.

6.2.3 The Extent of Leaching

Although the initial production rate of soluble COD by leaching from a new packed reactor may be affected by the liquid flow rate through the reactor, the total amount of COD produced by leaching should not be affected by the flow conditions or the initial COD concentration in the influent liquid. Therefore, the extent of leaching from the solid substrate should be independent of reactor configuration.

6.2.4 Operation Simplicity

As has stated in Chapter 3, when a new reactor is installed into the series reactor system, the connecting piping between the liquid reservoir, the newest reactor, the oldest reactor, the second oldest reactor, and the anaerobic filter (see Figure 3-5) have to be disconnected and then reconnected in the new positions such that the newest reactor is always located last in the flow stream and the effluent from the anaerobic filter can always flows into the oldest reactor. Unlike the laboratory system in which flexible tubing can be used and only two tubes (one influent and one effluent) are needed, the full scale system

has to use rigid plastic or metal pipes and four pipes (two influent and two effluent pipes) with associated valving are required for each packed reactor to connect to the liquid reservoir, the anaerobic filters and the adjacent packed reactors. However, for the parallel reactor configuration, the position of the newest packed reactor in the system is not important and only two pipes (one influent and one effluent pipe) are needed. A packed reactor needs only to be isolated with two valves while being emptied and refilled; it does not need to be relocated in the series flow pattern. Therefore, the piping, valving, and operational procedures are simplier when a parallel configuration is used.

6.3 Ultimate Usage of The Treated Solid Substrate

In lieu of disposal, the treated substrate may be used for pulp and paper making in which case it can be transported directly to the pulp and paper making facilities as a slurry without needing drying. The treated straw can be also used for animal bedding after the straw is dried. If the treated straw is not to be further utilized, it may be disposed by landfill or by spreading on farm land.

6.4 Operation of Packed Reactor at Short Solid Retention Time

The experimental results have shown that 65% of the soluble COD produced from a new packed reactor was contributed by rapid leaching of straw. Therefore, an alternative process would be to operate the packed reactors at a short solids retention time, to produce a high COD liquid simply by leaching without temperature control. The effluent

from the packed reactors can then be introduced to the temperature controlled anaerobic filter for conversion to methane gas.

6.5 Conclusion of The Engineering Application

Based on the above disscusion, the proposed process can be applied to a full scale system, using any of three types of reactor configurations. A summary of the comparsion between three reactor systems is shown in Table 6-1:

Table 6-1 Comparision of The Three Types of Reactor Systems

	Counter-current Series	Co-current Series	Parallel
Liquid Reservoir	Needed	Not Needed	Needed
Extent of Hydrolysis	Intermediate	Lowest	Highest
Extent of Leaching	Same	Same	Same
Operation Simplicity	More Complicated	More Complicated	Simpler

According to the information shown above, the parallel system is most suitable for a full scale system due to its simpler operation and greater extent of substrate hydrolysis.

CHAPTER SEVEN

CONCLUSIONS

The following conclusons about the coupled high solids anaerobic fermentation and anaerobic filtration of cellulosic residues can be drawn from the results of this research.

1. The proposed process of coupled high solids fermentation and anaerobic filtration has been successfully operated for an extended period of time. The use of packed reactors has successfully overcome the difficulties of handling substrate at the very high concentration of 34.4%. Recirculation of the liquid phase provided the buffer capacity to prevent pH inhibition of methanogenesis.
2. The major function for the packed reactors was the leaching and hydrolysis of the solid substrate and the production of organic acids. In addition, about 15% of the total methane production occured in the packed reactors, largely it is presumed, from utilization of the hydrogen produced during acid formation.
3. Initially, soluble COD production from a packed reactor is contributed mostly by rapid leaching of the straw. The effluent total soluble COD from a packed reactor reaches a peak concentration in about 12 to 14 hours after fresh substrate is introduced. After the peak occures, the soluble COD concentration decreased logarithmically with respect to time.

4. After the initial leaching, microbial activity was responsible for organic acid formation and further slow substrate degradation. The major fatty acids found in the packed reactor were acetic, propionic, and butryic acids. Acetic acid had the highest concentration, accounting for 55% of the total VFA COD, followed by propionic (31%) and butyric acids (14%). Other volatile fatty acids persisted in small concentrations of less then 20 mg/l.
5. The volatile fatty acid COD was produced at a slower rate than total soluble COD in the packed reactors, reaching a peak concentration approximately 24 hours for total soluble COD. This is additional evidence that initial soluble COD production was contributed by leaching rather than microbial activity.
6. The liquid reservoir served an important role as an equalization basin that reduced the variability of COD, preventing possible damage to the anaerobic filters due to shock loading. The hydraulic retention time in the liquid reservoir during Stage II was approximately 1.6 days which is not long enough for acid utilizing methanogens to grow as evidenced by negligible gas production in the liquid reservoir. Another important function of the liquid reservoir was to stored enough liquid volume for long term sampling.
7. The anaerobic filters were the major methane generators, producing 85% of the methane from the entire system. The highest specific methane production rate for one anaerobic filter was 2.12 liter CH_4/day/liter reactor volume during stable operation.

8. Total methane production per unit weight of substrate input was 104.3 ml methane/g substrate added in Stage I and 76.3 ml CH_4/g substrate input was obtained in Stage II. It is clear that the system produce more methane per gram of substrate input in Stage I than in Stage II. This was caused by the longer straw retention time of 40 days in Stage I compared with 18 days in Stage II. Therefore, a longer solids retention time would provide higher methane production per unit weight of solid substrate input due to increased straw degradation.
9. The VFA COD removal efficiency for Filter No.1 + No.2 in Stage II was 98.4% at a loading rate of 136 lb VFA COD/day per 10^3 ft^3 (2.19 g COD/day/liter) with a hydraulic retention time of 34 hours. The total soluble COD removal efficiency in Stage II for both reactors was 54.1% at a loading rate of 297 lb COD/day per 10^3 ft^3 (4.76 g COD/day/liter), with the hydraulic retention time of 34 hours.
10. Since the volatile fatty acids produced in the packed reactors were almost completely converted to methane and carbon dioxide in the anaerobic filters, no volatile acids were found to accumulate in the liquid phase. Therefore, the rate limiting step for the entire reactor system was the hydrolysis of solid substrate rather than the methanogenic step.
11. The extremely high VFA COD removal efficiency in both experiments suggests that efficient methane production may be accomplished at yet higher COD loading rates or at shorter hydraulic retention times.

12. More than 80% of the total soluble COD removed and over 88% of the volatile fatty acid COD removed was accomplished in Filter No.1. These data suggest that Filter No.1 was nearly large enough by itself. Filter No.2 essentially acted as a backup reactor which removed the remaining COD escaping from Filter No.1. With the recirculation of the liquid phase, the residual COD could be removed on the next pass.
13. The extent of straw substrate degradation was 34.6% in Stage I and 25.2% in Stage II obtained by mass balance calculation from methane production. These two values quite favorably agreed with the results obtained by fiber analysis, 36.0% in Stage I and 25.4% in Stage II. The extent of degradation obtained by weight loss measurements were 27.5% in Stage I and 20.3% in Stage II, lower than the data obtained from the other two methods. This discrepancy was most likely attributed by dissolved solids in liquid phase retained on the fermented straw when it was dried for weighing. The percent of cellulose and hemi-cellulose degrdation were 43.1% and 41.1% respectively in Stage I, and 26.2% and 30.8% respectively in Stage II.
14. When the liquid reservoir was excluded from the system so that the anaerobic filters received a transient substrate loading, the effluent soluble COD from Filter No.1 rose sharply. This result indicated that the existing microbial population was not able to completely utilize the sudden increase in substrate concentration. However, the effluent pH did not drop to a value lower than 6.5, suggesting that the methanogenic

bacteria could sustain methane production during transient loading.

15. A mathematical model was developed based on the mass balance concept expressing all parameters in terms of equivalent COD. The model includes terms for inflow, diffusion, COD production and COD utilization in a series of reactor segments. The results obtained from the mathematical model agreed very favorably with the experimental data. This model can be used to predict the soluble COD concentration inside the packed reactor as well as the effluent soluble COD concentration, provided the proper constants are given.
16. The proposed process can be applied to a full scale system using any of the three types of reactor configurations: (1) counter-current series, (2) co-current series, (3) parallel. The parallel system is most suitable for a full scale system due to its simpler operation and greater extent of substrate hydrolysis.

