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Abstract:

Biogas production is regarded as the best energy recovery process from wet organic solid wastes (WOSW). Feed composition, storage conditions and time will influence the compositions of feed to biogas processes. In this study, apple juice from Meierienes Juice factory was used as the model substrates to mimic the liquid phase that can be extracted from fruit or juice industry WOSW.

A series of batch experiments were carried out with different initial feed concentrations (0, 1, 2, 5, 10 %) of apple juice mixed with a biogas culture from the local anaerobic digester (AD). The initial feed content of 2 % and 5 % (weight %) gave the high biogas yields 55 (mL biogas per mL feed consumed), compared to 1 % and 10 %, which gave the biogas yields of 42 (mL biogas per mL feed consumed) perhaps due to substrate inhibition. The biogas yield data from 2 % and 5 % feed case were used for simulation in ADM1 to estimate the substrate compositions. Measured and estimation data from ADM1 are similar and show that the apple juice included mainly sugar, some protein, fat and organic acids with the total sCOD = 120 g COD/L. It implies that ADM1 can be used as a soft sensor method to estimate the substrate composition quite accurately from simple biogas measurements in batch experiments.

Three different inoculum preparation methods were used in this study: Slurry from outlet of AD reactor (unfiltered); Leachate from sieve filtered (pore size: 500 μm); Leachate from belt filter process after AD reactor. The study show that the inoculum prepared from belt filter leachate was suitable for biogas production studies and had some advantages compared to unfiltered cultures.

Micro-aeration treatment in the AD process was also tested in batch experiments. The results show that suitable amounts of air (oxygen) supply requires careful control for micro-aeration treatment to have a positive effect on biogas production in AD processes.

Abstract:

An experiment of continuous flow bioreactor (CFB) for acidogenesis was also carried out to study the feed buffer capacity/pH effect on H_2 yields and consumptions. The H_2 production rate was 8 mmol/L /d at pH =7.3, and 4 mmol/L /d at pH =3.7. H_2 was both produced and consumed in the reactor. pH also influenced the acid accumulation and therefore the metabolic pathways.

Five main hypotheses were tested in this study leading to the following conclusions: (1) Apple juice can be used as a model substrate for the study of fermentation and biogas production; (2) Food to microorganism (F/M) ratios can be used to identify the substrate overload issues or the substrate inhibition problems; (3) Leachate from the belt filter process after the AD reactor can be used as the inoculum for the study of biogas production processes; (4) Biogas substrate composition can be estimated from simple batch tests and ADM1 simulations; (5) pH influences the metabolic pathway selection of glucose fermentation.

Preface

This thesis is submitted to the Faculty of Technology, Telemark University College (TUC), as a partial fulfillment of the requirements for the Degree of Master of Science in Energy and Environmental Technology, under the subject code FMH606.

This thesis describes the study of the small scale biogas production systems where storage and feed composition are key issues. The aim is to establish a procedure that we can use to measure and predict the feed composition to obtain stable operating conditions for biogas processes.

I would like to present my sincere gratitude to my main supervisor Professor Rune Bakke for his great guidance of this thesis, for believing in my abilities, and also for his kind helps both in my academic career and my life during the two-year-master study at Porsgrunn (Norway). Thank you so much for a great experience.

I want to give my warmest thanks to the following friends for providing assistances and suggestions during this study: Lab. Chief H.H.Haugen, Carlos.Dinamarca-Røed, P.Deshai C. Botheju, exchange student Ganan Maitane and fellow graduate student Yuan Li.

Especially my sincere gratitude goes to my cherished and beloved friend Kari Margrethe S.Bakke for her love and care during my two years staying in Norway. She was always there to comfort me when I was in troubles.

Finally I want to present my thanks to all the faculties of Technology at TUC. Last two years have been quite an experience for me and you have all made it a memorable time in my life.

Dedication

This thesis is dedicated to my beloved parents in China, for their unconditional love and supports.

To my father:
the special friend/supervisor/constant source of motivation and inspiration in my life, the person I am always proud of and respect for!

To my mother:
the researcher of her own style/shopping mate/mirror of optimism/constant source of endless love in my life; the person by whom I am always spoiled in a positive way; the person who encourages me to follow my dreams, pursue my goals and look for the joys in life!

Thank you Mom and Dad for bringing me up to be who I am today! I love you both!

