Kinetics of Anaerobic Digestion of Selected C1 to C4 Organic Acids

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

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And hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Anaerobic digestion involves multiple bacterial and archaea species to convert organic matter into volatile fatty acids and finally into methane and carbon dioxide. In this study, the effect of six selected C1 to C4 organic acids on biogas production and microbial growth in anaerobic digestion was evaluated using batch assays. These C1 to C4 compounds included formic acid, acetic acid, propionic acid, lactic acid, pyruvic acid and butyric acid. Each organic acid was tested as a sole substrate in individual batch experiment at different concentrations range from 250 mg COD/L to 8000 mg COD. The cumulative biogas volume was recorded automatically by a respirometer every ten min. The biogas generation rate was calculated from the cumulative biogas generation curve while the specific microbial growth rate was estimated using Monod and Andrews equations based on the coupled stoichiometric reactions between biogas production and microbial growth. The tested organic acids were divided into two groups based on substrate self-inhibitory characteristics in anaerobic digestion. Anaerobic digestion of acetic acid and propionic acid presented self-inhibition at high acid concentrations while formic acid, lactic acid, pyruvic acid and butyric acid did not inhibit anaerobic digestion at the highest concentrations tested. The microbial growth rates using butyric acid, pyruvic acid and acetic acid were 0.170, 0.167, and 0.396 d^{-1}, respectively. For comparison, the growth using

propionic acid, lactic acid and formic acid was relatively slower with the growth rates of 0.136, 0.133 and 0.133 d^{-1}, respectively.

CHAPTER 1

INTRODUCTION

1.1 Anaerobic digestion and essential microbial groups in anaerobic digestion

Anaerobic digestion plays an important role in wastewater treatment processes. It includes a series of biochemical processes by different microorganisms to degrade organic matter under anaerobic conditions. Methane, the digestion byproduct, is a rich source of renewable energy, which can help to replace fossil fuel to contribute to environmental conservation and sustainability. Therefore, anaerobic digestion is widely used as an attractive means for wastewater treatment around the world while more and more new process configurations are continuously being developed (Pavlostathis and Giraldo-Gomez 1991b).

There are multistep reactions involved in anaerobic digestion for degrading complex biodegradable materials. Generally three major steps are involved in anaerobic digestion: particulate hydrolysis, acidogenesis, and methanogenesis. The coordinated steps or chemical reactions start with hydrolysis, in which complex organic materials are hydrolyzed and reduced to small size and soluble organic substrates. Fermentative

reactions are then carried out to convert amino acids, simple sugars and long chain fatty acids into short-chain fatty acids including acetic acid. A notable example is anaerobic degradation of glucose. In the process of glucose fermentation, it was reported that 75% of glucose could be fermented to lactic acid by *streptococcus agalactiae* (Mickelson 1972). Co-generative products included formic acid, acetic acid, propionic acid and ethanol (Oh et al. 2003). Pyruvic acid is regarded as a key intermediate product during glucose degradation, while lactic acid, acetic acid and formic acid are fermentative products from pyruvic acid (Mickelson 1972). Anaerobic oxidation of long chain fatty acids resulted in production of acetic acid, propionic acid and H_2 (Shin et al. 2003) . Another pathway for acetic acid generation is the acetogenesis from short-chain fatty acids (McCarty and Mosey 1991). Short-chain fatty acids such as formic acid, propionic acid, lactic acid, butyric acid and pyruvic acid are resources for acetic acid formation. While carbon dioxide and hydrogen are generated as initial biogas components, methane becomes the dominant gas later due to methanogensis, which include acetotrophic methanogensis to convert acetate to methane and hydrogenotrophic methanogensis to convert carbon dioxide and hydrogen gas to methane. Details of anaerobic digestion processes are presented in Figure 1.1.

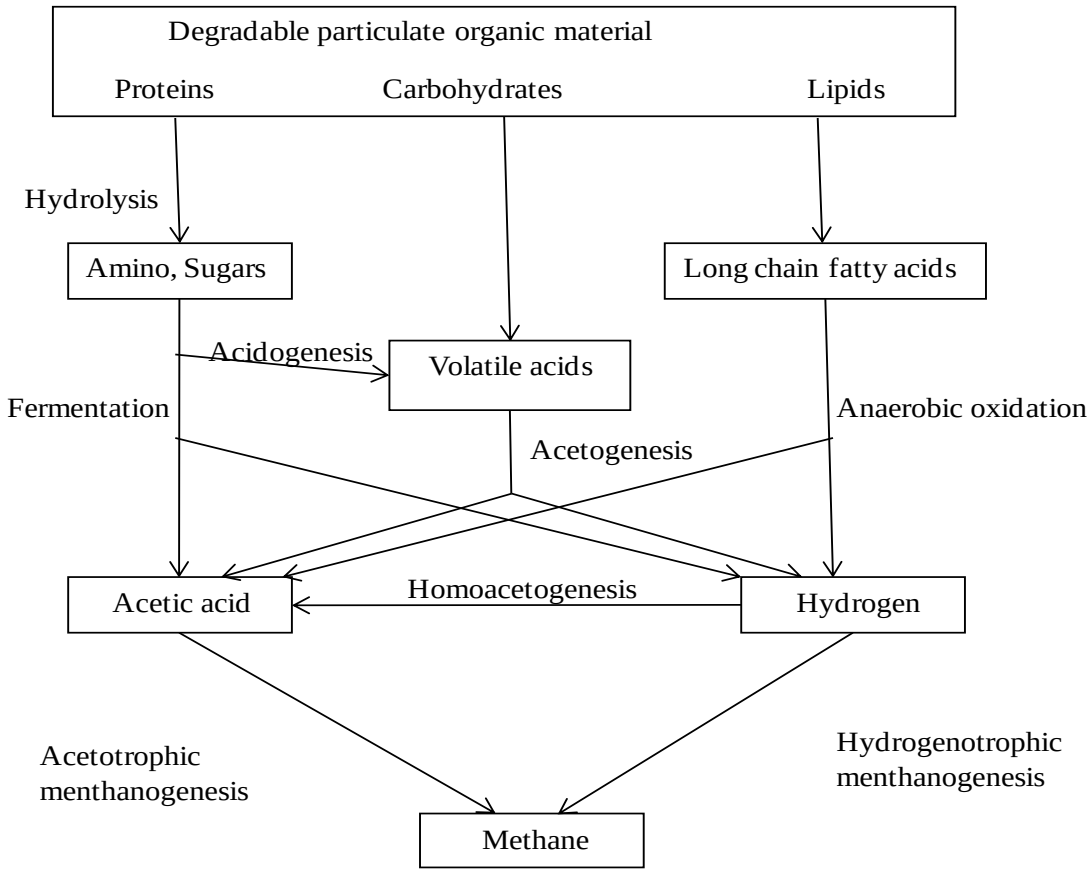


Figure 1.1 A scheme of anaerobic digestion involving multiple biochemical reactions

Anaerobic digestion is a complex process that is performed by a variety of microorganisms. Both Archaea and Bacteria are involved in anaerobic digestion. At least five different groups of microorganisms are involved in methane production. They are fermentative bacteria, hydrogen-producing acetogenic bacteria, hydrogen-consuming acetogenic bacteria, hydrogenotrophic methanogens and acetotrophic methanogens (Parkin and Owen 1986).

Biogas (mainly methane and carbon dioxide) producing microorganisms are divided into two major groups based on their use of elector donors (Grady et al. 1999). The

hydrogenotrophic methanogens obtain energy from the oxidation of H_2 and use CO_2 as elector acceptor (Equation 1.1.1).

$$4H_2 + CO_2 \xrightarrow{\text{hydrogenotrophic methanogen}} CH_4 + 2H_2O \quad (1.1.1)$$

While acetotrophic methanogens grow on acetic acid, consequently generating methane and carbon dioxide (Equation 1.1.2).

$$CH_3COOH \xrightarrow{\text{acetotrophic methanogen}} CH_4 + CO_2 \quad (1.1.2)$$

Representative kinetic values the growth of hydrogenotrophic methanogens and acetotrophic methanogens have been reported under mesophilic condition (Table 1.1) (Siegrist et al. 2002).

Table 1.1 Average kinetic values of mesophilic growth of hydrogenotrophic methanogens and acetotrophic methanogens

Kinetic parameters	Values	Hydrogenotrophic methanogens	Acetotrophic methanogens
Maximum growth rate	μ_{max} (d^{-1})	2.0	0.37
Half-saturation const	Ks (mg COD/L)	0.001	40
Decay rates	k_d (d^{-1})	0.3	0.05

Sulfate-reducing bacteria (SRB) which are commonly present in anaerobic digestion are a competitor of methanogens for substrates. Hydrogen, acetate, alcohols and

fermentation intermediates such as short-chain fatty acids are substrates preferred to use by SRB (Omil et al. 1998). The growth of SRB in anaerobic digestion depends on COD/sulfate ratio of the wastewater. Wastewater with COD/sulfate ratio at 0.67 provides an favorable environment for SRB growth and sulfate reduction while the growth of methanogens is inhibited (Rinzema 1988).

Due to the complexity of anaerobic digestion, studies on all subprocesses and the essential microbial groups involved in each step of anaerobic digestion are needed. A detailed understanding of the overall process and microbial activity is useful to improve and optimize anaerobic digestion process through new engineering and biotechnological approaches. Several key environmental factors such as temperature, pH, and exposure of toxic materials are discussed first in the following section.

Volatile fatty acids such as acetic acid, propionic acid, and butyric acid have been studied in anaerobic digestion for many times (Aguilar et al. 1995, Boltes et al. 2008, Ozturk 1991). Formic acid, pyruvic acid and lactic acid are also important metabolites in anaerobic digestion. However, whether all these VFAs are inhibitory to methanogens at high concentrations or not is still not clear (Parkin and Owen 1986). Besides the impacts of pH and temperature, high concentrations of VFAs are generally believed to inhibit methanogens (Grady et al. 1999). The non-ionized form of VFAs is as actually the true form causing inhibition. The inhibition is often

associated with the H_2 concentration that can control acetogenesis and methanogensis (Grady et al. 1999). A mixture of substrate using acetic acid, propionic acid and butyric acid has been tested in batch and continuous stirred tank reactor (Boltes et al. 2008). High acetic levels in reactor inhibited methanogensis. Therefore understanding different biochemical reactions of VFAs is crucial to anaerobic digestion (Vavilin and Lokshina 1996). The impact of organic acids on anaerobic digestion is therefore discussed in Section 1.3.

Kinetic modeling of anaerobic digestion is a useful tool to design and optimize anaerobic digestion process. It helps describe biological treatment processes in a series of quantitative equations (Pavlostathis and Giraldo-Gomez 1991b), and help simplify or optimize the further study based on simulation results. The kinetic constants were often used in default values. Although the process of anaerobic digestion involves multiple biochemical pathways and different microorganisms, kinetic models can be expressed in several simplified kinetic approaches, which is described in more details in Section 1.4.

1.2 Environmental Factors Affecting Anaerobic Digestion

Temperature--- The effects of temperature on anaerobic digestion are well recognized. Mesophilic (25-45^{0}C) and thermophilic (45-65^{0}C) anaerobic digestion are commonly applied in the field (O'Reilly et al. 2009). Most full-scale anaerobic digesters are operated at mesophilic temperature (Parkin and Owen 1986). Previous studies revealed several advantages of thermophilic digestion, including high organic removal rate, high degree of degradation and excellent solids stabilization (Buhr and Andrews 1977). Since wastewater and biosolids is discharged at relatively low temperature (e.g., 18 ^{0}C), recent research toward anaerobic treatment under psychrophilical condition becomes attractive. For instance, microbial communities involved in digestion are sensitive to temperature changes. However, researchers discovered that anaerobic digestion at low temperature showed reproducible microbial community structure and operational performance, suggesting that optimal cultivation of hydrogenotrophic methanogens is a effective way to improve process efficiency (O'Reilly et al. 2009).