CHAPTER EIGHT

SUGGESTIONS FOR FUTURE RESEARCH

As a result of this investigation, several ideas are suggested as possible topics for future research:

1. Investigation of the behavior of different substrate solids concentrations, different solids retention times, and different liquid flow rates in the packed reactors and anaerobic filters in order to determine the relationships between COD production and hydraulic retention time, the extent of substrate degradation and solids retention time. Also, more kinetic parameters can be determined to refine and extend the mathematical model.
2. Improvement of anaerobic filter design to determine the optimum organic loading.
3. Operation of packed reactors at lower (or ambient) temperatures and at short hydraulic retention times to study the feasibility of methane production from leachate of residues.
4. Further investigation of the effects of transient substrate loading on the performance of the anaerobic filters over an extended period.
5. Investigation of suitable pretreatment methods for lignocellulosic substrate to enhance the extent of substrate degradation.
6. Study of COD production from different types of cellulosic residues for comparsion with wheat straw.

APPENDICES

APPENDIX A

LIST OF SYMBOLS

A_j = Reactor cross-sectional area in segment j

B_1 = Parameter in mathematical model

B_2 = Parameter in mathematical model

B_3 = Parameter in mathematical model

b = Microorganism decay coefficient

C_j = Soluble COD concentration in segment j of packed reactor

D_{ij} = Dispersion coefficient, into segment j of packed reactor

D_{oj} = Dispersion coefficient, out from segment j of packed reactor

$\frac{dF}{dt}$ = Rate of substrate utilizaton

f = Constant for mathematical model

HRT = Hydraulic retention time

K = Maximum rate of substrate utilization

K_1 = Constant for mathematical model

K_2 = Constant for mathematical model

K_3 = Constant for mathematical model

L = Longitudinal segment length of packed reactor

N = Number of segments in one packed reactor

NCOD = Nonbiodegradable COD

P = COD production term in mathematical model

Q = Liquid flow rate

R = Rate of COD utilization in packed reactor

SRT = Solids retention time

S = Substrate concentration

S_j = Substrate weight in segment j of a packed reactor

S_t = Total substrate weight in one packed reactor

t = Time

V_j = Packed reactor volume in segment j

V_t = Total packed reactor volume

VFA = Volatile Fatty Acids

X = Longitudinal length of one segment in packed reactor

x_t = Active microbial mass in system

Y = Growth yield coefficient

α = Constant for COD production term in mathematical model

ϕ_c^m = Limiting minimum solids retention time

ϕ_c = Solids retention time

Appendix B

Mathematical Calculation for the Mass of COD Produced by Leaching

(A) Flow Through Leaching Test

Area under COD-Time curve in Figure 4-4 (by using Simpson's Rule)

$= 99{,}273 \frac{\text{mg hr.}}{\text{liter}}$

Flow Rate = 0.0365 liter/hour

Mass of COD Produced = 99,237 x 0.0365 = 3623 mg COD

Weight of straw in the reactor : 60 grams.

Mass COD per gram straw = 3623/60 = $60.4 \frac{\text{mg COD}}{\text{gm straw}}$

Hydraulic retention time = 12.5 hours

Elapsed time of flow through leaching test = 48 hours

T = 48/12.5 = 3.84

(B) Batch Leaching Test

The maximum soluble COD concentration in Figure 4-5 : 2,880 mg/l

Liquid volume in the batch reactor = 1,500 ml

Mass of COD produced = 2,880 (mg/l) x 1.50 (liter)

= 4,320 mg COD

Weight of straw = 50 grams

Mass of COD produced per gram of straw = 4320/50

$= 86.4 \frac{\text{mg COD}}{\text{gm straw}}$

(C) Packed Reactor

Flow Rate = 0.628 liter/day

Hydraulic Retention Time = 17.2 hours

Elapsed time = 17.2 x 3.84 (from A) = 66 hours

Area between solid curve and dashed curve in Figure 4-2 (by Simpson's Rule) in 66 hours = 213,463 $\frac{\text{mg hr.}}{\text{liter}}$

Mass of COD produced per gram of straw :

$$213{,}463 \times \frac{0.628}{24} \times \frac{1}{60} = 93.1 \frac{\text{mg COD}}{\text{gm straw}}$$

APPENDIX C

COD CONVERSION FACTORS

COD equivalent of the volatile fatty acids, CH_4, and H_2 can be calculated as the methods described bellow.

C.1 Acetic Acid

Molecular Weight: 60.05

$$CH_3COOH + 2\ O_2 \longrightarrow 2\ CO_2 + 2\ H_2O \qquad \text{(C-1)}$$

$$COD = \frac{2 \times 16 \times 2}{60.05} = 1.066\ g/g\ HAc$$

C.2 Propionic Acid

Molecular Weight: 74.0801

$$CH_3CH_2COOH + {}^{7}/_{2}\ O_2 \longrightarrow 3\ CO_2 + 3\ H_2O \qquad \text{(C-2)}$$

$$COD = \frac{16 \times 2 \times 3.5}{74.0801} = 1.512\ g/g\ HP$$

C.3 Butyric Acid

Molecular Weight: 88.1072

$$CH_3CH_2CH_2COOH + 5\ O_2 \longrightarrow 4\ CO_2 + 4\ H_2O \qquad \text{(C-3)}$$

$$COD = \frac{16 \times 2 \times 5}{88.1072} = 1.816\ g/g\ HB$$

C.4 Valeric Acid

Molecular Weight: 102.1343

$$CH_3(CH_2)_3COOH + {}^{13}/_2\ O_2 \longrightarrow 5\ CO_2 + 5\ H_2O \qquad (C\text{-}4)$$

$$COD = \frac{16 \times 13}{102.1343} = 2.037\ g/g\ HC$$

C.5 Caporic Acid

Molecular Weight: 116.1613

$$CH_3(CH_2)_4COOH + 8\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O \qquad (C\text{-}5)$$

$$COD = \frac{16 \times 8 \times 2}{116.1613} = 2.204\ g/g\ HC$$

C.6 Methane

Molecular Weight: 16

$$CH_4 + 2\ O_2 \longrightarrow CO_2 + 2\ H_2O \qquad (C\text{-}6)$$

$$COD = \frac{16 \times 2 \times 2}{16} = 4.0\ g/g\ CH_4$$

At standard conditions one mole of CH_4 occupied 22.4 liters, or one gram of CH_4 = ${}^{22.4}/_{16}$ = 1.40 liters, therefore, one liter of CH_4 is equal to ${}^{4.0}/_{1.4}$ = 2.86 gram COD destroyed. At 36°C, one gram of CH_4 is equal to 1.585 liters and one liter of CH_4 is equal to 2.52 grams COD destroyed.

C.7 Hydrogen

Molecular Weight: 1.008

$$2\ H_2 + O_2 \longrightarrow 2\ H_2O \qquad (C\text{-}7)$$

$$COD = \frac{16 \times 2}{1.008 \times 4} = 7.94\ g/g\ H_2$$

At standard temperature and pressure,

$$1 \text{ g } H_2 = \frac{22.4}{1.008 \times 2} = 11.11 \text{ liters}$$

Therefore, 1 liter of H_2 is equal to $^{7.94}/_{11.11} = 0.7146$ g COD destroyed.

C.8 Carbohydrate (Cellulose)

Molecular Weight: 162.0 n

$$(C_6 H_{12} O_6)_n + 6_n O_2 \longrightarrow 6_n CO_2 + 5_n H_2 O \qquad \text{(C-8)}$$

$$COD = \frac{32 \times 6_n}{162.0_n} = 1.185 \text{ g/g cellulose}$$

Appendix D

Data for Figures

Appendix D-3-1 Integrated Area Counts for Volatile Fatty Acids Standard Solution, Data for Figure 3-7.

Concentration (mg/l)	Area Counts (1)	(2)	(3)	Average	S.D.
Acetic Acid	M.W. = 60.05	1.066 g COD/g HAc			
6972.5	907367	870703	865420	881163	22846
3486.2	443550	434930	--	439240	
1743.1	212289	206525	205320	208045	3724
871.6	99825	102064	98313	100067	1887
435.8	49066	47190	48822	48359	1020
217.9	23454	24633	--	24044	
108.9	11792	12031	12031	12020	223
Propionic Acid	M.W. = 74.08	1.512 g COD/g HP			
4983.8	1072597	1073717	1098282	1081532	14517
2491.9	544382	546451	--	545417	
1246.0	266081	258349	261657	262029	3879
623.0	129712	129688	124916	128105	2762
311.5	64345	61214	63273	62944	1591
155.8	30984	30370	--	30677	
77.9	14699	14602	15236	14846	342
iso-Butyric Acid	M.W. = 88.11	1.816 g COD/g iHB			
997.1	277484	274885	282389	278253	3811
498.6	140705	140460	--	140533	
249.3	68949	66837	68635	68140	1140
124.6	34427	33963	32842	33744	815
62.3	17081	15946	16604	16544	570
31.2	8292	8045	--	8169	
15.6	3834	3872	4080	3929	132
Butyric Acid	M.W. = 88.11	1.816 g COD/g HB			
2988.1	791948	809559	818484	806664	13503
1494.1	400403	407570	--	403987	
747.0	195524	190216	191265	192335	2811
373.5	97241	97577	89815	94878	4388
186.5	48026	46197	47721	47315	980
93.2	23059	22717	--	22888	
46.6	11047	10898	11241	11062	172