Contents

Abbreviations

Anaerobic Digestion	AD
Anaerobic Digestion Model No.1	ADM1
Continuous Flow Bioreactor	CFB
Food to Microorganism Ratio	F/M ratio
Gas Chromatography	GC
Long Chain Fatty Acids	LCFAs
Soluble Chemical Oxygen Demand	sCOD
Total Chemical Oxygen Demand	tCOD
Total Organic Carbon	TOC
Total Solid	TS
Total Suspended Solid	TSS
Volatile Fatty Acids	VFAs
Volatile Solid	VS
Volatile Suspended Solid	VSS
Wet Organic Solid Waste	WOSW

1 Introduction

Bioenergy is regarded as one of the most important renewable energy. The biogas production can be used to recover energy from wet organic solid waste (WOSW) such as food industry residues, agriculture wastes and so on. Methods for treating WOSW should obtain mass reduction, reuse and recycling. Anaerobic Digestion (AD) process can produce biogas for energy recovery and fulfill the requirement for sustainable development. AD process requires low energy considerations, lower biomass yield, fewer nutrients and smaller reactor volume (Tchobanoglous *et al.,* 2003).

AD process includes three main steps: hydrolysis, acidogenesis, and methanogenesis (**Figure 1.1**). Normally the hydrolysis process takes a relatively long time for the particles and macromolecules in the WOSW to be converted into the smaller dissolved molecules. The separation of hydrolysis step from the biogas production is determined as the two steps AD process. In the two steps AD process, the feed into the reactor is considered as the particles hydrolyzed into to smaller organic molecules suitable for the biogas production.

The biogas production potential from different feed substrates varies, as does process operation conditions and process stability. The feed composition is a key issue in biogas production plants, but it can be difficult and expensive to measure the composition directly due to their inadequate biological stability, potentially pathogenic nature, potential for rapid autoxidation (Russ *et al.,* 2004).

The production of biological H_2 was also influenced by the substrate compositions. The H_2 production yields depend stoichiometrically on the range of fermentation volatile fatty acids (VFA) products formed (Rodríguez *et al.,* 2006). The metabolic pathways of H_2 production bacteria (**Figure 1.2**) are regulated by environmental factors such as pH, temperature and H_2 partial pressure (Liu *et al.*, 2006).

In this study, the apple juice produced from Meierienes factory in Norway was chosen as the model substrate to mimic the liquid phase that can be extracted from juice industry WOSW. This research was focused on studying the small scale biogas production systems where storage and feed composition are key issues. The aim is to establish a procedure that we can use to measure and predict the feed compositions to obtain stable operating conditions for biogas processes.

A series of batch experiments together with the simulations in Anaerobic Digestion Model No.1 (ADM1) were carried out to study the biogas production potential and estimations of substrate compositions in ADM1. A continues flow bioreactor (CFB) was setup to study the substrate buffer capacity/pH effect on H_2 yields and consumptions.

Five main hypotheses were tested in this study:

(1) apple juice can be used as the model substrate for the study of biogas production process;

(2) analyze the F/M ratios to can be used to identify the substrate overload issues or the substrate inhibition problems;

(3) leachate from the belt filter process after the AD reactor can be used as the inoculum for the study of biogas production process;

(4) Biogas substrate composition can be estimated from simple batch tests and ADM1 simulations;

(5) pH influences the metabolic pathway selection of glucose fermentation.