The rate of anaerobic degradation of organic substrates generally increases in the order of psychrophilic, mesophilic and thermophilic digestion. However, anaerobic digestion was traditionally operated in mesophilic range (25–45^{0}C) because of heat generation through methane combustion (Donoso-Bravo et al. 2009). In order to

compare the dynamic behavior of anaerobic digester under different temperature conditions, a mathematic model was developed at different temperatures (Siegrist et al. 2002). Temperature effects on kinetic formulation: specific microbial growth rate, half-saturation constant, and inhibition constants were presumed to be exponential equation:

$$F(T) = e^{\theta (T-To)} \qquad (1.2.1)$$

Where θ = temperature coefficient in the mesophilic (30−40 ^{0}C) and thermophilic (50−60 ^{0}C) temperature range

pH control in anaerobic digestion-- pH is an important factor for keeping functional anaerobic digestion. A typical pH is in the range of 6.5-7.6 (Parkin and Owen 1986). The accumulation of intermediate acids leads to pH drop during fermentation. In order to maintain stable operation, it is necessary to add bicarbonate or carbonate as an alkalinity buffer to neutralize volatile fatty acids and carbon dioxide (Parkin and Owen 1986). Although acetate and ammonia are two alternative buffers for anaerobic systems, these chemicals are unlikely to provide sufficient buffering capacity in anaerobic digestion systems. Acetate is a weak acid (K_a =1.75×10^{-5} at 25 ^{0}C) while ammonia is a weak base (K_a = 5.69×10^{-10} at 25 ^{0}C) (McCarty and Mosey 1991).

Inhibition on anaerobic digestion occurs while pH value is too high or too low. Therefore, a pH-inhibition coefficient I_{pH} was incorporated for modeling (McCarty and Mosey 1991). Monod Equation was modified with pH-inhibition variable I_{pH} as shown in Equation (1.2.2):

$$\mu = \hat{\mu} \frac{S \cdot I_{pH}}{K_S + S} \tag{1.2.2}$$

Where I_{pH} = pH-inhibition coefficient, ranges from 0.1 to 1

1.3 Impact of organic substrates on Anaerobic Digestion

Knowledge of organic substrate utilization in anaerobic digestion is essential. It can be used to assess the impact of organic compounds on anaerobic microbial growth in both engineered and natural systems. A better understanding of the anaerobic degradation of organic compounds also helps to design and optimize anaerobic digestion systems.

Glucose---Glucose from the hydrolysis of macromolecules such as polysaccharides and proteins is the most common substrate in anaerobic digestion. Together with the degradation by several microbial groups, glucose degradation in anaerobic digestion

include multiple reactions such as these listed in the following (Mickelson 1972, Oh et al. 2003):

$$C_6H_{12}O_6 \longrightarrow CH_3COCOOH + CH_3COOH + HCOOH + C_2H_5OH \quad (1.3.1)$$

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (1.3.2)$$

$$C_6H_{12}O_6 + 2H_2 \longrightarrow 2CH_3CH_3COOH + 2H_2O \quad (1.3.3)$$

$$C_6H_{12}O_6 \longrightarrow CH_3(CH_2)_2COOH + 2H_2 + 2CO_2 \quad (1.3.4)$$

Fermentative bacteria initially break down glucose to generate several types of short chain fatty acids. In one of the studies, the fermentation products of glucose degradation (after 99% removal) in batch tests included butyrate (6.4-29.4%), ethanol (15.4-28.8%), acetate (3.4-24.1%), propionate (0.3-12.8%), and hydrogen (4.0-8.1%), with slight amounts of acetone, propanol, and butanol (Oh et al. 2003). All of the products can be converted to methane by methanogens.

Short chain fatty acids--- VFAs that are commonly studied are acetic acid (HAc), propionic acid (HPa) and butyric acid (HBu) while other fatty acids such as formic acid, lactic acid and pyruvic acid are less understood in anaerobic digestion systems. The dissociation of short chain fatty acids and the stoichiometric reactions of them converted to methane are listed in Table 1.2

Table 1.2 Stoichiometric and redox reactions of short chain fatty acids in anaerobic digestion processes

	Stoichiometric reaction	Ionization constant(pKa)	Reactions(Methanogenesis)	E^0 (kJ/mol)
Formic Acid	$CHO_2(aq)=CHO_2^-+H^+$	3.75[a]	$4HCOO^-+4H^+=CH_4+3CO_2+2H_2O$[b]	-144.5[b]
Acetic Acid	$C_2H_4O_2(aq) = C_2H_3O_2^- + H^+$	4.78[a]	$CH_3COO^-+H_2O= CH_4+HCO_3^-$[c]	-31.0[c]
Propionic Acid	$C_3H_6O_2(aq) = C_3H_5O_2^- + H^+$	4.86[a]	$CH_3CH_2COO^- +1/2H_2O= CH_3COO^-+ 3/4CH_4+1/4CO_2$[b]	-26.4[b]
Pyruvic Acid	$C_3H_4O_3(aq)= C_3H_3O_3^-+H^+$	2.39[a]	$CH_3COCOO^-+H^++1/2H_2O = 5/4CH_4+7/4CO_2$[d]	-96.0[d]
Lactic Acid	$C_3H_6O_3(aq)= C_3H_5O_3^-+H^+$	3.08[a]	$2CH_3CH(OH)COO^-+H_2O= HCO_3^-+2CH_3COO^-+H^++CH_4$	-34.3[e]
Butyric Acid	$C_4H_8O_2(aq) = C_4H_7O_2^- + H^+$	4.83[a]	$CH_3CH_2CH_2COO+H_2O+1/2 CO_2=2CH_3COO^-+H^++1/2CH_4$[b]	-17.1[b]

[a](ZirChrom Separations 2006); [b](Boone et al. 1989); [c](Wang et al. 1999); [d](Bock et al. 1994); [e](Bryant et al. 1977)

Acetic acid has been reported as a precursor in methane production, resulting in 70% of total methane production in anaerobic digesters (Mountfort and Asher 1978). HAc is converted into carbon dioxide and methane by acetotrophic methanogens, and all other VFAs have to be converted to HAc before methane production (McCarty and Mosey 1991). It is generally believed that methane generated from HAc is the most sensitive process, therefore, this step is always considered as a rate limitation step in anaerobic digestion (Pavlostathis and Giraldo-Gomez 1991a).

A lot of studies reported that HAc is an easy degraded substrate. No significant inhibition to methanogens was observed at acetate concentration of 2400 mg/L in a

recent study (Wang et al. 2009). This result is in contradictory with earlier studies which suggested concentration of VFAs above 2000 mg/L could inhibit methane production (Buswell and Mueller 1952). In another batch experiment using mixed substrate consisting of acetic, propionic and butyric acid at a ratio of 2:1:1 COD basis (Boltes et al. 2008), high HAc concentrations again inhibited anaerobic digestion. The level of unionized HAc could explain the inhibition, because the unionized HAc at the concentration above 30 mg/L inhibit methanogenesis (Andrews and Pearson 1965). The HAc concentration of 2000 mg/L acetic acid can produce more than 30 mg/L HAc in the unionized form at pH 7. So inhibition could be severe when HAc concentration is high, especially under relatively low pH conditions in the digesters.

Propionic acid is a common intermediate fermentation product in anaerobic digestion. It inhibits methanogensis in anaerobic digestion although the results from researches vary (Hyun et al. 1998, Ozturk 1991, Wang et al. 2009). The accumulation of HPa inhibits the growth of some microbial species and methane production, which is often attributed to thermodynamically unfavorable reactions of HPa oxidation (Ozturk 1991). In another study (Hyun et al. 1998), a broad range of HPa doses in batch anaerobic digestion systems was tested. The results showed significant inhibition on methane production occurred at HPa concentrations of 5000 and 6000 mg COD/L. Similar results were reported that at HPa concentration of 900 mg/L, inhibition on both bacterial activities and cumulative methane yield occurred (Wang et al. 2009).

The fermentation product inhibition is linked with high partial pressure of hydrogen. When partial pressure of hydrogen exceeds about 10^{-4} atm, the rate of methane generation is reduced, meanwhile organic acids such as HPa increases (McCarty 1982).

Butyric acid is often examined simultaneously with HAC and HPa during anaerobic digestion (Lawrence and McCarty 1969, Ozturk 1991, Vavilin and Lokshina 1996, Wang et al. 2009). HBu could be a intermediate product from fermentation of carbohydrate, protein or lipid (Vavilin and Lokshina 1996). HBu at the concentration of 1800 mg/L was shown to be beneficial to methane yield (Wang et al. 2009). In biological hydrogen production applications, HBu at the concentrations between 4180 and 6270 mg/L only imposed slightly inhibition on hydrogen production, and a moderate inhibition occurred at the concentrations of 8360–12540 mg/L (Zheng and Yu 2005).

The rate of VFA conversion to methane usually follows the order of acetic acid>butyric acid>propionic acid (Ren et al. 2003). Kinetic studies of VFA degradation have been reported earlier, which are summarized in Table1.3 (Rittmann and McCarty 2001, Vavilin and Lokshina 1996)

Table 1.3 Kinetic constants for selected VFA degradation in anaerobic digestion

Substrate	T (^{0}C)	K_s(mg COD/L)	μ_{max} (d^{-1})	Y(mg VSS/mg COD)	Reference
Acetic acid	25	869	0.23	0.050	(Lawrence and McCarty 1969)
	35	154	0.32	0.040	(Lawrence and McCarty 1969)
	37	393	0.43		(Massey and Pohland 1978)
	55	820			(Van Lier et al. 1996)
Propionic acid	25	613	0.50	0.051	(Lawrence and McCarty 1969)
	35	32	0.40	0.042	(Lawrence and McCarty 1969)
	37	672			(Whitmore et al. 1985)
	55	86			(Van Lier et al. 1996)
Butyric acid	35	13	0.35	0.047	(Lawrence and McCarty 1969)
	55	16			(Van Lier et al. 1996)

Pyruvic acid is regarded as the most common intermediate in fermentation of glucose. It can be further converted to lactic acid, acetic acid and formic acid (Mickelson 1972). With the existent of pyruvate-utilizing menthanogens, only CO_2 and CH_4 were detected as fermentation products (as shown in Equation 1.3.5). However, while cell concentration of pyruvate-grown methanogens was low at 0.5 mg protein/mL, pyruvate was converted to CH_4, CO_2 and H_2 at ratio of 1 : 2 : 1 (Equation 1.3.6) (Bock et al. 1994).

$$\text{Pyruvate}^- + 0.5H_2O + H^+ \longrightarrow 1.25CH_4 + 1.75CO_2 \qquad (1.3.5)$$

$$\text{Pyruvate}^- + H_2O + H^+ \longrightarrow CH_4 + H_2 + 2CO_2 \qquad (1.3.6)$$

Lactic acid is often viewed as an undesigned terminal fermentation product (Wang et al. 2009). It provides “high-energy” food for many microorganisms (Laanbroek et al. 1982). As a result, lactate-fed SRB have advantage to compete with other microorganisms in mixed cultures (Oyekola et al. 2009). Reactions involved in anaerobic digestion of lactic acid depend on lactate concentration. It generates more propionate at high lactate concentration while produces more acetate at low concentration. Equations 1.3.7 and 1.3.8 represent the pathways of lactate degradation in anaerobic digestion at low and high concentration, respectively.

$$2\,\mathrm{Lactate} \rightarrow 3\,\mathrm{acetate} \tag{1.3.7}$$

$$3\,\mathrm{Lactate} \rightarrow \mathrm{acetate} + 2\,\mathrm{propionate} + \mathrm{H^+} + \mathrm{HCO_3^-} \tag{1.3.8}$$

Formic acid is a carbon source for hydrogenotrophic methanogenesis to produce methane (Boone et al. 1989). Formic acid can be detected in anaerobic digestion processes. It is an important intermediate although formate concentration in anaerobic digestors is extremely low due to the nature of organic degradation by mainly heterotrophs. Although fermentation of glucose and pyruvate can produce formate (Mickelson 1972), conversion of propionate or butyrate to formic acid is thermodynamically unfavorable when formate concentration is high (Boone et al. 1989).

Long-chain fatty acids (LCFA) also affect anaerobic digestion. It has been reported that methane production rate was inversely proportional to concentration of the LCFA containing 16 to 18 carbon atoms (Shin et al. 2003).

Results of kinetic studies of anaerobic digestion of these fatty acids along with regular substrates used in methanogensis are listed in Table 1.4 (Pavlostathis and Giraldo-Gomez 1991b).