Appendix D-3-1 Continued

Concentration (mg/l)	Area Counts (1)	(2)	(3)	Average	S.D.
iso-Valeric Acid	M.W. = 102.13	2.037 g COD/g iHV			
997.8	306012	316335	318756	313701	6768
498.9	155961	158818	--	157390	
249.5	77220	75168	--	75642	1063
124.7	37474	37425	36225	37041	707
62.4	18432	17617	18239	18096	426
31.2	8878	8558	8338	8591	272
15.6	3887	4167	4301	4118	211
Valeric Acid	M.W. =102.13	2.037 g COD/g HV			
1990.7	607809	633102	640895	627269	17297
995.3	309392	316318	--	312855	
497.7	152659	149024	147831	149838	2515
248.8	72704	73410	70460	72191	1540
124.4	34794	44956	35407	34719	728
62.2	16811	16535	--	16673	
31.1	7757	7754	8185	7899	248
Caporic Acid	M.W. = 116.16	2.204 g COD/g HC			
994.6	335074	346071	351875	344340	8533
497.7	167969	171050	--	169510	
248.7	81356	78389	77139	78961	2166
124.3	36110	37744	36481	36778	867
62.2	16682	17561	18291	17515	806
31.1	8656	8412	--	8534	
15.6	3581	3602	4000	3728	236

* S.D. = Standard Deviation

Appendix D-4-1 Effluent Total Soluble COD From the Packed reactors (Stage II)

Time* (hr.)	Total Soluble COD (mg/l) Reactor No. 4	Reactor No. 6	Reactor No. 7	Reactor No. 8	Reactor No. 9	Average (mg/l)	S.D.
2.0	--	--	880	1070	902	951	104
4.0	--	6612	--	--	6796	6704	
6.0	--	9565	9860	--	8338	9254	807
6.5	--	--	--	10695	--	10695	
8.0	9373	9123	9915	--	9860	9568	384
9.0	--	10476	--	--	--	10476	
10.0	11315	--	9591	11662	9395	10491	1164
11.0	--	10700	--	--	--	10700	
12.0	--	--	--	12707	9252	10980	
19.0	--	8220	--	--	--	8220	
20.0	--	--	--	9252	--	9252	
21.0	8798	--	--	--	--	8798	
24.0	--	--	7762	7628	--	7695	
26.0	--	--	7650	--	--	7650	
27.0	--	7024	7474	--	--	7249	
28.0	7548	--	--	7540	--	7544	
34.0	--	--	--	7430	--	7430	
36.0	--	--	--	--	7650	7650	
40.0	--	--	--	--	7303	7303	
43.0	--	6333	--	--	--	6333	
44.0	7240	--	6382	--	--	6811	
48.0	--	--	--	6775	--	6775	
49.0	--	6352	--	--	6953	6653	
50.0	--	--	6472	--	--	6472	
54.5	--	--	--	6412	--	6412	
56.0	6892	--	6412	--	--	6652	
58.0	--	--	--	6572	--	6572	
68.0	--	6042	6119	--	--	6081	
68.5	--	--	--	6432	--	6432	
72.0	--	--	--	--	6496	6496	
75.5	--	5814	--	--	--	5814	
79.0	--	5965	--	--	--	5965	
80.0	--	--	5852	--	--	5852	
91.0	--	5628	--	--	--	5628	
92.0	--	--	5908	6216	--	6062	
95.0	--	--	--	--	5891	5891	
96.0	--	--	5683	--	--	5683	
98.0	5536	--	--	--	--	5536	

Appendix D-4-1 Continued

Time (hr.)	No. 4 Reactor	No. 6 Reactor	No. 7 Reactor	No. 8 Reactor	No. 9 Reactor	Average (mg/l)	S.D.
			Total Soluble COD (mg/l)				
100.0	--	--	6157	--	--	6157	
102.0	--	--	--	--	5928	5928	
106.0	--	--	--	6099	5991	6045	
115.0	--	5558	--	--	--	5558	
118.0	--	--	--	--	5836	5836	
121.0	--	5628	--	--	--	5628	
124.0	--	--	--	--	5836	5836	
126.5	--	--	5758	--	--	5758	
130.0	5628	--	5814	--	5781	5741	99
139.0	--	5390	--	--	--	5390	
142.0	--	--	--	5600	--	5600	
144.0	--	--	--	--	5405	5405	
149.5	--	--	5536	--	--	5536	
161.0	--	--	5445	--	--	5445	
168.0	--	--	--	--	5426	5426	
175.0	--	--	--	5370	--	5370	
177.0	--	5053	--	--	--	5053	
186.0	--	--	--	5481	--	5481	
187.0	--	--	5354	--	--	5354	
191.0	--	5247	--	--	--	5247	
198.0	--	--	--	5076	--	5076	
204.0	--	--	--	5370	5053	5212	
223.0	--	5070	5127	--	--	5099	
224.0	--	--	--	5059	--	5059	
235.0	--	4948	--	--	--	4948	
249.0	--	4890	--	--	--	4890	
273.0	--	4707	--	4573	--	4640	
281.0	--	--	4510	--	--	4510	
285.0	--	4414	--	--	--	4414	
321.0	--	4238	--	--	--	4238	
337.0	--	4275	--	--	--	4275	
357.0	--	4210	--	--	--	4210	
381.0	--	4082	--	--	--	4082	

* Time zero was installation of reactor in the system
S.D. = Standard Deviation

Appendix D-4-2 Individual Volatile Fatty Acid Concentration In The Packed Reactors. (Stage II)

Elapsed Time(hr)	Volatile Acids Concentration (mg/l as Acetic Acid)					Total VFA COD (mg/l)
	HAc	HP	iHB	HB	iHV	
Packed Reactor No.8						
6.5	816	44	137	84	--	1849
12.0	1169	223	47	343	--	2748
20.0	1485	528	4	304	--	3450
28.0	1743	658	8	237	--	3935
48.0	1715	572	7	98	--	3566
58.0	1668	531	8	51	--	2998
68.5	1361	415	13	58	16	2505
130.0	1097	340	12	27	14	1978
142.0	1017	326	8	22	12	1833
165.0	931	307	5	15	10	1670
188.0	835	275	4	10	--	1485
214.0	773	260	3	15	--	1396
238.0	715	252	2	15	--	1301
263.0	748	260	3	6	--	1341
Packed Reactor No.9						
12.0	1259	374	35	92	--	2422
22.0	1521	563	12	202	--	3354
40.0	1510	504	17	103	20	2991
60.0	1488	449	17	68	20	2756
72.0	1558	468	16	107	20	2998
82.0	1320	394	15	48	25	2420
95.0	1183	362	10	31	14	2112
106.0	1109	257	9	12	13	1782
118.0	1091	337	10	20	11	1932
144.0	926	298	5	23	10	1671
168.0	901	306	5	22	9	1658
193.0	800	275	3	20	7	1673

* samples taken from reactor effluent

Note: HAc = Acetic Acid
HP = Propionic Acid
iHB = ios-Butyric Acid
HB = Butyric Acid
iHV = iso-Valeric Acid
VFA = Volatile Fatty Acid

Appendix D-4-3 Individual Volatile Fatty Acid Concentrations In the Liquid Equilization Reservoir (Stage II)

Date		Volatile Acid Concentration (mg/l as HAc)						VFA COD
1982	Time	HAc	HP	iHB	HB	iHV	HV	(mg/l)
10/5	1600	1347	454	10	182	14	18	2905
10/6	1000	1534	545	10	175	15	18	3263
10/7	1000	1312	486	7	98	11	12	2667
10/8	1000	1476	481	8	204	14	19	3153
10/9	1400	1568	520	10	211	14	21	3349
10/10	1030	1501	482	8	127	13	14	2952
10/11	1000	1546	463	11	191	14	33	3212
10/12	0400	1509	471	6	181	17	32	3157
10/12	2400	1576	487	11	138	19	24	3137
10/13	1200	1416	418	16	57	18	--	2548
10/15	1000	1403	409	14	175	16	20	2887
10/16	1200	1498	440	15	246	18	--	3235
10/17	1200	1575	430	16	223	18	23	3262
10/18	1300	1369	403	14	196	16	19	2891
Average		1474	464	11	172	16	21	3044
S.D.		89	41	3	51	2	6	240

* S.D. = Standard Deviation

Appendix D-4-4 Effluent COD Concentration From The Liquid Reservoir and Anaerobic Filters. (Stage II)