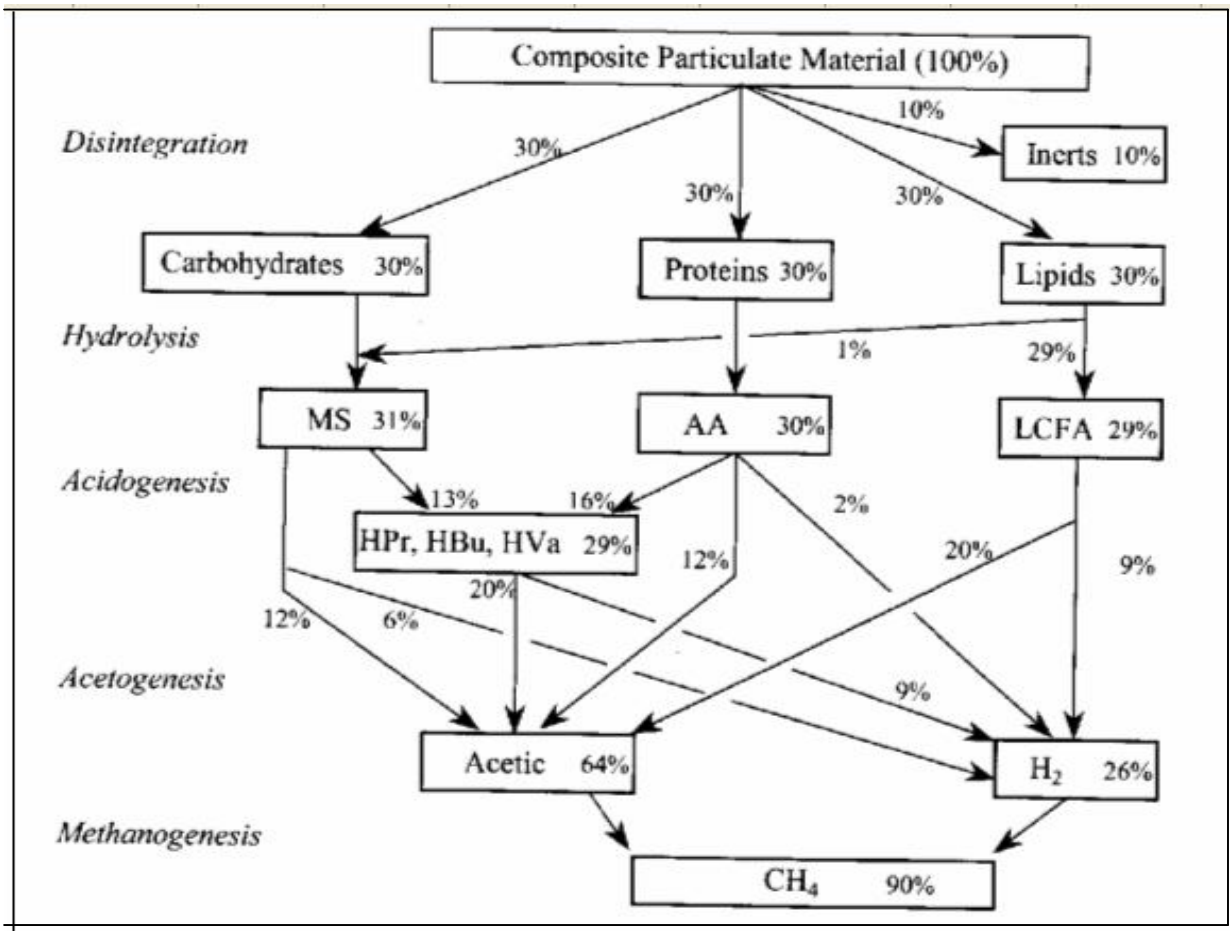


Figure 1.1 Three main steps: hydrolysis, acidogenesis, and methanogenesis in AD process (*Bastone et al.,* 2002)