Table 1.4 Kinetic constants of anaerobic degradation of fatty acids in mesophilic anaerobic digestion

Substrate	Process	Ks (mg COD/L)	μ_{max} (d^{-1})	Y(mg VSS/mg COD)
Long-chain fatty acid	Anaerobic oxidation	105-3180	0.085-0.55	0.04-0.11
Short-chain fatty acid	Anaerobic oxidation	12-500	0.13-1.20	0.025-0.047
acetate	Acetotrophic methanogenesis	11-421	0.08-0.7	0.01-0.054
Hydrogen/carbon dioxide	H_2-oxidizing methanogenesis	4.8×10^{-5}-0.60	0.05-4.07	0.017-0.045

Lastly, 2-Bromoethanesulfonate (BES, $BrCH_2CH_2SO_3$) is a specific inhibitor of methanogenic growth because of its comparability with Coenzyme M (CoM; $HSCH_2CH_2SO_3$), which is a cofactor involved in the final step of methane biosynthesis (Gunsalus, 1978, Vogels, 1988). Other chemical reactions involved in anaerobic digestion are generally not affected by BES (Chiu and Lee 2001).

1.4 Kinetic Models used in Anaerobic Digestion

Process kinetic studies of anaerobic degradation of organic substrates can contribute to basic process analysis, optimal design and operation, and maximum substrate utilization rate in anaerobic digestion (Pavlostathis and Giraldo-Gomez 1991b). Modeling of biological processes in anaerobic digestion is a good tool to describe and convert complex biological problems and extensive experimental data into simple formula (McCarty and Mosey 1991). The kinetic description of anaerobic digestion is based on coupled relationship between microbial growth and substrate utilization as described in details below.

1.4.1 Fundamental of Kinetics and Stoichiometry

Microbial growth rate---The microbial growth rate in anaerobic digestion is described by the following autocatalytic equation based on the nature of microbial binary fission:

$$\frac{dX}{dt} = \mu X \qquad (1.4.1)$$

Where $\frac{dX}{dt}$ = microbial growth rate, mg VSS/L·h^{-1}

X = biomass concentration, mg/L

t = time, h

μ= specific growth rate, h^{-1}

Growth yield and substrate utilization rate---Stoichiometric reactions between substrate utilization and microbial growth suggest that the relationship between microbial growth rate and substrate growth rate is coupled as follows:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} \tag{1.4.2}$$

Where $\frac{dS}{dt}$ = substrate utilization rate, mg substrate/L·h^{-1}

S = substrate concentration, mg/L

Y = microbial growth yield coefficient, mg substrate/mg VSS·h^{-1}

Relationship between substrate concentration and microbial growth rate--- The changes of substrate and biomass concentration in batch reactor must be solved simultaneously with equations (1.4.1) and (1.4.2). To do so, Monod equation is needed to link substrate concentration with bacterial growth. Over the years, the empirical based Monod equation was verified experimentally. The Monod equation is described as follows:

$$\mu = \hat{\mu}\frac{S}{K_S + S} \tag{1.4.3}$$

Where $\hat{\mu}$ =maximum specific growth rate, h^{-1}

Ks = substrate concentration at one half the maximum rate, mg/L

The Monod equation is similar to the equation from Michaelis-Menten Model.

Combined equation 1.4.2 and 1.4.3 results in:

$$\frac{dS}{dt} = -\frac{\hat{\mu} \cdot X}{Y} \frac{S}{K_S + S} \tag{1.4.4}$$

This equation describes that the rate of substrate utilization rate depends on multiple factors including substrate concentration. However, some organic substrates show significant inhibition on anaerobic reactions at high substrate concentrations. Consequently, a modified Monod equation-Andrews equation including an inhibition coefficient is introduced as follows:

$$\mu = \hat{\mu} \frac{S}{K_S + S + S^2 / K_I} \tag{1.4.5}$$

Where K_I = inhibition coefficient, mg/L

1.4.2 Kinetic Models for Anaerobic Digestion

This section describes several commonly used models in anaerobic digestion.

Growth kinetics of two types of methanogenic species---Two acetate-fed completely stirred tank reactors (CSTR) were established using different feeding rates (Conklin et al. 2006). Because of the difference of feeding rate, specific acetotrophic methogenic species, namely *Methanosarcina* and *Methanosaeta* were enriched. Based

on the estimated microbial growth of *Methanosarcina* and *Methanosaeta* and substrate utilization kinetics, a better feeding method was summarized for each species. Several kinetic equations are shown in equation (1.4.6), (1.4.7) and (1.4.8), and the kinetic constants are listed in Table1.1:

$$X_a = \frac{Y(\Delta S)}{1 + k_d SRT} \tag{1.4.6}$$

$$X_{a+i} = \frac{Y(\Delta S)}{1 + k_D SRT} \tag{1.4.7}$$

$$\text{active fraction} = \frac{1 + k_D SRT}{1 + k_d SRT} \tag{1.4.8}$$

Where X_a = active biomass, mg/L

X_{a+i} = total biomass, mg/L

Y = biomass yield, mg substrate/mg VSS·h^{-1}

ΔS = used biomass, mg/L

K_d = decay rate, d^{-1}

SRT = solids retention time, d

K_D = total decay rate, d^{-1}

1.4.3 Kinetic models of Substrate Utilization

Eastman and Ferguson Model

A simple model for substrate utilization is Eastman and Ferguson Model (Eastman and Ferguson 1981), which was set up based on a continuous stirred tank reactor (CSTR) treating primary sewage sludge. In this model, the hydrolysis and fermentation phase are assumed to be combined in acidogenic phase.

This model described hydrolysis of particulate substrate to soluble biodegradable substrate to follow a first-order reaction. At constant temperature and pH, coupled with the use of biomass growth first-order endogenous decay, the reaction rates can be expressed. The hydrolysis of particulate COD in influent (F) is as follows:

$$\rho_F = k_h F \tag{1.4.9}$$

Both hydrolysis of particulate COD and substrate utilization affect soluble biodegradable substrate in the effluent (S):

$$\rho_S = k_h F - \frac{1}{Y}\hat{\mu}\frac{SX}{K_s + S} \tag{1.4.10}$$

On the rate of formation of fermentation products (P) and the rate of active biomass concentration in the effluent of CSTR were (Zheng and Yu).

$$\rho_P = (1-Y)\ \frac{1}{Y}\hat{\mu}\frac{SX}{K_s + S} + k_d X \tag{1.4.11}$$

$$\rho_X = \hat{\mu}\frac{SX}{K_s + S} - k_d X \qquad (1.4.12)$$

Where ρ = rate, g COD L^{-1} h^{-1} , k_h= Hydrolysis constant,h^{-1}; μ=Maximum specific growth rate, h^{-1}; Ks=Saturation constant, g COD/L; Y= Growth yield coefficient, g COD/g COD; k_d= Decay coefficient, h^{-1}; F= Particulate degradable substrate in influent, g COD/L; S= Soluble degradable substrate in the effluent, g COD/L; X= Active biomass concentration in the effluent, g COD/L.

Siegrist Model

More complex substrate model (Siegrist et al. 2002) has been established to describe the dynamic behavior of mesophilic and thermophilic digestion in a CSTR. The model included acetotrophic methanogenesis, propionate degradation and hydrolysis, and inhibition factors affecting organic degradation. A first-order equation was also used to describe the kinetics of hydrolysis of particulate substrate. This model provided equations to describe the dynamic behavior of fermentation and methanogensis (Table 1.5) coupled with the use of different inhibition factors (Table 1.6).

Table 1.5 Specific process rates (ρ_j) of substrate degradation in anaerobic digestion

Process	Rate ρ_j/(g COD / mol m^{-3} d^{-1})
Hydrolysis	$K_H X_S$
Amino acids fermentation	$\mu_{max,3} \frac{S_{aa}}{K_{S,aa} + S_{aa}} I_{pH,3} X_{aa}$
Fatty acid anaerobic oxidation	$\mu_{max,5} \frac{S_{fa}}{K_{S,fa} + S_{fa}} I_{ac,5} I_{H_2,5} I_{pH,5} X_{fa}$
Propionate anaerobic oxidation	$\mu_{max,6} \frac{S_{pro}}{K_{S,pro} + S_{pro}} I_{ac,6} I_{H_2,6} I_{pH,6} I_{NH_3,6} X_{pro}$
Acetotrophic methanogenesis	$\mu_{max,7} \frac{S_{ac}}{K_{S,ac} + S_{ac}} I_{pH,7} I_{NH_3,7} X_{ac}$
Hydrogenotrophic methanogenesis	$\mu_{max,8} \frac{S_{H_2}}{K_{S,H_2} + S_{H_2}} I_{pH,8} X_{H_2}$

ρ_j= process rate, $I_{i,j}$ = inhibition functions, S_i = soluble components, X_i = particulate components,

Table 1.6 Process inhibition factors involved in anaerobic digestion

Process	Equation
Consumption of the LCFA and propionic acid	$I_{ac,j} = \frac{K_{I,ac,j}}{K_{I,ac,j} + S_{ac}}$
Consumption of the LCFA and propionic acid	$I_{H_2,j} = \frac{K_{I,H_2,j}}{K_{I,H_2,j} + S_{H_2}}$
Propionic acid degradation and acetotrophic methanogenesis	$I_{NH_3,j} = \frac{K_{I,NH_3,j}^{\ 2}}{K_{I,NH_3,j}^{\ 2} + S_{NH_3}^{\ 2}}$
Fermentation, aerobic oxidation, and methanogenesis	$I_{pH,j} = \frac{K_{I,H,j}^{\ 2}}{K_{I,H,j}^{\ 2} + S_H^{\ 2}}$

$I_{ac,j}$ = inhibition coefficient caused by acetate, $I_{H2,j}$= inhibition coefficient caused by hydrogen, $I_{NH3,j}$ = inhibition coefficient caused by free ammonia, $I_{pH,j}$ = inhibition coefficient caused by pH

Laboratory reactors fed with acetate, propionate and formate as substrates were used for model calibration. Consequently A set of kinetic constants was therefore calculated (Table 1.7).

Table 1.7 Kinetic values of Mesophilic (35 ± 5 ^{0}C) temperature

process	coefficient			process		coefficient
	k_H (d^{-1})	μ_{max} (d^{-1})	K_s (g COD/m^3)			K_I
hydrolysis	0.25			acetate (g COD/m^3)		1500
amino acid ferment		4.0	50	H_2 (mg COD /m^3)	LCFA degradation	3
					propionate degradation	1
					fermentation	0.01
LCFA degradation		0.60	1000	pH (mol/m^3)	methanogensis /anaerobic oxidation	0.0005
propionate degradation.		0.60	20	NH_3 (g N/m^3)	propionate degradation	25
					acetotrophic methanogensis	17
acetotrophic methanogensis		0.37	40			
hydrogenotrophic methanogensis		2.0	0.001			

METHANE Model

Generalized ‹METHANE› model of anaerobic digestion was constructed (Noike et al. 1985). This model was improved three year after it published (Vavilin and

Lokshina 1996), with the modification to include a sum of three types of VFAs (acetic, propionic and butyric acids) and the inhibition factors.

Evaluation of VFA degradation and microbial activity through batch studies was conducted using different substrates. The model assume high initial biomass concentration (X_0) with low biomass change (ΔX), high substrate concentration (S) and low half-saturation coefficient (K_s), and higher maximum specific growth rate of biomass ($\hat{\mu}$) compared with low biomass decay coefficient (k_d). Kinetic equations and rate constants are shown as following：

$$S = S_0 - \frac{X_0}{Y}(e^{\hat{\mu}t} - 1) \tag{1.4.13}$$

$$\hat{\mu} = \frac{1}{\Delta t}\ln\frac{r_2}{r_1} \tag{1.4.14}$$

$$\frac{X_0}{Y} = \frac{S_0 - S(t)}{e^{\hat{\mu}t} - 1} \tag{1.4.15}$$

Where r_1,r_2 = substrate removal rates; t = current time.

The improved ‹METHANE› model included the inhibition factor, but it became more complex:

$$S = S_0 - \frac{X_0}{Y}\left(\frac{1 + I_0 K_i}{1 + I_0/K_i - k/K_i t}\right)^{\hat{\mu}K_i/k} \tag{1.4.16}$$

Where I_0 =initial inhibitor concentration

Finally, the Monod and Andrews equations were tested through the use of ‹METHANE› model by fitting the data from a continuous–flow reactor. The kinetic constants were obtained and presented in Table 1.8. Results from this model simulation showed Monod and Andrews kinetics failed to describe the system of treating acetate at the concentration of 65 g/L, likely because of pH inhibition and non-ionized acetate inhibition that were not considered in the equations (Vavilin and Lokshina 1996).