Date	Time	Elapsed	L.R.		Filter No.1		Filter No.2	
		Time	TSCOD	VFACOD	TSCOD	VFACOD	TSCOD	VFACOD
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
10/3	2400	0	--	--	--	--	--	--
10/4	2200	22	6353	--	--	--	2728	--
10/5	1000	34	7024	--	--	--	3014	--
10/5	1600	40	6775	2905	--	--	2899	113
10/6	1000	58	6940	3263	5229	1445	3014	67
10/6	2200	70	6940	--	--	--	--	--
10/7	1000	82	6294	2667	4983	--	3161	70
10/7	1400	86	6003	--	--	--	--	--
10/8	1000	104	6612	3153	4034	907	2899	39
10/8	1800	112	6472	--	--	--	--	--
10/9	1400	132	7300	3349	4540	901	3044	32
10/9	1600	134	6633	--	--	--	--	--
10/9	2100	139	6432	--	--	--	--	--
10/10	1100	153	6796	2952	4194	776	3146	27
10/10	1200	155	6157	--	--	--	--	--
10/10	2200	165	6392	--	--	--	--	--
10/11	1000	177	6372	3212	3611	567	2942	38
10/11	2400	191	6892	--	--	--	--	--
10/12	0400	195	6796	3157	3844	602	3015	42
10/12	1300	204	7172	--	--	--	--	--
10/12	2400	215	7003	3137	--	--	--	--
10/13	1200	227	6612	2548	--	--	--	--
10/13	1800	233	5965	--	--	--	--	--
10/14	1100	250	6392	--	3719	--	3043	--
10/14	1800	257	6796	--	--	--	--	--
10/14	2200	261	--	--	3750	472	3161	12
10/15	1000	273	6633	2887	3907	305	2986	20
10/15	2200	285	7172	--	--	--	--	--
10/16	1200	299	6592	3235	3766	264	3161	13
10/16	1600	303	5909	--	--	--	--	--
10/17	1200	323	7172	3263	3642	247	3384	104
10/18	1300	348	6492	2891	3596	195	3161	50
Average			6636	3044	3729[a]	253[b]	3047	48
Standard Deviation			372	240	111	46	150	32

a Calculated from data after hour 177
b Calculated from data after Hour 273
* COD Unit = mg/l; Time Unit= Hour

Appendix D-4-5 Effluent Volatile Fatty Acid COD Concentration of Anaerobic Filters and Liquid Reservoir. (Stage I)

Date	Liquid Reservoir	Filter No.1	Filter No.2
4/07	5039	458	32
4/08	4762	319	28
4/09	--	--	56
4/10	5146	199	252
4/11	4692	1048	138
4/12	5908	--	--
4/13	5333	1394	207
4/14	5042	--	--
4/15	5272	--	54
4/17	6038	--	--
4/18	5532	376	249
4/19	5422	--	--
4/22	6148	--	--
4/23	5613	--	--
4/24	5450	--	--
4/26	5832	477	135
4/19	5119	--	342
5/02	5582	1144	68
5/04	4529	842	35
5/06	5309	199	23
Average	5356	682	125
S. D.	434	453	106