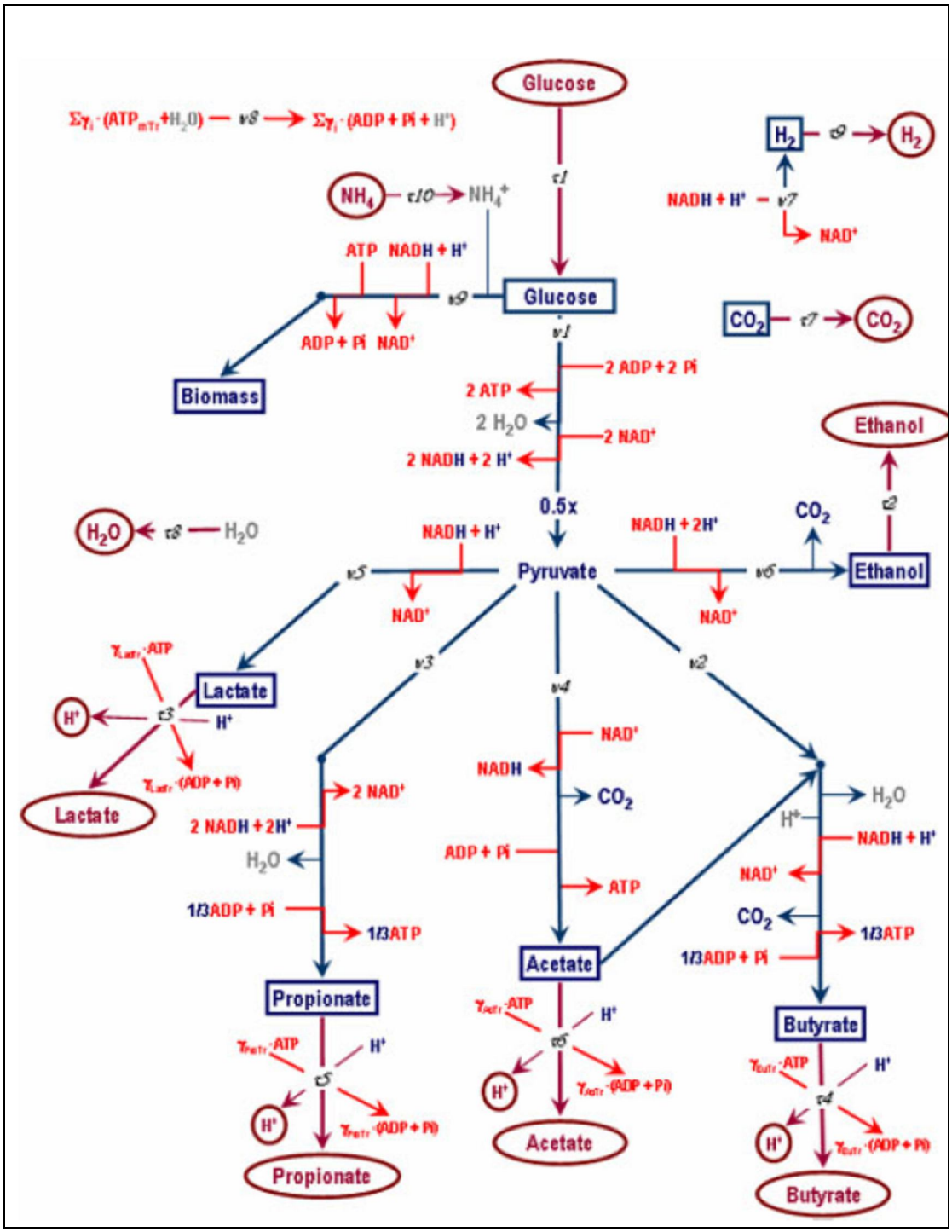


Figure 1.2 Metabolism pathways of the fermentation of glucose.(Rodríguez et al., 2006)

2 Materials and Methods

This chapter includes the following information: the materials used in the experiment; the experimental setup; the sampling and analytical methods.

2.1 Inoculum and Substrate

The inoculum used in this experiment was mesophilic sludge from a suspended-one-stage AD reactor treating primary sludge at Porsgrunn wastewater treatment plant. The inoculum was pretreated before the experiment. Three different inoculum pretreatment methods were used in this study (**Table 2.1**).

Table 2.1 Inoculum pretreatments for the biogas production experiment

Reactor	Identification name of the Inoculum	Pretreatment
Methanogenesis Bioreactor for CH_4 production	Unfiltered	Slurry from outlet of AD reactor
	Belt filter	Leachate from belt filter after the AD reactor
	Sieve filter	Leachate from sieve filter (sieve pore size: 500 μm)
Continuous Flow Bioreactor for H_2 production	Inoculum for H_2	(1) sieve filtered with the effluents from AD reactor (sieve pore size: 500 μm) (2) "heat shock" treatment to inactivate the methanogenesis bacteria: $40^0C \rightarrow 60^0C \rightarrow 80^0C \rightarrow 104^0C \xrightarrow{overnight} 35^0C$ time difference between each heating is ~2 h (3) adding 1 g coffee granules (instant coffee) to form the biofilms

Substrate used in this experiment is the apple juice (Brand: TINE) made from Meierienes Juice factory in Norway. The composition of the apple juice can be seen from the following Chapter 3.1.

2.2 Experimental Setup

Two different experiments were setup: the batch experiment for CH_4 production and the continuous flow bioreactor (CFB) for H_2 production.

2.2.1 Batch Experimental Setup for CH_4 Production

The batch experiment was performed in series of 60 mL medical syringes (Termuro) used as small anaerobic digesters (**Figure 2.1**). The content in the reactor was a mixture of apple juice and inoculum. Each syringe was connected to a needle blocked by a rubbery stopper to stop the leakage from gas and liquid. The syringes were kept on a laboratory shaker (Aqua Produktor LV-1). The batch experiment setup is placed in an incubator (Forma Scientific Steri-Cult Incubator), at a temperature of 35±1°C, which was within the optimal temperature range for the mesophilic bacteria (Henstra *et al.,* 2007).