Table 1.8 Kinetic constants of acetate degradation using Monod and Andrews model

Model	μ_{max} (d^{-1})	K_s (mg/L)	Y (mM /mM)	K_i (g/L)	K_{pH1}	K_{pH2}
Monod	0.30	200	0.015		3.15	2.95
Andrews	0.45	20	0.015	50		

Anaerobic Digestion Model, No.1 (ADM1)

A recent development of anaerobic digestion modeling is the landmark model named Anaerobic Digestion Model, No.1 (ADM1) that was developed by International Water Association (IWA) Task Group (Boltes et al. 2008). This model supplied a fundamental basis for kinetic modeling of anaerobic digestion with a set of default kinetic parameters. Because of its wide applicability, it was modified to fit experimental data for VAF degradation (Boltes et al. 2008).

A set of kinetic constants from VFA degradation was obtained using the modified ADM1 (Table 1.9) (Boltes et al. 2008). In this research, mixed substrates containing acetate, propionate and butyrate at 2:1:1 COD ratio were added in both batch and continuous stirred tank reactors. Biogas and mixed biomass generation rate were obtained from the estimated VFA degradation rate. Finally kinetic parameters were estimated for both batch and continued reactors. Two modified Monod equations of substrate consumption in batch reactors were used in this modified model:

Propionate and butyrate consumption

$$q_i = q_{mi}\left(1-\frac{HAc}{HAc^*}\right)^n \frac{S}{S+K_{Mi}\left(1-\frac{HAc}{HAc^*}\right)^m} \qquad (1.4.17)$$

Acetate consumption

$$q_a = q_{ma}\frac{S}{K_{Ma}+HAc+\left(\frac{HAc^2}{K_{ia}}\right)} \qquad (1.4.18)$$

Where q_i = specific substrate consumption rate, d^{-1}

q_a = specific acetate substrate consumption rate, d^{-1}

q_{mi} = maximum specific substrate consumption rate, d^{-1}

q_{ma} = maximum specific acetate consumption rate, d^{-1}

HAc = inhibitor concentration, mg COD/L

HAc* = critical inhibitor concentration above which reaction stops, mg COD/L

K_{Mi} /K_{Ma}= Monod saturation constant, mg/L

m = order of reaction

S = substrate concentration, mg/L

Table 1.9 Kinetic constants of degradation of acetic, propionic and butyric acid in anaerobic digestion

Substrate	Continuous feeding		Batch assays			
	q_m (g COD/g VSS d)	K_M (g COD/L)	K_i (g COD/L)	n (g COD/g VSS d)	m(g/COD/L)	I (g COD/L)
HAc	0.682	0.892	0.667			
HPa	0.082	0.426		0	-6.405	4.633
HBu	0.239	0.055		0	0	

I = acetic acid concentration

Application of the ADM1 model was applied to determine propionate degradation in anaerobic digestion (Hyun et al. 1998). Because of the complex pathway of propionate degradation, the kinetic model was set up with modifications. A steady-state anaerobic Master Culture Reactor (MCR) was operated to enrich propionate degraders. MCR provided test culture for serum bottles, and then cumulative gas volume, gas components, and relationship between acetate accumulation and propionate degradation were analyzed. For modeling substrate self inhibition, the Haldane (Andrews) equation was used for modeling (Hyun et al. 1998).

1.5 Summary and Research Perspectives

Since the various results of VFA biodegradation kinetics in anaerobic digestion exist in the literature, the exact substrate concentration affecting anaerobic digestion remains to be determined in a controlled system using digested sludge. Moreover, few studies address the impact of formic acid, lactic acid and pyruvic acid on anaerobic digestion. The purpose of this research was to determine the impact of selected C1 to C4 organic acids on anaerobic digestion. Results of this research could therefore contribute to anaerobic digestion modeling, especially when the influent waste contains high amounts of specific organic acids.

CHAPTER 2

MATERIALS AND METHODS

2.1 Reactor setup

Batch tests were conducted using sixteen 500-mL serum bottles. Each bottle was seeded with 300 mL digestion sludge that was collected from Columbia Wastewater Treatment Plant and had been digested for 20 days. Prior to seed each bottle, sludge was fed with feedstock including the followings: Carbonate buffer (Shimada et al. 2008), a combination of $NaHCO_3$ (3450 mg/L) and $KHCO_3$ (1725 mg/L); $CaCl_2 \cdot 2H_2O$, 100 mg/L; Macronutrients including NH_4Cl, 114mg/L; $NaHPO_4$, 28mg/L.$MgSO_4$, 44 mg/L; and trace metals (Choi et al. 2008) which contained $FeCI_2 \cdot 4H_2O$, 2 mg/L; $MnSO_4 \cdot H_2O$, 3.4 mg/L; $(NH_4)_6MoO_{24} \cdot 4H_2O$, 1.2 mg/L; $CuSO_4$, 0.8 mg/L; $Zn(NO_3)_2 \cdot 6H_2O$, 1.8 mg/L; $Ni(NO_3)_2 \cdot 6H_2O$, 0.3 mg/L. These anaerobic digestion tests of selected six organic acid materials (formic acid, acetic acid, propionic acid, pyruvic acid, lactic acid and butyric acid), all in sodium form were then conducted in separate batches. Detailed experimental designs for the six chemicals are shown in Table 2.1. The concentration of substrates added to batch

reactors was expressed in COD units. All experiments were conducted in triplicate, unless elsewhere specified.

Table 2.1 Design of batch anaerobic digestion experiments

Organic chemical	Materials added
	300 mL Sludge + 100 mL DI water
Formate	300 mL Sludge + 100 mL formate at a final concentration ranging from 500 to 8000 mg COD/L
	300 mL Sludge + 100 mL DIwater
Acetate	300 mL Sludge + 100 mL acetate at a final concentration ranging from 500 to 5000 mg COD/L
	300 mL Sludge + 100 mL DIwater
Propionate	300 mL Sludge + 100 mL propionate at a final concentration ranging from 500 to 5000 mg COD/L
	300 mL Sludge + 100 mL DIwater
Pyruvate	300 mL Sludge + 100 mL pyruvate at a final concentration ranging from 250 to 4000 mg COD/L
	300 mL Sludge + 100 mL DIwater
Lactate	300 mL Sludge + 100 mL lactate at a final concentration ranging from 250 to 4000 mg COD/L
	300 mL Sludge + 100 mL DIwater
Butyrate	300 mL Sludge + 100 mL butyrate at a final concentration ranging from 250 to 4000 mg COD/L

Duplicate blank samples were prepared for these experiments. Before each anaerobic digestion test, to maintain anaerobic environment, nitrogen gas was flushed for 1 min before the bottle was closed. Gas production from anaerobic digestion in each serum bottle was directly recorded using Challenge AER-200 Respirometer. The equipment recorded the cumulative biogas volume every ten minutes for about 6 days, after which gas generation from the remaining substrate was insignificant. Aliquots (15 mL)

of mixed liquor were taken from each batch reactor before and after digestion for pH (using OAKLON pH meter) and biomass COD (using HACH high-range vials). The anaerobic digestion was conducted at room temperature (23 ± 1 ^{0}C) throughout the whole test.

Biogas from anaerobic digestion is mainly composed of 60 to 75 percent methane, followed by 25-45% carbon dioxide (Haug et al. 1978, Parkin and Owen 1986). The fractions of methane and CO_2 in biogas were inferred from the batch anaerobic test using BES ($BrCH_2CH_2SO_3^-$) as a specific methanogen inhibitor. BES a structural analogue of Coenzyme M and can completely inhibit methanogenic activity at high concentrations (Nollet et al. 1997). The volume of methane in total biogas was estimated by comparing the difference from the cumulative biogas volume in anaerobic digestion in the presence or absence of BES. Four different concentrations of BES were examined and the cumulative biogas production was recorded. The methane fraction of biogas was further determined by measuring carbon dioxide concentration in headspace gas of the bottle using a CO_2 detection tube (GASTEC No.2H), and result was compared with that from the BES test.

2.2 Kinetic studies

A stoichiometric equation of substrate utilization coupled with methane production and microbial growth depends on the chemical composition of the substrate. The

following Equation 2.1 (McCarty and Mosey 1991) has been developed and applied for the overall conversion of substrate to methane, carbon dioxide, biomass, ammonia and bicarbonate.

$$C_nH_aO_bN_c + (2n + c - b - \frac{9sd}{20} - \frac{ed}{4})H_2O \rightarrow \frac{de}{8}CH_4 + (n - c - \frac{sd}{5} - \frac{de}{8})CO_2 + \frac{sd}{20}C_5H_7O_2N + (c - \frac{sd}{20})NH_4^+ + (c - \frac{sd}{20})HCO_3^- \quad (2.1)$$

Where d = 4n + a - 2b - 3c; s = fraction of waste converted to cells; e = fraction of waste converted to methane gas for energy (s + e = 1); $C_nH_aO_bN_c$ = empirical formula of waste being digested; $C_5H_7O_2N$ = empirical formula of bacterial dry mass (i.e., VSS)

This equation was used for all the six organic acid substrates in this research. The substrate utilization rate ($\frac{dS}{dt}$) was determined by monitoring biogas production rate ($\frac{dV}{dt}$) (Equation 2.2) (specifically methane production rate after taking methane fraction in total biogas into consideration):

$$\frac{dS}{dt} = -\frac{Y}{1-Y}\frac{dV}{dt} \quad (2.2)$$

Biogas generation rate was obtained from cumulative biogas volume figures. The substrate degradation in anaerobic digestion was assumed following a first order rate process at low substrate concentration. Consequently, biogas generation as follow:

$$V = V_m(1 - e^{-kt}) \quad (2.3)$$

Where: V = cumulative gas volume at time t, mL; V_m = ultimate gas yield, mL; k = the first order rate constant, /d

Parameters of anaerobic biodegradation, V_m and k, were estimated using a nonlinear regression fitting the experimental data. The regression was performed by Marquardt-Levenberg algorithm by Sigma Plot 10.0. Figure 2.1 is giving an example of resulted methane production rate as a function of substrate concentration.

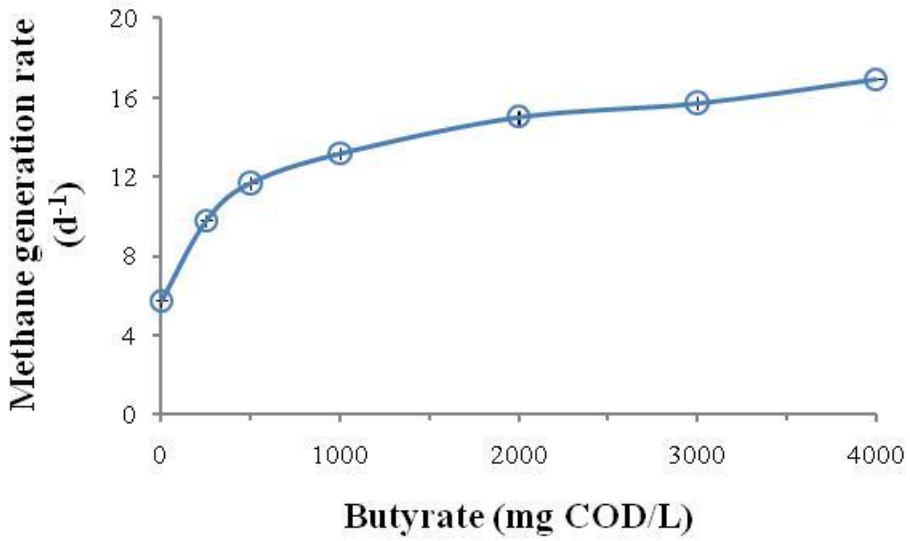


Figure 2.1 Biogas production rate as a function of substrate (butyrate) concentration.

However, not all of the substrates could be completely degraded at high substrate concentrations after 6 days anaerobic digestion. In such cases, experimental data were not be fitted by the first order equation. Instead, biogas generation rate was assumed to follow zero order reaction and calculated directly from cumulative biogas curves as indicated by their slopes.