* S. D. = Standard Deviation
Data for Figure 4-17

APPENDIX E

FORTRAN PROGRAM LISTINGS

Appendix E-1 PROGRAM CUVFIT

```
C       PROGRAM CUVFIT
C       LEAST-SQUARES POLYNOMIAL CURVE FITTING FOR EXPERIMENTAL DATA.
C       FOR HIGH SOLIDS ANAEROBIC FERMENTATION AND ANAEROBIC FILTRATION
C       OF CELLULOSIC MATERIAL
C       BY YOW-MING LIN, OCTOBER 1982, AT MICHIGAN STATE UNIVERSITY
C
C ********************************COMMENTS*********************************
C       A     = STARTING MATRIX OR INVERSE MATRIX
C       DETER = DETERMINENT
C       EPS   = THE MINIMUN PIVOT MAGNITUDE PERMITTED
C       ITER  = 0, READ NEW DATA SET FOR ANOTHER RUN.
C       ITER  = 1, READ N,M,EPS,XSTART AND USE OLD X, AND Y VALUES
C       ITER  = 2, STOP PROGRAM EXECUTION.
C       IPLOT = 0, NO CURVE PLOTING DATA FILE IS WANTED.
C       IPLOT = 1, DATA FILE(S) WILL BE CREATED.
C       KCUV  = 1, TO PLOT ARITHMETIC SCALE CURVE.
C       KCUV  = 2, TO PLOT LOG. SCALE CURVE.
C       INDIC = NEGATIVE VALUE, DO N*N MATRIX INVERSE ONLY
C       INDIC = 0, N*N+1 MATRIX INVERSE AND SOLVE POLYNOMIAL EQU. COEF.
C       M     = DEGREE OF POLYNOMIAL.
C       N     = NUMBER OF DATA POINTS.
C       X     = VALUES FOR X AXIS.
C       Y     = VALUES FOR Y AXIS.
C       FNAME = FILE NAME
C       XSTART= STARTING X VALUE
C ************************************************************************
C
        PROGRAM CUVFIT
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION A(41,41),B(41),X(41),Y(41),C(41,41),BX(41)
       *,IROW(41),JCOL(41),JORD(41),T(41),CNY(360),TIME(360)
        LOGICAL*1 FNAME(10)
        ITER=0
        KOUNT=1
15      PRINT*,'INPUT N,M,INDIC,EPS,XSTART'
        READ(5,*)N,M,INDIC,EPS,XSTART
        IF(N .EQ. 0)GO TO 99
        WRITE(7,200)N,M,INDIC,EPS,ITER,XSTART
200     FORMAT(1H1,9X,'N      =',I4/1H ,9X,'M      =',I4/1H ,9X
       *,'INDIC  =',I4/1H ,9X,'EPS    =',E10.2/1H ,9X,'ITER   ='
       *,I4/1H ,9X,'XSTART =',F8.3)
        IF(ITER .NE. 0)GO TO 25
        PRINT*,'INPUT X(I),I=1,N'
        READ(5,*)(X(I),I=1,N)
        PRINT*,'INPUT Y(I),I=1,N'
        READ(5,*)(Y(I),I=1,N)
        WRITE(7,210)
210     FORMAT(1H ,///,9X,'GIVEN DATA'/1H ,//,12X,' TIME (HR)',14X,'C O D
       *(MG/L)')
        WRITE(7,220)(X(I),Y(I),I=1,N)
220     FORMAT(1H0,9X,F11.4,14X,F11.4)
25      PRINT*,'INPUT IPLOT;  IPLOT=0, NO CURVE PLOT IS WANTED'
        PRINT*,'              IPLOT=1, DATA FILE(S) FOR CURVE(S) PLOTING'
```

```
      PRINT*,'                        WILL BE CREATED'
      READ(5,*)IPLOT
      PRINT*,'INPUT KCUV; KCUV=1, FOR ARITHMETIC SCALE'
      PRINT*,'            KCUV=2, FOR LOG. SCALE CURVE'
      READ(5,*)KCUV
      IF(IPLOT .EQ. 0)GO TO 65
      WRITE(5,74)KOUNT
74    FORMAT(1H ,'THE NEXT DATA FILE WILL BE CUVNO',I1,'.DAT'/1H ,
     *'YES OR NO ? (INPUT Y OR N)')
      READ(5,76)ANS
76    FORMAT(A1)
      IF(ANS .EQ. 'Y')GO TO 65
      PRINT*,'DATA FILE NO. ?, INPUT KOUNT;'
      PRINT*,'VALUE OF KOUNT WILL BE THE NO. FOR DATA FILE CUVNO_.DAT'
      PRINT*,'VALUES OF KOUNT: 1,2,3,4,5, KOUNT=6, WILL STOP EXECUTION'
      READ(5,*)KOUNT
C
C     TO CREAT STARTING MATRIX FROM EXPERIMENTAL DATA
C
65    DO 10 I=1,N
10    C(I,1)=0.1D01
      NOR=M+1
      DO 20 J=2,NOR
      DO 20 I=1,N
20    C(I,J)=C(I,J-1)*X(I)
      DO 30 I=1,NOR
      DO 30 J=1,NOR
      A(I,J)=0.
      DO 30 K=1,N
30    A(I,J)=A(I,J)+C(K,I)*C(K,J)
      DO 40 I=1,NOR
      B(I)=0.
      DO 40 K=1,N
40    B(I)=B(I)+C(K,I)*Y(K)
      MAX=NOR
      IF(INDIC .GE. 0)GO TO 50
      GO TO 60
50    MAX=NOR+1
      DO 70 I=1,NOR
70    A(I,MAX)=B(I)
      WRITE(7,310)
310   FORMAT(1H ,///,9X,'THE STARTING MATRIX IS:')
60    DO 35 I=1,NOR
      WRITE(7,230)I,(A(I,J),J=1,MAX)
230   FORMAT(1H0,3X,I3,2X,7E16.7/9X,7E16.7)
35    CONTINUE
C
C     DO MATRIX INVERSING
C
      CALL MTXINV(NOR,A,BX,EPS,INDIC,DETER)
C
C     INDIC = "-" NUMBERS, DO MATRIX INVERSE ONLY
C
```

```
      IF(INDIC .GE. 0)GO TO 80
      WRITE(7,240)DETER
240   FORMAT(1H ,///,9X,'THE DETERMINENT IS:',E20.9/1H ,///,9X,'THE
     *INVERSE MATRIX IS:')
      DO 45 I=1,NOR
      WRITE(7,250)I,(A(I,J),J=1,MAX)
250   FORMAT(1H0,3X,I3,2X,7E16.7/9X,7E16.7)
45    CONTINUE
255   ITER=ITER+1
      GO TO 15
C
C     INDIC .GE. 0, DO MATRIX INVERSE AND SOLVE POLYNOMIAL COEFFICIENTS
C
80    WRITE(7,260)DETER
260   FORMAT(1H ,///,9X,'THE DETERMINENT IS',E20.9/1H ,///,9X,'THE SOLUT
     *IONS OF POLYNOMIAL COEFFICIENTS ARE:')
      DO 420 I=1,NOR
      II=I-1
420   WRITE(7,261)II,BX(I)
261   FORMAT(/,1H ,9X,I3,2X,'DEGREE COEFICIENT =',F17.10)
      IF(INDIC .NE. 0)GO TO 90
      WRITE(7,270)
270   FORMAT(1H ,///,9X,'THE INVERSE MATRIS IS:')
      DO 430 I=1,NOR
      WRITE(7,280)I,(A(I,J),J=1,MAX)
280   FORMAT(1H0,3X,I3,2X,7E16.7/9X,7E16.7)
430   CONTINUE
C
C     FIND THE LARGEST NUMBER OF Xs
C     AND COMPUTE Y VALUES FROM SOLVED NUMERICAL EQUATION
C
90    XBIG=BIG(X,1,N)
      IEND=IFIX(XBIG)
      IST=IFIX(XSTART)
      ISTP=IST+1
      DO 55 I=ISTP,IEND
      IFOR=I
      RI=FLOAT(IFOR)
      TIME(I)=RI
      CNY(I)=BX(1)
      MP=M+1
C     POLYNOMIAL EQUATION
      DO 55 J=2,MP
55    CNY(I)=CNY(I)+BX(J)*RI**(J-1)
      WRITE(7,295)
295   FORMAT(1H1,12X,'TIME(HR)   COD(MG/L)   TIME(HR)   COD(MG/L)   TIME
     *(HR)   COD(MG/L)   TIME(HR)   COD(MG/L)   TIME(HR)   COD(MG/L)')
C     GENERATE NUMBER OF ROWS TO BE PRINTED OUT
      CROW=(BIG(X,1,N)-SMALL(X,1,N))/5.0+1.0
      KR=IFIX(CROW)
      DO 95 K=1,KR
      WRITE(7,290)K+IST,CNY(K+IST),(KR*(I-1)+K+IST,CNY(KR*(I-1)+K+IST)
     *,I=2,5)
```

```
290     FORMAT(1H ,/,13X,I5,4X,F10.3,4(4X,I5,4X,F10.3))
95      CONTINUE
C       IPLOT = 0, NO CUVE PLOTTING
        IF(IPLOT .EQ. 0)GO TO 145
        PRINT*,'INPUT LAST, INCRM, FOR DATA FILES'
        PRINT*,'      LAST=THE MAX. TIME OF THE DATA FILE'
        PRINT*,'      INCRM=TIME INCREMENT BETWEEN TWO DATA POINTS'
        READ(5,*)LAST,INCRM
C       TO GENERATE DATA FILE AND ASSIGN DATA FILE NAME
        ENCODE(10,146,FNAME)KOUNT
146     FORMAT('CVFT',I2,'.DAT')
        CALL TRANSL(FNAME,FNAME,'0',' ')
        PRINT 147,(FNAME(I),I=1,10)
147     FORMAT(1H ,'FILE NAME IS: ',10A1)
        OPEN(UNIT=1,NAME=FNAME,TYPE='NEW')
C       TO WRITE THE DATA FILE
        CALL CUVWRT(ISTP,LAST,INCRM,TIME,CNY,KCUV)
        KOUNT=KOUNT+1
145     PRINT*,'INPUT ITER; ITER=0, READ NEW DATA SET;'
        PRINT*,'            ITER=1, READ N,M,EPS,XSTART USE OLD X AND Y'
        PRINT*,'            ITER=2, STOP'
        READ(5,*)ITER
        IF(ITER .EQ. 2)GO TO 99
        GO TO 15
99      STOP
        END
```

```
C
C       SUBROUTINE TO WRITE DATA IN DATA FILE
C
        SUBROUTINE CUVWRT(ISTP,LAST,INCRM,TIME,CNY,KCUV)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION TIME(360),CNY(360)
        DO 105 IG=ISTP,LAST,INCRM
        IF(KCUV .EQ. 1)GO TO 300
        TIME(IG)=DLOG(TIME(IG))
        CNY(IG)=DLOG(CNY(IG))
300     WRITE(1,320)TIME(IG),CNY(IG)
320     FORMAT('RD',2G15.7)
105     CONTINUE
        WRITE(1,325)
325     FORMAT('ED')
        CLOSE(UNIT=1)
        RETURN
        END
```

```
C
C       SUBROUTINE FOR MATRIX INVERSE
C
        SUBROUTINE MTXINV(N,A,BX,EPS,INDIC,DETER)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION IROW(41),JCOL(41),JORD(41),T(41),A(41,41),BX(41)
        IROW(1)=0
        JCOL(1)=0
        NOC=N
        IF(INDIC .GE. 0)NOC=N+1
        IF(N .LE. 50)GO TO 5
        WRITE(7,200)
200     FORMAT(10H0N TOO BIG)
        DETER=0.
        RETURN
5       DETER=0.1D01
        DO 10 K=1,N
        KM1=K-1
        PIVOT=0.
        DO 15 I=1,N
        DO 15 J=1,N
        IF(K .EQ. 1)GO TO 20
        DO 25 ISCAN=1,KM1
        DO 25 JSCAN=1,KM1
        IF(I .EQ. IROW(ISCAN))GO TO 15
        IF(J .EQ. JCOL(JSCAN))GO TO 15
25      CONTINUE
20      IF(DABS(A(I,J)) .LE. DABS(PIVOT))GO TO 15
        PIVOT=A(I,J)
        IROW(K)=I
        JCOL(K)=J
15      CONTINUE
        IF(DABS(PIVOT) .GT. EPS)GO TO 30
        DETER=0.
        RETURN
30      IROWK=IROW(K)
        JCOLK=JCOL(K)
        DETER=DETER*PIVOT
        DO 35 J=1,NOC
35      A(IROWK,J)=A(IROWK,J)/PIVOT
        A(IROWK,JCOLK)=1.0/PIVOT
        DO 10 I=1,N
        AIJCK=A(I,JCOLK)
        IF(I .EQ. IROWK)GO TO 10
        A(I,JCOLK)=-AIJCK/PIVOT
        DO 40 J=1,NOC
40      IF(J .NE. JCOLK)A(I,J)=A(I,J)-AIJCK*A(IROWK,J)
10      CONTINUE
        DO 45 I=1,N
        IROWI=IROW(I)
        JCOLI=JCOL(I)
        JORD(IROWI)=JCOLI
45      IF(INDIC .GE. 0)BX(JCOLI)=A(IROWI,NOC)
```

```
      INTCH=0
      NM1=N-1
      DO 50 I=1,NM1
      IP1=I+1
      DO 50 J=IP1,N
      IF(JORD(J) .GE. JORD(I))GO TO 50
      JTEMP=JORD(J)
      JORD(J)=JORD(I)
      JORD(I)=JTEMP
      INTCH=INTCH+1
50    CONTINUE
      IF(INTCH/2*2 .NE. INTCH)DETER=-DETER
      IF(INDIC .LE. 0)GO TO 55
      RETURN
55    DO 60 J=1,N
      DO 65 I=1,N
      IROWI=IROW(I)
      JCOLI=JCOL(I)
65    T(JCOLI)=A(IROWI,J)
      DO 60 I=1,N
60    A(I,J)=T(I)
      DO 70 I=1,N
      DO 75 J=1,N
      IROWJ=IROW(J)
      JCOLJ=JCOL(J)
75    T(IROWJ)=A(I,JCOLJ)
      DO 70 J=1,N
70    A(I,J)=T(J)
      RETURN
      END
```

```
C
C       FUNCTION TO FIND THE LARGEST VALUE.
C
        FUNCTION BIG(XMAT,M,N)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION XMAT(M,N)
        BIG=XMAT(1,1)
        DO 20 I=1,M
        DO 30 J=1,N
        IF(BIG .LT. XMAT(I,J))BIG=XMAT(I,J)
30      CONTINUE
20      CONTINUE
        RETURN
        END
```

```
C
C       FUNCTION TO FIND THE SMALLEST VALUE
C
        FUNCTION SMALL(XMAT,M,N)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION XMAT(M,N)
        SMALL=XMAT(1,1)
        DO 20 I=1,M
        DO 30 J=1,N
        IF(SMALL .GT. XMAT(I,J))SMALL=XMAT(I,J)
30      CONTINUE
20      CONTINUE
        RETURN
        END
```