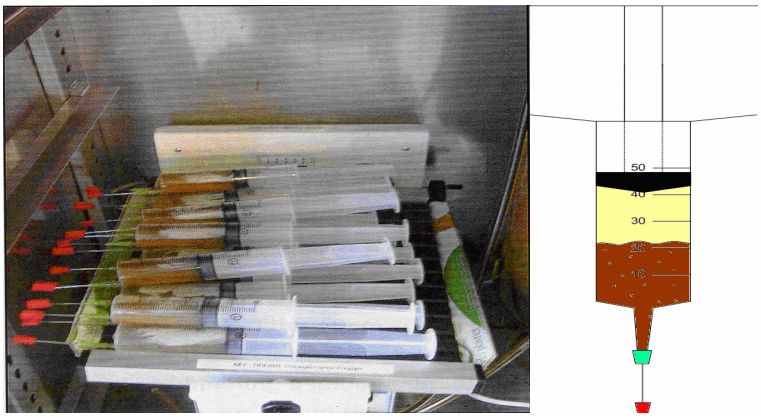


Figure 2.1 Batch experimental setup. The left figure shows the overview of batch experiment setup with syringes on a shaker in an incubator; the right one shows a single syringe as an anaerobic digester with a rubber stopper.

Table 2.2 Quantitative dosing of inoculums, apple juice in the batch experiments for the study of substrate content effect on biogas yields

Reactor	Apple juice Content (weight%)	Parallels	Inoculum [mL]	Apple Juice [mL]
1	0	3	30	0
2	1	3	30	0.3
3	2	3	30	0.6
4	5	3	30	1.5
5	10	3	30	3.0

Five different load levels: 0, 1, 2, 5 and 10 weight percentage of apple juice, were tested with 3 parallels for each load level (**Table 2.2**). Three different inoculums were used separately in each load level. (**Table 2.1 and Table 2.2**)

Similar batch experiments were also carried out to study the biogas yield in micro-aeration conditions. (**Table 2.3**)

Table 2.3 Quantitative dosing of inoculums, apple juice in the batch experiments for the study of micro-aeration effect on biogas yields

Reactor	Apple juice Content (weight%)	Parallels	Inoculum [mL]	Apple Juice [mL]	Headspace Of air [mL]
6	2	3	30	0.6	5
7	5	3	30	1.5	10

The estimations of the amount of air as the headspace for micro-aeration were calculated based on the sCOD values of the substrates. (**Appendix B**)

The detailed information of batch experimental procedures can be found in **Appendix D.**

2.2.2 Continuous Flow Reactor Setup for Acidogenesis

The schematic diagram of continuous flow bioreactor used in this study is shown in Figure 2.2. The reactor with volume of 270 mL was submerged in a water batch with temperature controlled at 35 °C. The substrates were fed into the reactor by the pump with the average liquid speed 33 mL/h (HRT=8 h). The inoculum used for this experiment (**Table 2.1**) were pretreated by "heat shock" method (Oh *et al.*, 2003; Okamoto *et al*., 2000) to inactivate the methanogenesis bacteria and retain the thermophilic bacteria for hydrogen production.

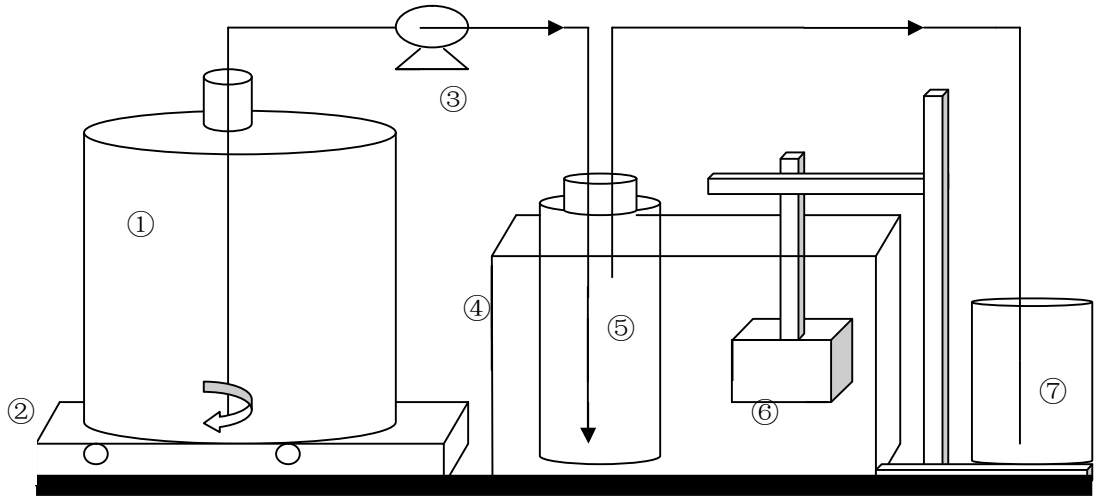


Figure 2.2 Schematic diagram of the continuous flow bioreactor:
1. feed tank with 10 g COD/L apple juice; 2. Magnetic stir controller; 3. Pump with tube Φ=0.95 μm; 4. Water bath (constant T=35 ℃); 5. Bioreactor (V=270 mL;
6. Heater with temperature controller; 7. Waste container