The equation of biomass generation follows a first order reaction with respect to biomass concentration (X). Previous studies have shown that organic portion of biomass represents 85% of the total biomass (Grady et al. 1999). With an assumed microbial growth yield, the biomass specific growth rate (μ) at different substrate concentrations was calculated using Equation 2.5 (Grady et al. 1999).

$$\frac{dX}{dt} = \mu X \tag{2.4}$$

$$\mu = (\frac{Y}{1-Y})(\frac{dV}{Xdt}) \tag{2.5}$$

Where: $\frac{dV}{dt}$ = biogas (methane) generation rate, mL/h

X=biomass concentration, mg COD/L

Y= methanogenic growth yield coefficient, mg VSS/mg COD

μ= specific growth rate, h^{-1}

V= biogas volume, mL

Based on available literature information (e.g. Table 1.4), a same average value of Y (0.03 mg VSS/mg COD) was used for the six organic acid substrates in microbial kinetic parameter estimation.

Monod kinetic has been proved powerful in describing microbial growth (Noike et al. 1985, Zehnder et al. 1980). It has been successfully used for modeling anaerobic digestion processes (Pavlostathis and Giraldo-Gomez 1991b). Hence, Monod Equation 2.6 was used to fit the data of anaerobic degradation of VAFs in this study.

$$\mu = \hat{\mu}\frac{S}{K_S + S} \tag{2.6}$$

Where: Ks= substrate concentration at one-half the maximum rate, mg/L

Maximum specific growth rate ($\hat{\mu}$) and substrate half saturation constant (Ks) were obtained using a nonlinear regression fit to the data that contained the estimated specific growth rates as a function of substrate concentration. Since substrate self-inhibition behavior was observed in some of the anaerobic digestion experiments, Andrews Equation 2.7 was applied to estimate additional parameter --inhibition coefficient (Fukuzaki et al. 1990a, Fukuzaki et al. 1990b).

$$\mu = \hat{\mu}\frac{S}{K_S + S + S^2 / K_I} \tag{2.7}$$

Where: K_I = inhibition coefficient, mg/L

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Digested sludge property and pH condition

Digested sludge from the Columbia Wastewater Plant was used and characterized in each anaerobic digestion experiment to obtain comparable experimental results of anaerobic digestion of six selected C1 to C4 organic substrates. As can be seen from Figure 3.1, the measured total COD of the digested sludge was closed to 10,000 mg COD/L. There was a slight decrease of biomass COD after 6-d digestion for all blank samples tested in different batch tests, indicating that the concentrations of residual VFAs or other soluble biodegradable substrates in the digested sludge is very low and would not affect the anaerobic digestion of C1 to C4 organic acids in this study .

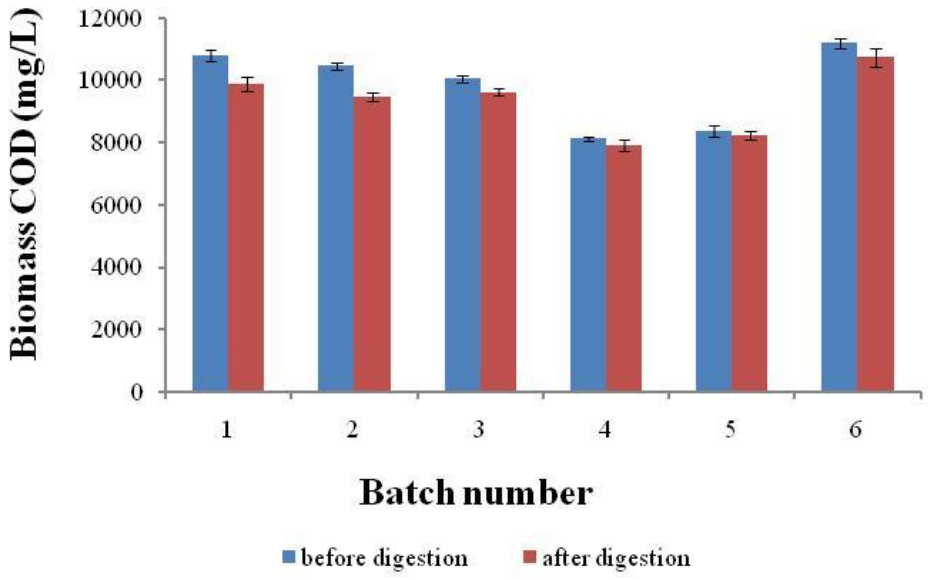


Figure 3.1. Total Biomass COD concentration of the blank samples in different batch experiments. The blue and red columns represent biomass COD values before and after anaerobic digestion.

The measured pH values of blank samples were between 7.5-8.0. For comparison, the measured pH values of the sludge samples treated with C1 to C4 organic acids at the highest concentrations were also between 7.5 and 8.0. Because of the use of organic acid salts and the addition of carbonate buffering source in the digestion bottles, little change of pH was expected.

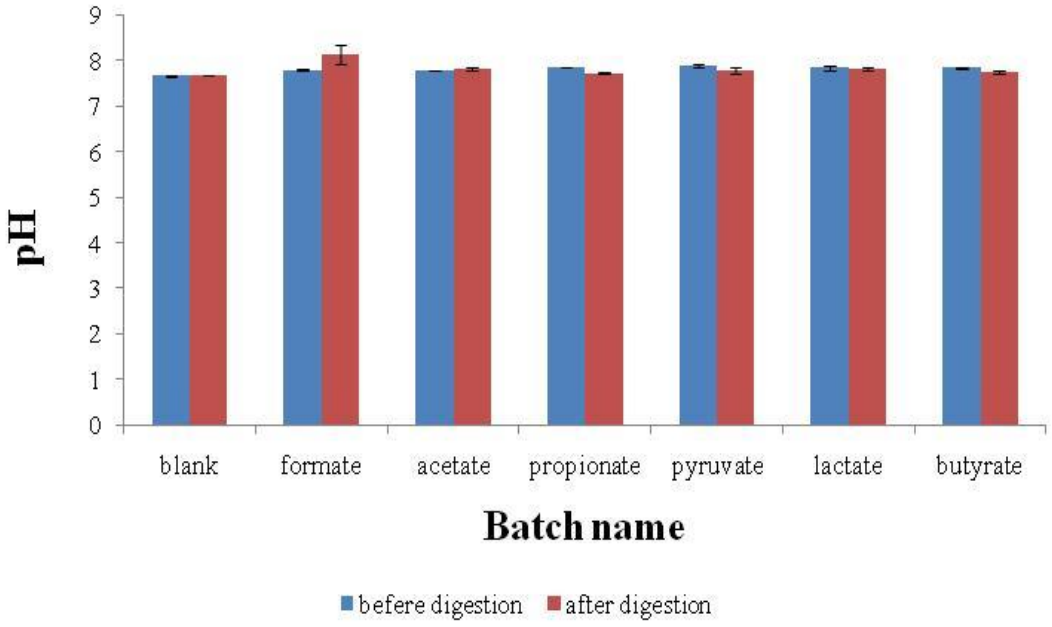


Figure 3.2 The change of pH of the blank sludge samples and sludge fed with 4000 mg COD/mL C1 to C4 organic acid salts. The blue and red columns represent pH values before and after anaerobic digestion.

3.2 Biogas composition estimation using glucose as a sole substrate

Glucose was used as sole substrate in conjunction with the use of BES to infer methane fraction from cumulative biogas production in anaerobic digestion. Prior to that, a separate experiment was conducted to determine the optimal glucose concentration for maximum biogas production in anaerobic digestion. Glucose concentration at 1000 mg COD/L was verified to result in the highest biogas generation rate (Figure 3.3). Based on this observation, anaerobic sludge was fed with 1000 mg COD/L glucose and amended with BES at different concentrations. The results demonstrated that BES completely inhibited methane production at the concentrations of 30 mM and above (Figure 3.4). From Figure 3.4, it is estimated that methane represented about 60% of the total biogas. Meanwhile, direct CO_2 measurements of headspace gas at the end of anaerobic digestion resulted in 35-40% of CO_2 in the total biogas, consistent with the results from the BES study.

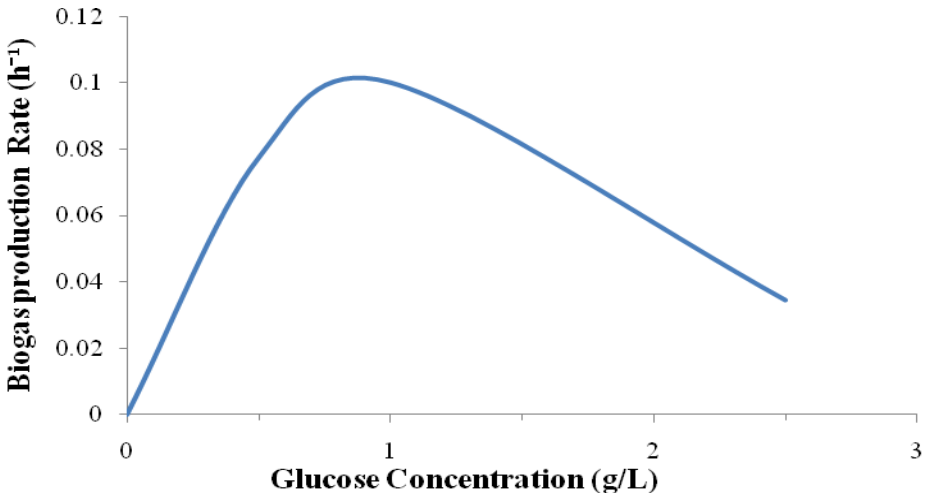


Figure 3.3 Biogas production rate as a function of glucose concentration.

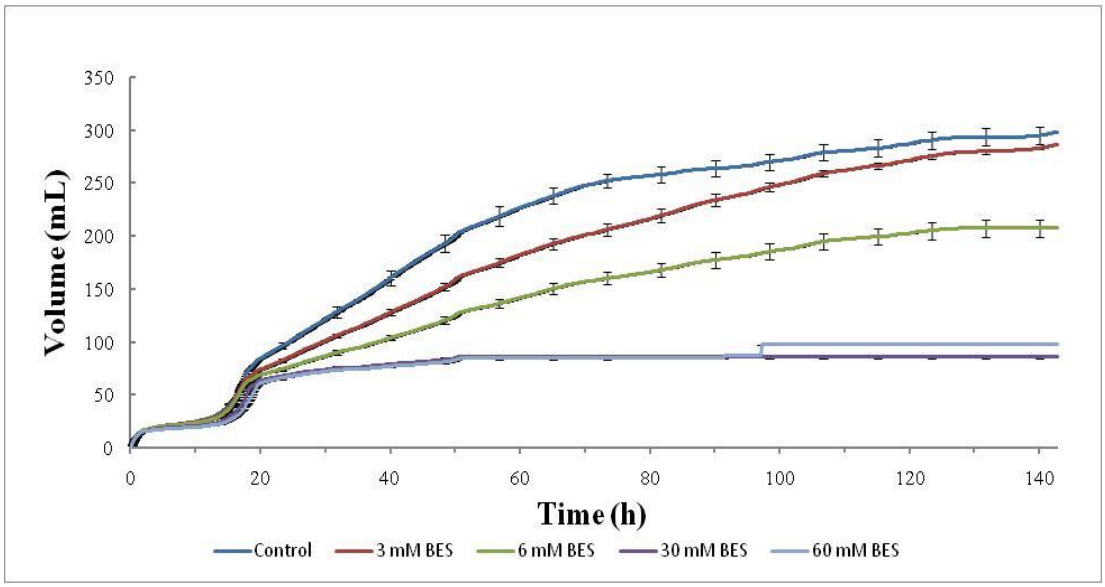


Figure 3.4 Cumulative biogas production at different BES concentrations using glucose (1000 mg COD /L) as sole substrate.

3.3 Impact of organic acid substrates on anaerobic digestion

3.3.1 Substrates showing no inhibition on anaerobic digestion

Figure 3.5 shows the experimental data of cumulative biogas production fed with butyrate at a concentration range from 250 to 4000 mg COD/L. The rate of biogas produced was directly proportional to substrate concentration, as can be seen from Figure 3. 6.