Appendix E-2 PROGRAM MODEL

```
FORTRAN IV     V02.5-2    Wed 20-Jul-83 06:55:35

C       PROGRAM MODEL
C       MATHEMATICAL MODEL FOR SUBSTRATE DEGRADATION IN HIGH SOLIDS
C       ANAEROBIC FERMENTATION AND ANAEROBIC FILTRATION OF CELLULOSIC
C       AGRICULTURAL RESIDUES.
C       PROGRAM TO SOLVE PARTIAL DIFFERENTIAL EQUATIONS
C       BY YOW-MING LIN, OCTOBER 1982, AT MICHIGAN STATE UNIVERSITY
C
C       **********COMMENTS**********
C       ALF  = EXPONENTIAL CONSTANT OF COD PRODUCTION TERM IN EQUATION TWO.
C       AR   = CROSS-SECTIONAL AREA OF PACKED REACTOR. (CM**2)
C       C1   = CONSTANT FOR COD PRODUCTION TERM IN EQUATION ONE.
C       C2   = CONSTANT FOR COD PRODUCTION TERM IN EQUATION ONE.
C       DL   = TOTAL LENGTH OF A SINGLE REACTOR. (CM)
C       DPI  = DISPERSION COEFFICIENT INTO A SEGMENT IN THE REACTOR.
C       DPO  = DISPERSION COEF. OUT FROM A SEG. IN THE REAC.(CM**2/HR)
C       DTOUT= TIME INTERVAL THAT Y VALUE TO BE PRINTED OUT. (HR.)
C       IPLOT= 0, NO DATA FILE FOR CURVE PLOTING WILL BE CREATED.
C       IPLOT= 1, CREATE ONE CURVE PLOTING DATA FILE FOR THE LAST SEG.
C       IPLOT= 2, NOYS PLOTING DATA FILES FOR ALL SEGS. WILL BE CREATED.
C       ITYPE= 1, TO STOP THE PROGRAM.
C       ITYPE= 2, CHANGE NOYS FOR ANOTHER RUN.
C       ITYPE= 3, CHANGE YCOD1,ALF,RU,C1,C2,Y0 FOR ANOTHER RUN.
C       KCUV = 1, TO PLOT ARITHEMATIC SCALE CURVE FOR THE LAST SEGMENT.
C       KCUV = 2, TO PLOT LOG SCALE CURVE FOR THE LAST SEGMENT.
C       KCUV = 3, TO PLOT BOTH ARITHMETIC AND LOG. SCALE FOR THE LAST SEG.
C       NAME = TITLE FOR THE PRINTED RESULTS.
C       NAMEY= TITLE FOR THE PRINTED RESULTS.
C       NOYS = NUMBER OF YS, IN THIS PROGRAM Y IS THE NO. OF SEGMENTS.
C       NOBS = NUMBER OF BS  (IN DIFFERENTIAL EQUATIONS)
C       QF   = FLOW RATE OF THE LIQUID PHASE. (ML/HR)
C       RU   = RATE OF COD UTILIZATION IN A SINGLE REACTOR.(NOT CH4 REACTOR)
C       SGL  = LENGTH OF ONE DIVIDED SEGMENT IN THE REACTOR. (CM)
C       SGV  = VOLUME OF ONE DIVIDED SEGMENT IN THE REACTOR. (ML)
C       SUB  = WEIGHT OF SUBSTRATE IN ONE SINGLE REACTOR. (GM.)
C       T    = TIME. (HR)
C       TCHK = TIME CHECK TO USE SECOND DIFFERENTIAL EQUATION (HR)
C       T0   = TIME ZERO. (HR)
C       TMAX = TIME MAXMUM FOR CALCULATION. (HR)
C       VOL  = VOLUME OF ONE SINGLE REACTOR. (ML)
C       YCOD1= CONSTANT FOR COD PRODUCTION TERM IN EQUATION TWO (MG/L/HR)
C       Y0   = INITIAL Y VALUES AT TIME ZERO.
C       ************************************************************
        PROGRAM MODEL
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION NAMEY(10,10)
        DIMENSION F(15),Y(15),Y0(15),RK(4,15),B1(15),B2(15),B3(15),
       *DPO(15),DPI(15),TP(210),YP(210,12)
        LOGICAL*1 FNAME(10),PRIFX(4)
        PRINT*,'INPUT TITLE NAME'
        READ(5,35)NAME,((NAMEY(I,J),J=1,2),I=1,9)
35      FORMAT(2A2,9(2A2,2A2))
        PRINT*,'INPUT QF, AR, VOL, DL, SUB'
```

```
      READ(5,*)QF,AR,VOL,DL,SUB
20    PRINT*,'INPUT IPLOT;  IPLOT=0, NO CURVE PLOT IS WANTED'
      PRINT*,'              IPLOT=1, PLOT ONE CURVE(LAST SEG. ONLY)'
      PRINT*,'              IPLOT=2, PLOT CURVES FOR ALL SEGMENTS'
      PRINT*,'NOTE:WHEN USE IPLOT=1, NO. OF NOYS WILL BE THE  NO.'
      PRINT*,'FOR DATA FILE NOY_.DAT'
      READ(5,*)IPLOT
      IF(IPLOT .NE. 1)GO TO 30
      PRINT*,'INPUT KCUV; KCUV=1, ARITHMETIC SCALE FOR THE LAST SEG.'
      PRINT*,'            KCUV=2, LOG. SCALE FOR THE LAST SEG.'
      PRINT*,'            KCUV=3, BOTH ARITH. AND LOG. SCALE'
      READ(5,*)KCUV
30    PRINT*,'INPUT NOYS, NOBS'
      READ(5,*)NOYS,NOBS
      N1=NOYS+1
      IRUN=NOYS
      IF(NOYS .EQ. 0)GO TO 99
      PRINT*,'INPUT DISPERSION COEF. DPI(I)'
      READ(5,*)(DPI(I),I=1,N1)
      PRINT*,'INPUT DPO(I)'
      READ(5,*)(DPO(I),I=1,N1)
25    PRINT*,'INPUT DT, DTOUT, TCHK'
      READ(5,*)DT,DTOUT,TCHK
      PRINT*,'INPUT T0, TMAX'
      READ(5,*)T0,TMAX
      PRINT*,'INPUT YCOD1, ALF, RU, C1, C2'
      READ(5,*)YCOD1,ALF,RU,C1,C2
      PRINT*,'INPUT INITIAL CONDITIONS, Y0(I),I=1,NOYS+1'
      READ(5,*)(Y0(I),I=1,N1)
      YS=FLOAT(NOYS)
      SGL=DL/YS
      SGV=VOL/YS
      SGS=SUB/YS
      DO 100 I=2,N1
      B1(I)=(-QF-AR*DPI(I)/SGL-AR*DPO(I)/SGL)/SGV
      B2(I)=(QF+AR*DPO(I)/SGL)/SGV
      B3(I)=AR*DPI(I)/(SGV*SGL)
100   CONTINUE
      WRITE(7,40)NOYS,NOBS,T0,TMAX,DTOUT,DT,TCHK
40    FORMAT(1H1,7X,8HNOYS   =,I5/8X,8HNOBS   =,I5/8X,8HT0     =F11.5
     */8X,8HTMAX   =,F11.5/8X,8HDTOUT  =,F11.5/8X,8HDT     =,F11.5
     */8X,8HTCHK   =,F11.5)
      DO 60 I=1,N1
      WRITE(7,70)I,Y0(I)
70    FORMAT(1H ,7X,3HY0(,I2,3H) =,F11.5)
60    CONTINUE
      WRITE(7,85)QF,AR,NOYS,VOL,DL,SUB,YCOD1,ALF,RU
85    FORMAT(1H0,7X,8HFLOW   =,F11.5,8H (ML/HR)/8X,8HCS AREA=,F11.5,
     *8H (CM**2)/8X,8HNO. SEG=,I5,/8X,8HVOLUME =,F11.5,8H (CM**3)/8X,
     *8HLENGTH =,F11.5,10H (CM/RCT.)/8X,8HSUBST. =,F11.5,8H (GRAMS)/8X,
     *8HYCOD1  =,F11.5,11H (MG/L.G.H)/8X,8HALPHA  =,F11.5/8X,8HRATE UT=,
     *F11.5,10H (MG/L/HR))
      WRITE(7,75)C1,C2
```

```
75      FORMAT(8X,8HC1      =,F11.5/8X,8HC2      =,F11.5)
        WRITE(7,90)(DPI(I),I=1,N1)
90      FORMAT(1H ,7X,8HDPI(I) =,10F11.5)
        WRITE(7,95)(DPO(I),I=1,N1)
95      FORMAT(1H ,7X,8HDPO(I) =,10F11.5)
        WRITE(7,110)
110     FORMAT(1H0,//,8X,'SEGMENT NO.',10X,'B1',14X,'B2',14X,'B3')
        DO 115 K=2,N1
        JJ=K-1
        WRITE(7,120)JJ,B1(K),B2(K),B3(K)
120     FORMAT(1H0,11X,I3,2X,3F16.6)
115     CONTINUE
        WRITE(7,130)NAME,((NAMEY(I,J),J=1,2),I=1,5)