*Table 2.4 Compositions of substrates for continuous flow bioreactor (The detailed information on the preparations of stock solution A, B, vitamins and minerals can be found in **Appendix F**)*

Components	Concentration
Stock solution A	1 mL per 1 L feed
Stock solution B	2 mL per 1 L feed
Vitamin solution	1 mL per 1 L feed
Mineral solution	2 mL per 1 L feed
*Buffer solution ($NaHCO_3$)	3.5 g per 1 L feed
Organic source (apple juice)	10 g COD/L
* Two kinds of substrates were tested separately in this study: one with buffer solution, the other without buffer solution.	

The substrate solutions were pretreated in the sterilization equipment with T=121°C , 90 min. In this study, two kinds of substrates were tested to study the pH effect for the H_2 production process (acidogenesis and acteogenesis process). The main difference of the two substrates was the buffer solution (**Table 2.4**).

2.3 Samplings and Analytical Methods

This sub-chapter describes the sampling procedures, measurement methods, statistical analysis methods and simulation methods.

2.3.1 Samplings

The batch experiment was carried out within 15 days. Produced biogas accumulated inside the syringe by expanding the volume (piston moves). Biogas samples were taken regularly by removing the rubber stopper and pressing it through the needle. The volume of biogas produced was recorded by reading the volume scale of the syringe every day.

The continuous flow reactor experiment has been running for more than one moth. The gas samples collected every work day were analyzed by the gas chromatography (GC). The liquid samples were prepared for Volatile Fatty Acids (VFA) analysis the same time when collecting gas samples.

The detailed information on gas sample collection method can be found in **Appendix E.**

2.3.2 Experimental Analysis

The overall block diagram of the measurements for biogas process study is illustrated in Figure 2.3.