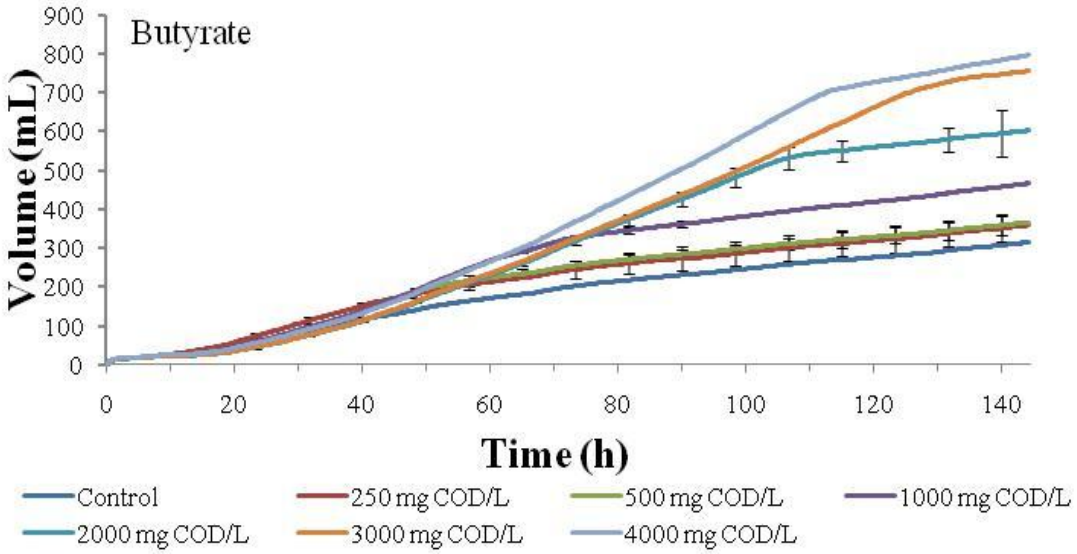


Figure 3.5 Cumulative biogas production curves of anaerobic digestion of butyrate at different butyrate concentrations.

A plot of specific growth rate verse substrate concentration (Figure 3. 6) showed no gas production inhibition by butyrate at the concentrations ranging from 250 to 4000 mg COD/L. This result was consistent with recent reports that butyrate has no inhibition on methanogensis (Wang et al. 2009).

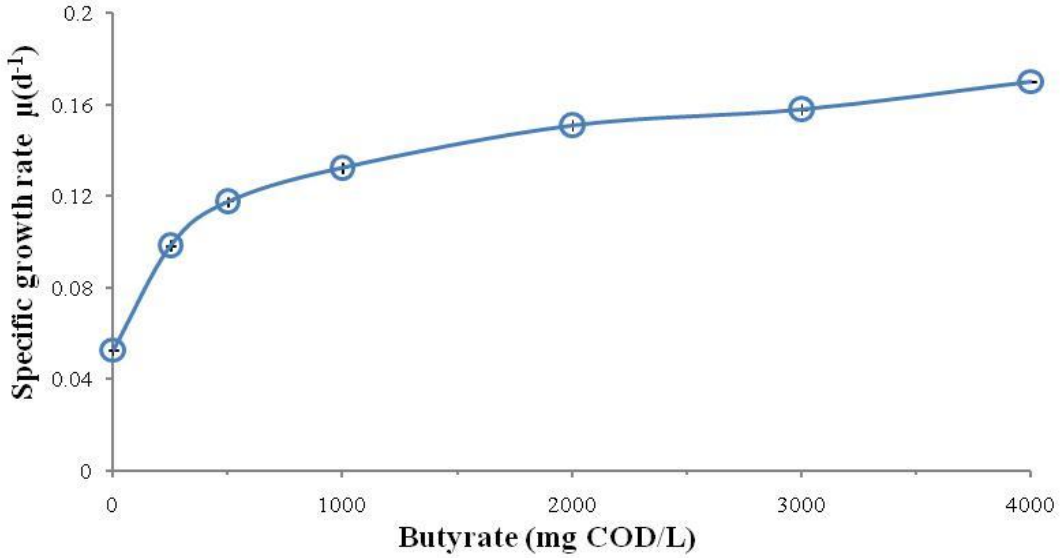
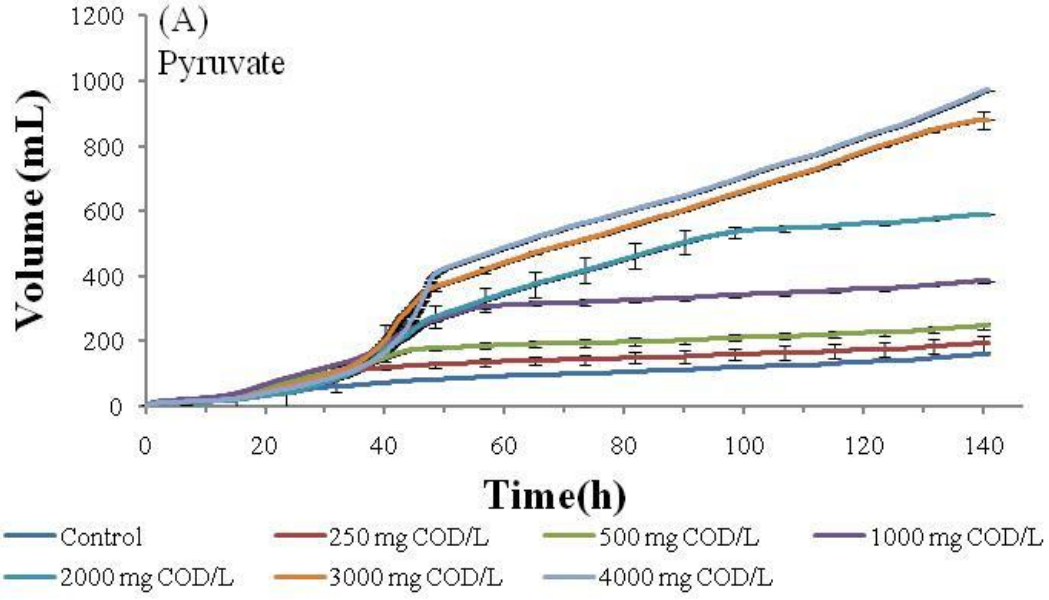


Figure 3.6 Value of biomass specific growth rate of reactor fed by butyrate, no inhibition was detected in the substrate concentration ranges experiments tested.

Similar trend was found in figures by plotting the cumulative biogas production with the concentration of substrates such as pyruvate, lactate and formate (Figure 3.7), which indicated no inhibition on methanogenesis by these substrates. The specific biomass growth rate increased with substrate concentration (Figure 3.8).



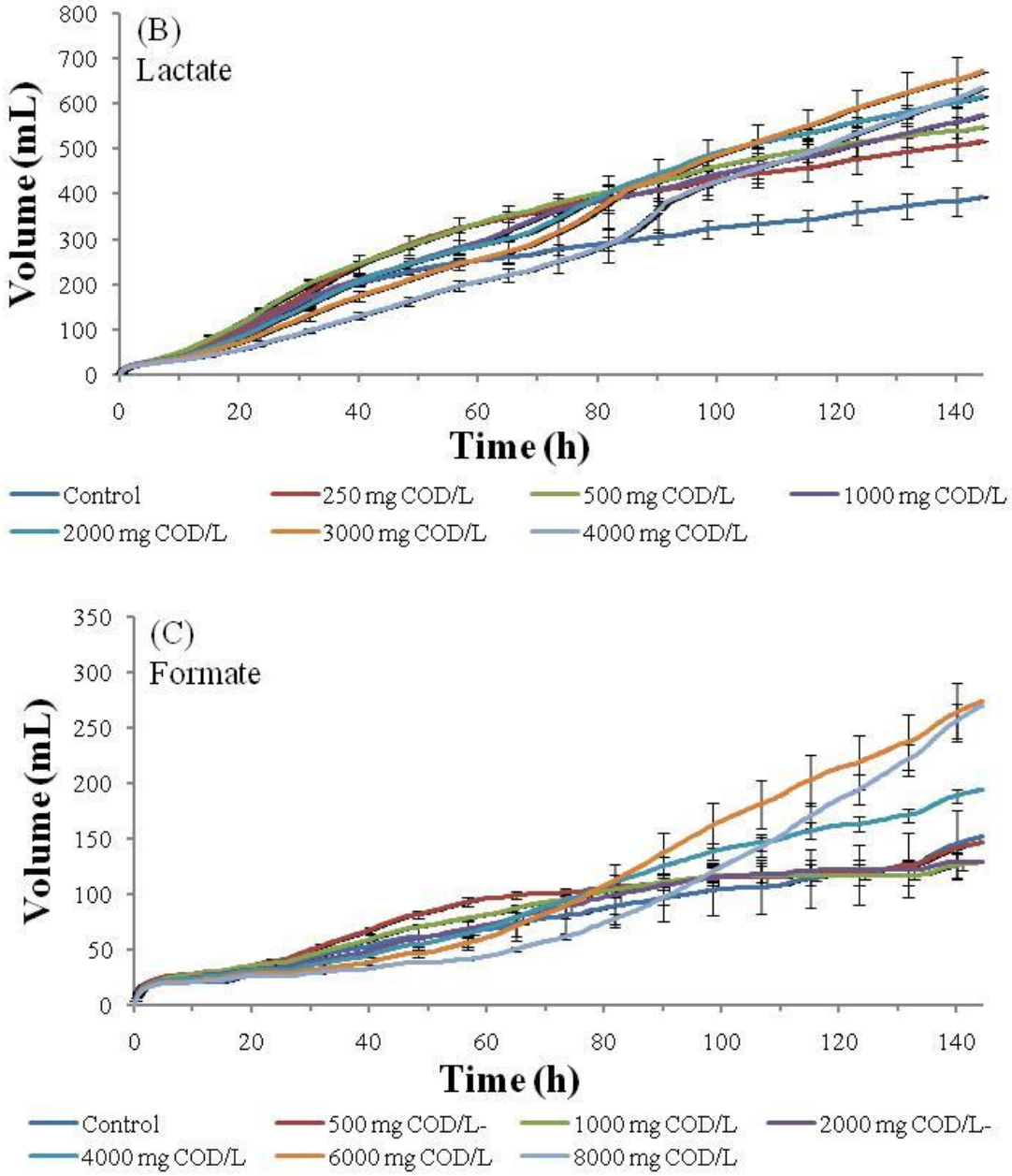
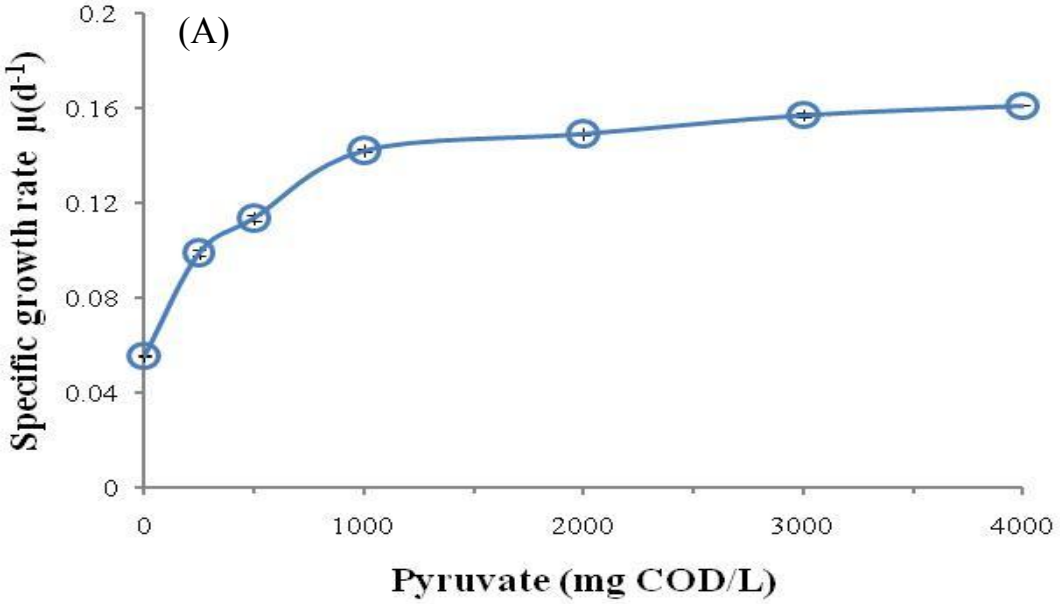
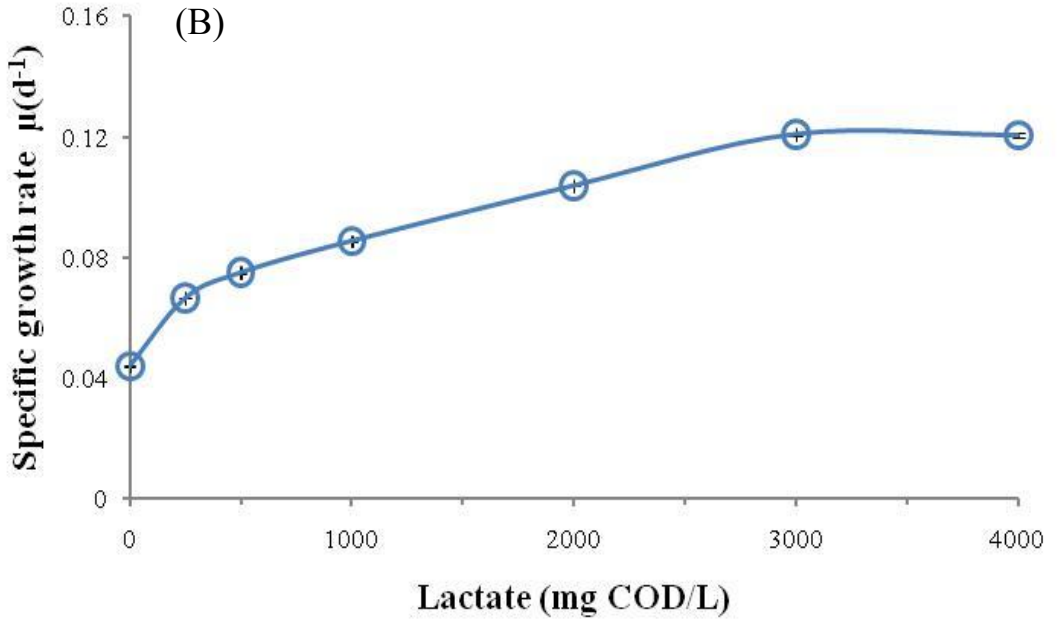