130     FORMAT(1H1,5X,2A2, 5(4X,2A2,2A2))
        T=T0
        TOUT=0.0
        DTOUT=DTOUT-0.000001
        DO 140 I=1,N1
140     Y(I)=Y0(I)
        TP(1)=T0
        DO 135 I=1,NOYS
135     YP(1,I)=Y0(I+1)
        KT=2
180     DO 150 ITER=1,4
        IF(T .GT. TCHK)GO TO 145
        CALL EQ1(F,Y,B1,B2,B3,NOYS,C1,C2,T,RU,SGS,SUB)
        GO TO 155
145     CALL EQ2(F,Y,B1,B2,B3,NOYS,YCOD1,ALF,T,RU,SGS)
155     CALL RUNKU(ITER,NOYS,DT,F,Y,T,T0,Y0,RK)
150     CONTINUE
        T0=T
        IF(T .GT. TMAX)GO TO 160
        DO 170 I=1,N1
170     Y0(I)=Y(I)
        TOUT=TOUT+DT
        IF(TOUT .LT. DTOUT)GO TO 180
160     TOUT=0.0
        WRITE(6,190)T,(Y(I),I=2,N1)
        WRITE(7,190)T,(Y(I),I=2,N1)
190     FORMAT(1H ,1X,F8.2,10F11.2)
        TP(KT)=T
        DO 200 I=1,NOYS
200     YP(KT,I)=Y(I+1)
        KT=KT+1
        IF(T .LT. TMAX)GO TO 180
        IF(IPLOT .EQ. 0)GO TO 230
        IF(IPLOT .EQ. 1)GO TO 240
C       SEG1.DAT IS THE DATA FILE FOR SEGMENT ONE.
C       NOY4.DAT IS THE DATA FILE FOR THE LAST SEG. WHEN A REACTOR IS
C       DIVIDED INTO FOUR SEGMENTS.
        DO 210 ICHK=1,NOYS
        CALL DATFIL('SEG',ICHK,TP,YP,KT)
210     CONTINUE
```

```
      GO TO 230
240   IF(KCUV .EQ. 1)GO TO 245
      IF(KCUV .EQ. 2)GO TO 260
245   CALL DATFIL('NOY',NOYS,TP,YP,KT)
      IF(KCUV .EQ. 1)GO TO 230
260   DO 175 I=1,KT-1
      TP(I)=DLOG(TP(I))
175   YP(I,NOYS)=DLOG(YP(I,NOYS))
      CALL DATFIL('LNNO',NOYS,TP,YP,KT)
230   PRINT*,'INTER ITYPE, ITYPE=1; TO STOP THE PROGRAM'
      PRINT*,'             ITYPE=2; CHANGE NOYS AND OTHER DATA'
      PRINT*,'             ITYPE=3; READ DT,DTOUT,TCHK'
      PRINT*,'             YCOD1,ALF,RU,C1,C2,Y0(I)'
      READ(5,*)ITYPE
      GO TO(99,20,25)ITYPE
99    STOP
      END
```

```
C
C       DIFFERENTIAL EQUATIONS 1
C
        SUBROUTINE EQ1(F,Y,B1,B2,B3,NOYS,C1,C2,T,RU,SGS,SUB)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION F(15),Y(15),B1(15),B2(15),B3(15)
C       ***************************EQUATION 1*********************************
        DO 10 J=2,NOYS
10      F(J)=B1(J)*Y(J)+B2(J)*Y(J-1)+B3(J)*Y(J+1)+SUB*C1+SGS*C2*DLOG(T)
       *-RU/NOYS
        M=NOYS
        F(M+1)=B1(M+1)*Y(M+1)+B2(M+1)*Y(M)+B3(M+1)*Y(M+1)+SUB*C1+SGS*C2
       1*DLOG(T)-RU/NOYS
C       ************************************************************************
        RETURN
        END
```

```
C
C       DIFFERENTIAL EQUQTIONS 2
C
        SUBROUTINE EQ2(F,Y,B1,B2,B3,NOYS,YCOD1,ALF,T,RU,SGS)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION F(15),Y(15),B1(15),B2(15),B3(15)
C
C       ***************************EQUATIONS 2*********************************
        DO 10 J=2,NOYS
10      F(J)=B1(J)*Y(J)+B2(J)*Y(J-1)+B3(J)*Y(J+1)+SGS*YCOD1*T**ALF-RU/NOYS
        M=NOYS
        F(M+1)=B1(M+1)*Y(M+1)+B2(M+1)*Y(M)+B3(M+1)*Y(M+1)+SGS*YCOD1*T**ALF
       *-RU/NOYS
C       ************************************************************************
        RETURN
        END
```

```
C
C       SUBROUTINE TO SOLVE DIFFERENTIAL EQUATIONS.
C
      SUBROUTINE RUNKU(ITER,NOYS,DT,FYT,YMOD,TMOD,T0,Y0,RK)
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION FYT(15),YMOD(15),Y0(15),RK(4,15)
      N1=NOYS+1
      GO TO(1,2,3,4)ITER
1     DTA=DT/3.0
      TMOD=T0+DTA
      DO 11 I=2,N1
      RK(1,I)=FYT(I)
11    YMOD(I)=Y0(I)+DTA*RK(1,I)
      RETURN
2     DTA=DT/3.0
      TMOD=T0+2.0*DTA
      DO 12 I=2,N1
      RK(2,I)=FYT(I)
12    YMOD(I)=Y0(I)-DTA*RK(1,I)+DT*RK(2,I)
      RETURN
3     TMOD=T0+DT
      DO 13 I=2,N1
      RK(3,I)=FYT(I)
13    YMOD(I)=Y0(I)+DT*(RK(1,I)-RK(2,I)+RK(3,I))
      RETURN
4     TMOD=T0+DT
      DTA=DT/8.0
      DO 14 I=2,N1
      RK(4,I)=FYT(I)
14    YMOD(I)=Y0(I)+DTA*(RK(1,I)+3.0*RK(2,I)+3.0*RK(3,I)+RK(4,I))
      RETURN
      END
```

```
C
C       SUBROUTINE TO CREAT DATA FILES AND ASSIGN DATA FILE NAME
C
        SUBROUTINE DATFIL(LINDA,LIT,TP,YP,KT)
        IMPLICIT REAL*8(A-H,O-Z)
        DIMENSION TP(210),YP(210,12)
        LOGICAL*1 FNAME(10),PRIFX(4)
        IF(LINDA .EQ. 'SEG')GO TO 20
        IF(LINDA .EQ. 'NOY')GO TO 30
        ENCODE(10,45,FNAME)LIT
45      FORMAT('LNNO',I2,'.DAT')
        GO TO 50
20      ENCODE(10,25,FNAME)LIT
25      FORMAT('SEG',I2,'.DAT')
        GO TO 50
30      ENCODE(10,35,FNAME)LIT
35      FORMAT('NOY',I2,'.DAT')
50      CALL TRANSL(FNAME,FNAME,'0',' ')
        OPEN(UNIT=1,NAME=FNAME,TYPE='NEW')
        PRINT 33,(FNAME(I),I=1,10)
33      FORMAT(1H ,'FILE NAME IS; ',10A1)
C
C       TP=TIME DATA FOR X AXIS.
C       YP=DATA FOR Y AXIS.
C
        DO 60 JP=1,KT-1
        WRITE(1,55)TP(JP),YP(JP,LIT)
55      FORMAT('RD', 2G15.7)
60      CONTINUE
        WRITE(1,65)
65      FORMAT('ED')
        CLOSE(UNIT=1)
        RETURN
        END
```

```
NOYS    =     5
NOBS    =     3
TO      =     0.00001
TMAX    =   202.00000
DTOUT   =     2.00000
DT      =     0.04000
TCHK    =     6.00000
YO( 1) = 3000.00000
YO( 2) = 1000.00000
YO( 3) = 1000.00000
YO( 4) = 1000.00000
YO( 5) = 1000.00000
YO( 6) = 1000.00000

FLOW    =    26.00000 (ML/HR)
CS AREA=   481.32000 (CM**2)
NO. SEG=     5
VOLUME  =   435.00000 (CM**3)
LENGTH  =    11.50000 (CM/RCT. )
SUBST.  =    60.00000 (GRAMS)
YCOD1   =     7.98000 (MG/L. C. H)
ALPHA   =    -0.23800
RATE UT=     4.52000 (MG/L/HR)
C1      =    16.70000
C2      =     9.86000
DPI(I)  =     0.00000     0.00482     0.00482     0.00482     0.00482     0.00000
DPO(I)  =     0.00000     0.00000     0.00482     0.00482     0.00482     0.00482
```