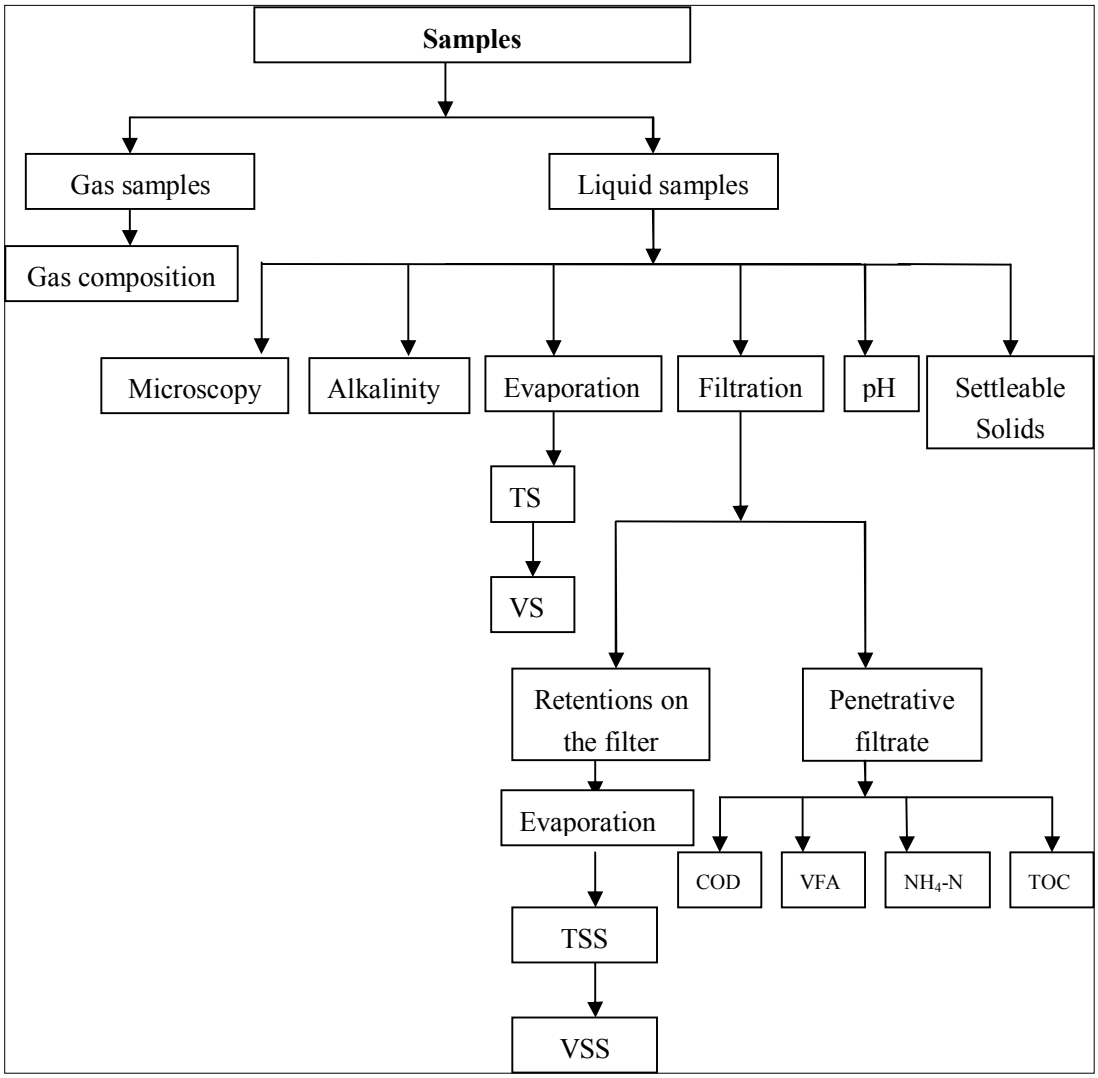


Figure 2.3 overall measurement block diagram of biogas production experiment

To obtain the information of substrate (apple juice) compositions, the following parameters were measured: pH, total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total organic carbon (TOC), Volatile Fatty Acids (VFA). (**Table 2.5**)

To obtain the information of the inoculum (**Table 2.1**), the following parameters were measured: Total Solid (TS), Volatile Solid (VS), Total Suspended Solids (TSS), Volatile Suspended Solid (VSS).

Table 2.5 Equipments and measurement methods used in this study

Parameters	Equipments and Measurement Methods
Gas Compositions	Gas Chromatography (Hewlett Packard, P series micro GC)
pH	pH meter (Metrohm 744, MT-00010)
VFA	Gas Chromatography
COD	Colorimetric Method (Hach DR 2000, modified method 962)
TS,TSS	Standard methods for the examination of water and wastewater
VS,VSS	(Eaton *et al.,* 1995)
Sterilization	Sterilizer (with T=121°C , 90 min)

2.3.3 Statistical Analysis Methods

The variation of the experimental data was analyzed as standard deviation and "t-testing" statistics method was used to determine the significance (Spiegel *et al.,* 1972). In this study, the significance was reported at the α of 0.05.

2.3.4 Simulation Method

The batch experiments were also simulated by the ADM1 (Bastone *et al.*, 2002) implemented in Aquasim as a "soft sensor" method to estimate the chemical composition of apple juice and biogas yield. This was done by simulating various possible feed compositions, assuming that the simulation that best matches the biogas production observed gave the approximate real substrate compositions.

3 Results

The experimental results described in this chapter are as follows: (1) chemical characterization of apple juice; (2) three different effects on biogas (CH_4) yield: feed contents, inoculum pretreatment methods and micro-aeration effect; (3) estimation of substrate (apple juice) compositions in ADM1; (4) continuous flow bioreactor (CFB) for H_2 production: pH effect on H_2 yields and consumptions; pH effect on VFA distributions in H_2 production process; fate of COD distributions in H_2 production process.

3.1 Chemical Composition of Apple Juice

Table 3.1 shows the chemical composition of apple juice used as the organic feed in this experiment. The calculation of COD equivalent coefficient can be found in **Appendix C**.

Table 3.1 Measured and reported composition of apple juice used as the organic feed

Data from the laboratory analysis			COD equivalent coefficient	g COD/L
pH		3.72		
tCOD	[g/L]	125		
sCOD	[g/L]	123		
TOC	[g/L]	43.5		
Acetic Acid	[g/L]	0.12	1.06	0.13
Propionic Acid	[g/L]	0.14	1.51	0.21
Isobutyric Acid	[g/L]	0.19	1.82	0.35
Butyric Acid	[g/L]	0.087	1.82	0.15
Isovaleric Acid	[g/L]	0.076	2.8	0.21
Valeric Acid	[g/L]	0.17	2.8	0.48
Isocaproic Acid	[g/L]	0.027	2.21	0.06
Caproic Acid	[g/L]	0.14	2.21	0.31
Data from the ingredient descriptions of the apple juice (MEIERIENES JUICE) Pr. 100 g (ca. 1dl)			COD equivalent coefficient	g COD/L
Protein	[g/L]	1	1.5	1.5
Carbohydrates	[g/L]	110		
Of which is sugars	[g/L]	105	1.07	113
Fat	[g/L]	<1	2.91	2.91
Saturated fats	[g/L]	< 1		
Fibers	[g/L]	< 10		

It can be seen from Table 3.1 that the tCOD and sCOD values of apple juice were close. It implies the soluble COD (sCOD) account for most COD content of apple

juice. This character of apple juice is a benefit for the hydrolysis process, indicating that apple juice is suitable as the organic feed to study biogas reactors. Table 3.1 also shows that sugar and carbohydrates, mainly as sugar, are the major components of apple juice.