Figure 3.7 Cumulative biogas production curves of anaerobic digestion of pyuvate (A) lactate (B), and formate (C) at different substrate concentrations.


(A)
Specific growth rate μ(d-1)
0.2
0.16
0.12
0.08
0.04
0
0
1000
2000
3000
4000
Pyruvate (mg COD/L)


(B)
Specific growth rate μ(d-1)
0.16
0.12
0.08
0.04
0
0
1000
2000
3000
4000
Lactate (mg COD/L)

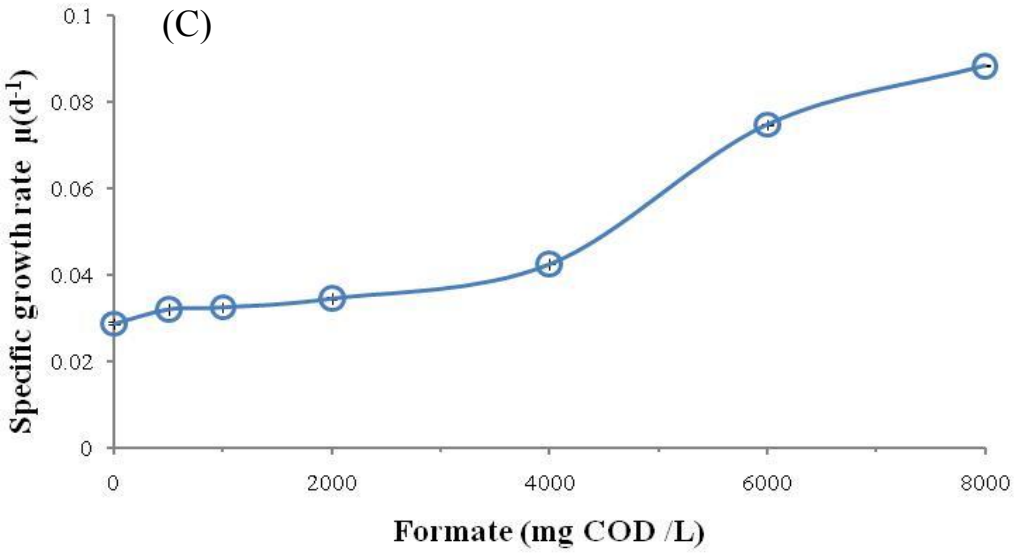


Figure 3.8 Specific growth rate (μ) of biomass as a function of organic acid substrate concentration in batch anaerobic digestion systems fed with pyruvate (A), lactate (B), Formate (C) .

3.3.2 Substrates showing inhibition on anaerobic digestion

Results from anaerobic digestion of acetate were totally different compared to those of the substrates reported earlier. Figure 3.9 provided experimental data collected from the batch assay. Biogas generation rate decreased when substrate concentration increased from 2000 to 5000 mg COD/L, which indicted self-inhibition by the substrate at high acetate concentrations. The result is consistent with previous findings that inhibition occurred at high acetate concentrations (Buswell and Mueller 1952, Grady et al. 1999). Specific biomass growth rate was calculated using Andrews equation and the results showed the growth rate reached the peak at low acetate concentration (1000 mg COD/L). The results indicated that higher acetate

concentrations inhibited the activities of methanogens. Previous studies suggested that unionized acetic acid at the concentration from 30 to 60 mg/L is actually inhibitory to methanogens (Andrews and Pearson 1965). The calculated unionized acetic acid concentration in this study was 2.3 mg/L at 1000 mg/L COD at pH 7.5-8, suggesting that self-inhibition may start at even lower concentration.

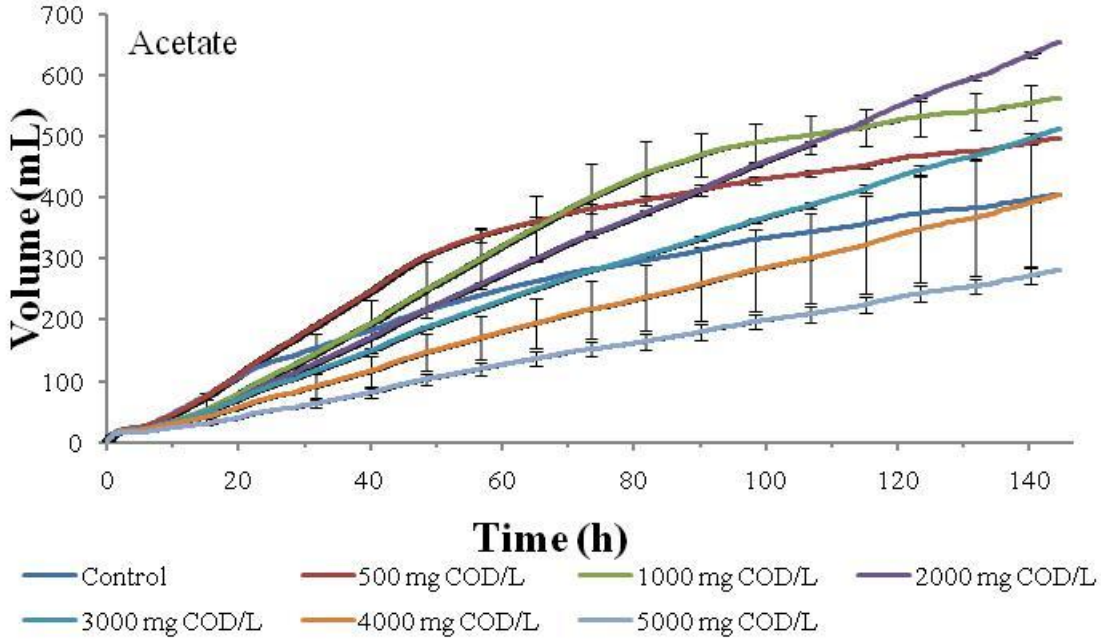


Figure 3.9 Cumulative biogas production curves of anaerobic digestion of acetate at different acetate concentrations.

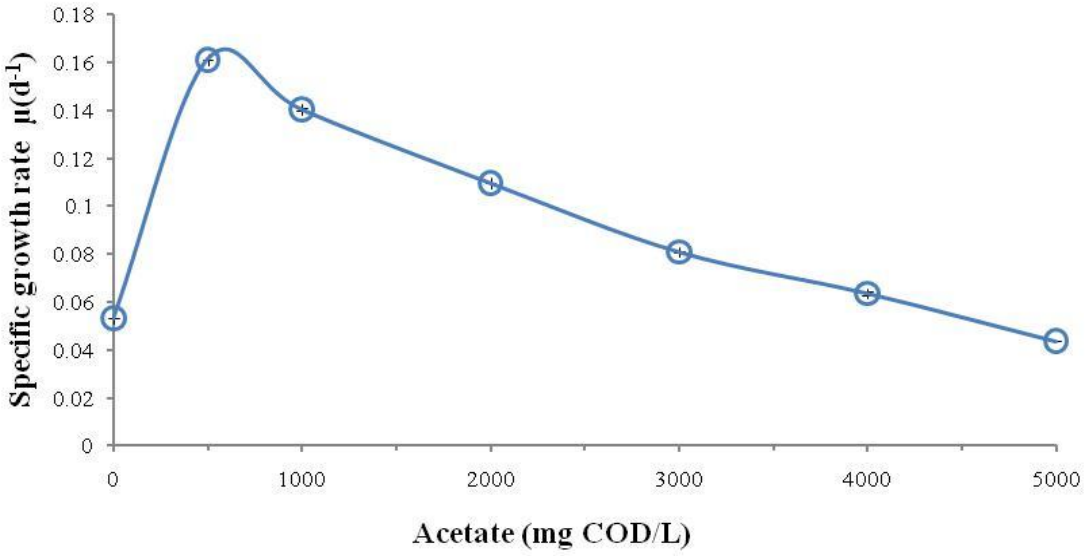


Figure 3.10 Specific growth rate (μ) of biomass as a function of acetate concentration in COD in anaerobic digestion.

Propionate also showed self-inhibition behavior in anaerobic digestion. Results from batch anaerobic digestion of propionate are shown in Figure 3.11. At the propionate concentrations of 500 COD/L, the maximum biogas production rate was observed. However, lower biogas generation rate was found in batch reactors at the propionate concentrations of 2000 mg COD/L or higher Moreover, a 30 hours lag phase was observed at the concentrations of 4000 and 5000 mg COD/L.

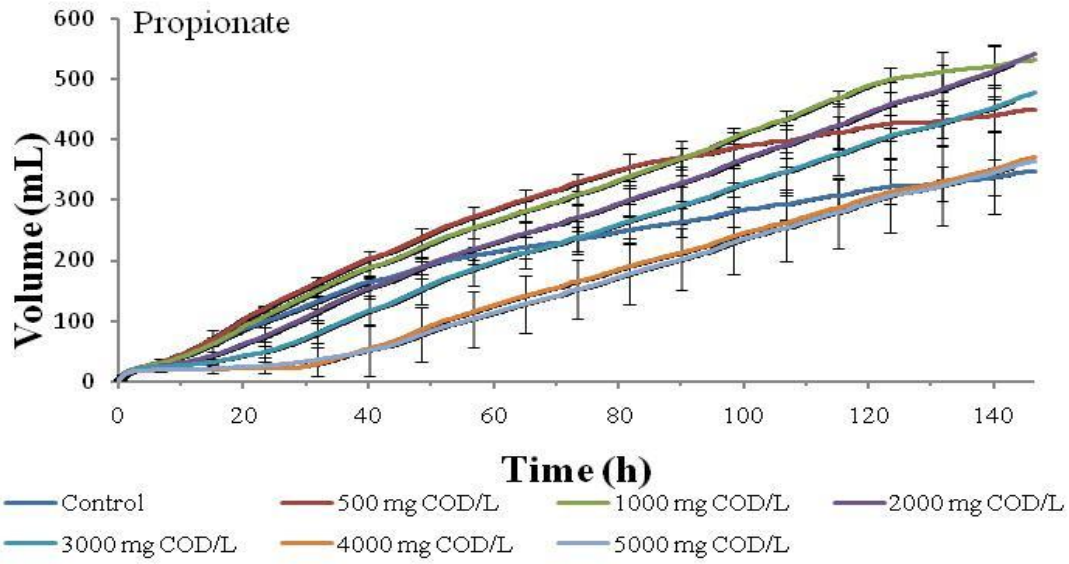


Figure 3.11 Cumulative biogas production curves of anaerobic digestion of pyruvate at different pyruvate concentrations.