SEGMENT NO.	B1	B2	B3
1	-0.310445	0.298851	0.011594
2	-0.322039	0.310445	0.011594
3	-0.322039	0.310445	0.011594
4	-0.322039	0.310445	0.011594
5	-0.310445	0.310445	0.000000

2.00	3247.55	2839.56	2670.45	2632.03	2625.82
4.00	5635.00	6131.12	6051.80	5957.05	5918.33
6.00	7414.73	9296.51	9825.44	9874.74	9836.57
8.00	5920.78	8476.05	9838.65	10323.45	10427.09
10.00	5075.75	7510.47	9393.88	10423.91	10839.32
12.00	4586.49	6685.22	8728.20	10193.82	11000.46
14.00	4295.54	6055.04	8028.64	9739.20	10903.06
16.00	4117.09	5597.60	7396.29	9178.35	10596.30
18.00	4003.67	5272.83	6869.31	8603.02	10154.59
20.00	3928.62	5043.50	6450.51	8069.79	9650.84
22.00	3876.70	4880.57	6126.67	7605.83	9142.25
24.00	3839.07	4763.03	5879.68	7218.39	8666.45
26.00	3810.51	4676.35	5691.95	6903.30	8243.65
28.00	3787.88	4610.64	5548.60	6651.06	7881.12
30.00	3769.26	4559.31	5437.87	6450.57	7577.76
32.00	3753.45	4517.95	5350.89	6291.26	7327.84
34.00	3739.66	4483.63	5281.15	6163.94	7123.68
36.00	3727.41	4454.37	5223.96	6061.11	6957.25
38.00	3716.35	4428.85	5175.99	5976.85	6821.17
40.00	3706.24	4406.18	5134.85	5906.65	6709.04
42.00	3696.92	4385.71	5098.87	5847.09	6615.61
44.00	3688.26	4367.02	5066.85	5795.65	6536.69
46.00	3680.17	4349.77	5037.94	5750.47	6469.01
48.00	3672.58	4333.73	5011.52	5710.15	6410.06
50.00	3665.42	4318.74	4987.15	5673.70	6357.93
52.00	3658.65	4304.64	4964.48	5640.36	6311.21
54.00	3652.23	4291.34	4943.27	5609.58	6268.80
56.00	3646.13	4278.75	4923.31	5580.93	6229.90
58.00	3640.32	4266.79	4904.46	5554.10	6193.89
60.00	3634.77	4255.40	4886.59	5528.85	6160.32
62.00	3629.47	4244.54	4869.60	5504.97	6128.82
64.00	3624.38	4234.15	4853.40	5482.30	6099.11
66.00	3619.51	4224.21	4837.93	5460.73	6070.98
68.00	3614.83	4214.67	4823.12	5440.15	6044.24
70.00	3610.32	4205.51	4808.93	5420.47	6018.76
72.00	3605.98	4196.70	4795.30	5401.61	5994.41
74.00	3601.80	4188.21	4782.19	5383.51	5971.09
76.00	3597.77	4180.03	4769.57	5366.12	5948.73
78.00	3593.87	4172.14	4757.40	5349.37	5927.23
80.00	3590.10	4164.51	4745.66	5333.23	5906.55
82.00	3586.45	4157.13	4734.32	5317.66	5886.63
84.00	3582.91	4150.00	4723.36	5302.62	5867.40
86.00	3579.48	4143.08	4712.74	5288.08	5848.83
88.00	3576.16	4136.37	4702.46	5274.01	5830.88
90.00	3572.93	4129.87	4692.50	5260.37	5813.51
92.00	3569.79	4123.55	4682.82	5247.16	5796.68
94.00	3566.74	4117.42	4673.43	5234.34	5780.37
96.00	3563.77	4111.45	4664.31	5221.89	5764.55
98.00	3560.89	4105.64	4655.44	5209.79	5749.18
100.00	3558.07	4099.99	4646.81	5198.03	5734.26
102.00	3555.33	4094.49	4638.41	5186.59	5719.74
104.00	3552.66	4089.13	4630.23	5175.46	5705.62
106.00	3550.05	4083.89	4622.25	5164.61	5691.88
108.00	3547.50	4078.79	4614.48	5154.04	5678.49
110.00	3545.02	4073.81	4606.89	5143.73	5665.44
112.00	3542.59	4068.95	4599.48	5133.67	5652.71
114.00	3540.22	4064.20	4592.25	5123.85	5640.30
116.00	3537.90	4059.55	4585.19	5114.27	5628.18
118.00	3535.63	4055.01	4578.28	5104.90	5616.35
120.00	3533.40	4050.57	4571.53	5095.75	5604.79
122.00	3531.23	4046.22	4564.93	5086.80	5593.49
124.00	3529.10	4041.97	4558.46	5078.04	5582.44
126.00	3527.02	4037.80	4552.14	5069.48	5571.63
128.00	3524.97	4033.72	4545.94	5061.09	5561.05
130.00	3522.97	4029.72	4539.87	5052.87	5550.70

132.00	3521.00	4025.80	4533.92	5044.83	5540.55
134.00	3519.08	4021.95	4528.09	5036.94	5530.62
136.00	3517.19	4018.18	4522.37	5029.21	5520.88
138.00	3515.33	4014.48	4516.77	5021.63	5511.34
140.00	3513.51	4010.85	4511.26	5014.19	5501.98
142.00	3511.72	4007.28	4505.86	5006.90	5492.79
144.00	3509.97	4003.78	4500.56	4999.74	5483.78
146.00	3508.24	4000.34	4495.35	4992.71	5474.94
148.00	3506.54	3996.96	4490.24	4985.80	5466.26
150.00	3504.88	3993.64	4485.21	4979.02	5457.73
152.00	3503.24	3990.38	4480.27	4972.35	5449.36
154.00	3501.62	3987.17	4475.42	4965.81	5441.13
156.00	3500.04	3984.01	4470.64	4959.37	5433.04
158.00	3498.48	3980.90	4465.95	4953.04	5425.09
160.00	3496.94	3977.85	4461.33	4946.81	5417.27
162.00	3495.43	3974.84	4456.78	4940.69	5409.58
164.00	3493.94	3971.88	4452.31	4934.66	5402.02
166.00	3492.48	3968.97	4447.91	4928.73	5394.57
168.00	3491.04	3966.10	4443.58	4922.90	5387.25
170.00	3489.61	3963.28	4439.31	4917.15	5380.04
172.00	3488.21	3960.49	4435.11	4911.49	5372.94
174.00	3486.83	3957.75	4430.97	4905.92	5365.95
176.00	3485.47	3955.05	4426.89	4900.43	5359.06
178.00	3484.13	3952.39	4422.87	4895.02	5352.28
180.00	3482.81	3949.76	4418.91	4889.69	5345.60
182.00	3481.51	3947.17	4415.00	4884.43	5339.01
184.00	3480.23	3944.62	4411.15	4879.25	5332.52
186.00	3478.96	3942.10	4407.36	4874.15	5326.13
188.00	3477.71	3939.62	4403.61	4869.11	5319.82
190.00	3476.47	3937.17	4399.92	4864.15	5313.60
192.00	3475.26	3934.76	4396.27	4859.25	5307.46
194.00	3474.05	3932.37	4392.68	4854.42	5301.41
196.00	3472.87	3930.02	4389.13	4849.65	5295.44
198.00	3471.70	3927.70	4385.63	4844.94	5289.55
200.00	3470.54	3925.40	4382.17	4840.30	5283.73
202.00	3469.40	3923.14	4378.76	4835.71	5277.99

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