Figure 3.12 presented the biomass specific growth rate changed with substrate concentration. Similar to the profile obtained from acetate degradation study, specific growth rate decreased when the propionate concentration increased. The degree of rate reduction was, however, much slower than that in the presence of acetate.

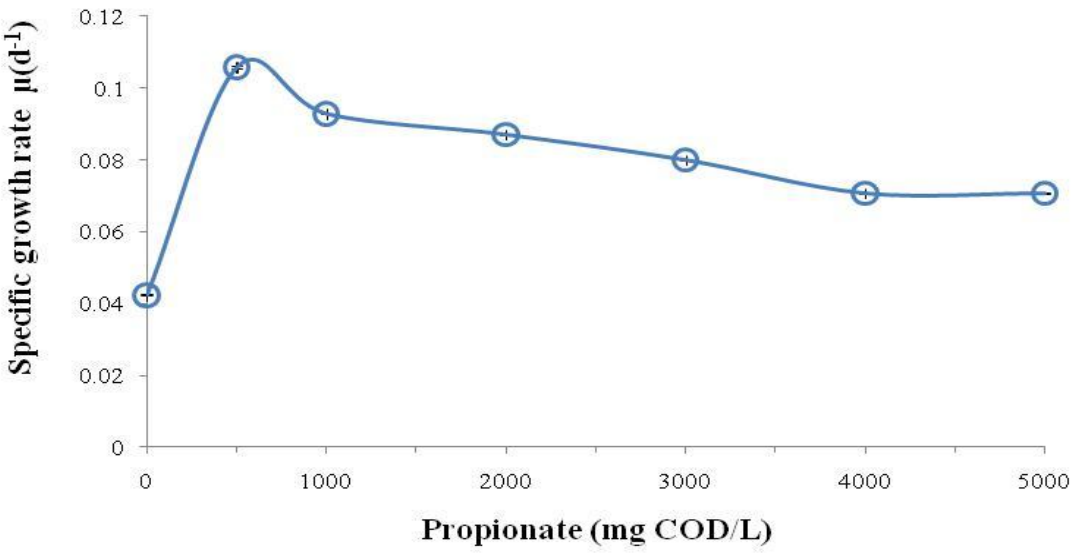


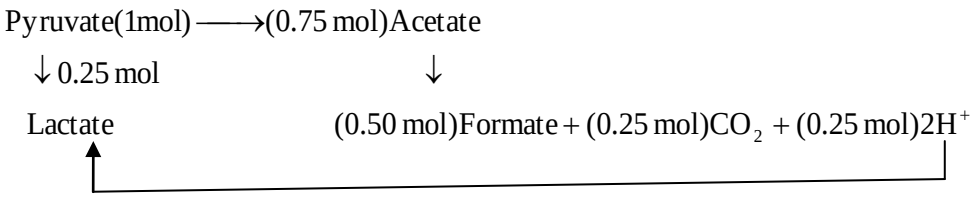
Figure 3.12 Specific growth rate (μ) of biomass as a function of propionate concentration in anaerobic digestion.

3.4 Anaerobic degradation of organic acid salts having three carbons – pyruvate, lactate and propionate

Pyruvate, lactate and propionate are important metabolites in anaerobic digestion. Propionate is easily formed from degradation of lactate and pyruvate, while pyruvate can be converted to lactate through fermentation. However, the slight distinction on chemical structure brought significant differences among the three organic acid substrates.

Both pyruvate and lactate did not inhibit anaerobic digestion at the highest concentration tested. However, the profiles of biogas production were quite different. From Figure 3.7 (A), it appeared that a lag phase lasting for a prolonged period of

time (40 h) at the beginning of anaerobic digestion. This was perhaps due to fermentation of pyruvate converting pyruvate to lactate temporary at the very beginning of pyruvate degradation. (Mickelson 1972). In addition, acetate and formate could be formed, although later on all these intermediates might be converted to methane and CO_2.



(3.1)

Figure 3.8 (B) showed the unique characteristic of specific biomass growth rate as a function of lactate. Compared with that of pyruvate, the rate was more sensitive to low concentration of lactate as μ increased quickly at substrate concentration range of 0-1000 mg COD /L. Such characteristics of the change of microbial growth rate with lactate concentration was possibly related to unique degradation pathways for lactate (Zellner et al. 1994).

Comparing with anaerobic digestion pyruvate, an insignificant lag phase was observed (Figure 3.7 (B)). It is reported that lactate is an easily degraded substrate in anaerobic digestion. According to Equations 1.3.7 and 1.3.8, as long as lactate concentration was high, propionate appeared to be the main product in the first step of lactate degradation (Zellner et al. 1994). When lactate concentration dropped, acetate

instead of propionate became the final product, which was directly used for methane production. Other degradation pathways such as the reaction expressed by Equation 3.2 coupled with hydrogen production might be also possible. Therefore, multiple pathways of lactate degradation might result in unique patterns of biogas production and sensitivity of microbial growth to lactate concentrations.

$$\text{Lactate} + H_2O \longrightarrow \text{acetate} + 4H_2 + CO_2 \qquad (3.2)$$

Propionate had different impact on anaerobic digestion compared to pyruvate and lactate. In Figure 3.11, the lag phase lasted for about 30 hours at high propionate concentration (>4000 mg/L). Furthermore, biomass specific growth rate decreased as substrate concentration increased. These results indicate that high concentration of propionate is very resistant to degradation in anaerobic digestion. High concentration of propionate inhibited anaerobic digestion as also reported earlier (Hyun et al. 1998).

A relatively lower microbial growth using propionate as a substrate compared to that of other C3 organic acids is possibly due to the intrinsic slow conversion of propionate to acetate (Equation 3.3). The free-energy required for this reaction is positive at 76.1 kJ/mol. Only at extremely low hydrogen concentration ΔG of this reaction became negative. This requires syntrophic interactions by quickly removing hydrogen by hydrogenotrophic methanogens (3.4 and 3.5).

$$\text{propionate} + 2H_2O \xrightarrow{\text{acetogenesis}} \text{acetate} + 3H_2 + CO_2 \qquad (3.3)$$

$$\text{acetate} \xrightarrow{\text{acetotrophic methanogenesis}} CH_4 + CO_2 \quad (3.4)$$

$$3H_2 + \frac{3}{4}CO_2 \xrightarrow{\text{hydrogenotrophic methanogensis}} \frac{3}{4}CH_4 + \frac{3}{2}H_2O \quad (3.5)$$

3.5 Formate

Figure 3.8 (C) revealed that the specific growth rate of biomass increased with formate at high concentrations. While it changed a little at low formate concentrations, the rate increased rapidly starting at 4000 mg COD/L. Comparing with other organic acid substrates, much higher COD concentration of C1 organic acid was need for methane generation.

Formate is a methanogenic precursor in anaerobic digestion (Equation 3.6). It is suggested that the reaction of formate to generate hydrogen is reversible (Thiele and Zeikus 1988). As described in Equation 3.7, H_2 consuming methanogens can also use formate as the electron donor for catabolic CO_2 reduction to CH_4 (Boone et al. 1989). In Figure 3.7(C), after a 20 hours lag phase, microorganisms adapted to the new environment in batch reactors with signal of biogas production. At low formate concentrations (500-2000 mg COD/L), biogas generation slightly increased with the concentration. When formate concentrations reached, 4000-8000 mg COD/L, biogas generation increased greatly. The reversible reaction expressed by Equation 3.6 might dominate at low formate concentrations resulting in limited hydrogen and CO_2 supply for methanogensis. While at high formate concentrations, both Equation 3.6 and 3.7

favor move to right direction. More hydrogen and CO_2 were generated and biogas generation rate increased significantly.

$$HCOOH \longleftrightarrow H_2 + CO_2 \quad (3.6)$$

$$4HCOO^- + 4H^+ \xrightarrow{hydrogenophilic\ methanogenesis} 2H_2O + 3CO_2 + CH_4 \quad (3.7)$$

3.6 Kinetic constants

Biomass specific growth rate were obtained through best fit of the experimental data using Monod and/or Andrews equations. Monod equation was used to fit the data from Figure 3.6 and 3.8 which showed no inhibition of substrates on anaerobic digestion, while Andrews equation was used in the studies of anaerobic digestion of acetate and propionate because both of these two substrates presented inhibition at high substrate concentrations.

Results of model simulation using Monod and Andrews equations were summarized in Table 3.1 where maximum biomass specific growth rate (μ_{max}), substrate half saturation constant (K_s) and inhibition coefficient (K_I) were presented. Anaerobic digestion of butyrate, pyruvate and acetate resulted in relatively high specific growth rates while the use of formate, lactate and propionate resulted in relatively low specific growth rates. These findings suggested that butyrate, pyruvate and acetate

were easily degraded in anaerobic digestion processes, while formate, lactate and propionate were relatively hard to break down in the similar environment. In addition, acetate and propionate presented self-inhibition on anaerobic digestion at high substrate concentrations.

Table 3.1 A summary of kinetic constants of anaerobic digestion of C1 to C4 organic acid salts under room temperature

Model/Equation	$\mu = \mu_{max} * S/(K_d + S)$				$\mu = \mu_{max} * S/(K_d + S + S^2/K_I)$	
	Butyrate	Pyruvate	Lactate	Formate	Acetate	Propionate
μ_{max} (/d)	0.170	0.167	0.136	0.133	0.396	0.133
Ks (mg/L)	209	189	393	5101	412	111
K_I (mg/L)					764	4707

Comparing with the results listed in Table 1.3 and 1.4, the kinetic constants of anaerobic digestion of butyrate were within the range reported earlier. Value of μ_{max} was in the range of 0.13-1.20 /d, and K_s value was also between 12-500 mg/L. Similar results were observed for anaerobic digestion of pyruvate. The maximum biomass specific growth rate was slightly low, possibly because the experiment was conducted at room temperature. The close parameters of anaerobic digestion of butyrate and pyruvate suggested that microbes could equally grow on butyrate and pyruvate very well.

Lactate had relatively lower μ_{max} value compared to pyruvate, probably due to its multiple degradation pathways involved in anaerobic digestion and accumulation of propionate that inhibited biomass growth.

Formate degradation in anaerobic digestion was unique among the six organic acids studied. Extremely high substrate half saturation constant of formate degradation indicated that methanogenic growth might not be sensitive to low concentrations of formate. It has been reported that only hydrogenotrophic methanogens can use formate as both an electron donor and acceptor for methane production.

Acetate and propionate inhibited biomass growth and biogas generation at high concentrations. From Table 3.1 acetate had the highest μ_{max} value compared to other substrates, suggesting that acetate is a most easily biodegradable substrate in anaerobic digestion. Results calculated in this study were in agreement with previous reports (Table 1.3). However, nonionized acetic acid formed by high concentrations of acetate still had negative impact on anaerobic digestion. The inhibition coefficient of acetate was much lower than that of propionic acid indicating strong self-inhibition by acetic acid. Kinetic data of propionate in Table 3.1 were consistent with the literature values.

CHAPTER 4

CONCLUSIONS

Throughout this study on kinetics of six selected organic acid salts in anaerobic digestion, we found that formic acid, lactic acid, pyruvic acid and butyric acid had no inhibition on anaerobic digestion, while acetic acid and propionic acid presented self-inhibition at high concentrations. A set of kinetic parameters was obtained which can be used for future research, process simulation and optimization. Digestion of butyric acid, pyruvic acid and acetic acid resulted in relatively high biomass specific growth rates at 0.170, 0.167, and 0.396 d^{-1}, respectively. On the other hand, the use of propionic acid, formic acid and lactic acid resulted in relatively low biomass growth rates at 0.136, 0.133 and 0.133 d^{-1}, respectively.. Other specific findings from this study were in the following:

- Low concentration (<4000 mg COD/L) formic acid did not substantially support biomass growth and biogas generation.
- Pyruvic acid and lactic acid had similar impacts on biogas generation and biomass growth. Pyruvic acid was relatively easier to be degraded by microorganisms than lactic acid.

- In the concentration range of butyric acid tested in this study, butyric acid was not inhibitory to anaerobic digestion. Kinetic results of butyric acid were similar to those of pyruvic acid.

- The inhibition coefficient (K_I) of acetic acid was lower than that of propionic acid indicating strong self-inhibition of microbial growth by acetic acid while propionic acid appeared to be highly resistant to degradation in anaerobic digestion.

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