3.2 Biogas Yield from Batch Experiment

The biogas yield was determined as the volume of biogas produced per 1 mL apple juice consumed. Different factors influence the biogas yield in the AD bioreactor. Three different influences: Feed content, inoculum pretreatment methods and micro-aeration effect, were studied in the batch experiment. The results are as follows.

3.2.1 Effect of Feed Contents on Biogas Yield

The final biogas yields in AD batch bioreactor with different initial feed content are shown in Table 3.1 and Figure 3.1.

Table 3.1 Accumulated 15 days biogas yield in different batch reactors with different organic feed content and with the inoculum pretreated by the sieve filters (pore size: 500μm). The variation of the data is analyzed at the significance level of 0.05

Reactor	Apple Juice Content (weight %)	Volume of apple juice as feed (mL)	Total volume of biogas recorded (mL)	Biogas produced from feed (total-blank) (mL)	Biogas yield (biogas produced [mL] per feed consumed [mL])
R1	0 (blank)	0	15±6		
R2	1	0.3	28±5	13±1	44±3
R3	2	0.6	49±5	34±1	57±2
R4	5	1.5	98±11	83±5	55±3
R5	10	3.0	100±3	85±3	42±1

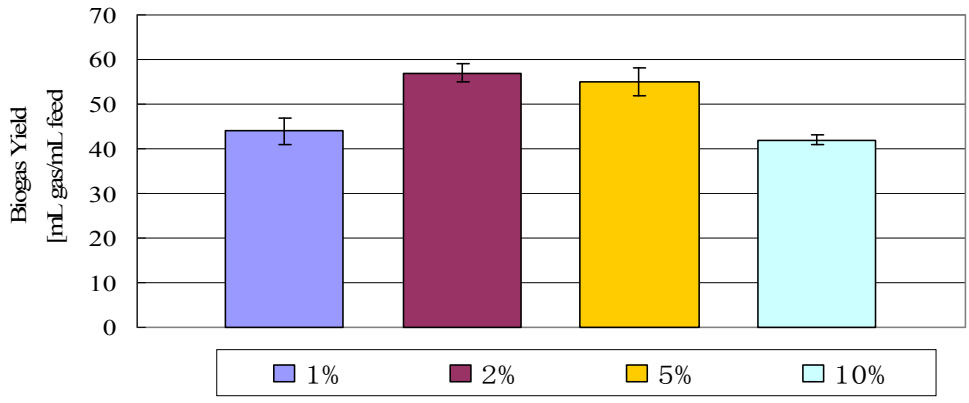


Figure 3.1 Accumulated biogas yields with different initial feed contents and with the inoculum pretreated by the sieve filters (pore size: 500μm)

Table 3.1 and Figure 3.1 illustrate that high biogas yields were observed in all AD bioreactors. The standard deviation shows there were no significant biogas yield differences between the case of using 2 % (weight %) feed and 5 % (weight %) feed, which gave the biogas yields about 55 [mL biogas per mL feed]. No significant differences were found between the lower biogas yield cases with 1 % and 10 % (weight %) feed content, which gave the biogas yields about 42 [mL biogas per mL feed].

Table 3.2 Alkalinity and pH values analyzed at the end of 15 experimental days, NH_4-N values analyzed at the 6th of the experimental day

Reactor	Apple Juice Content (Weight %)	Alkalinity mg/L $CaCO_3$	pH	NH_4-N [mg/L]
1	0 (blank)	3203	7.6	731
2	1	2948	7.5	641
3	2	4732	7.4	622
4	5	3215	7.7	597
5	10	7381	8.2	571

The robust AD reactor normally has a alkalinity of 2000~5000 mg/L as $CaCO_3$ to maintain the suitable pH range for the methanogenesis bacteria to grow (Tchobanoglous *et al.,* 2003). Except for the R5 with apple juice content of 10 %, all the other reactors were within the suitable alkalinity, which came from the inoculum.

At the end of experimental days, the initial feed content of 10 % (weight %) (**Figure 3.1**) gave the high alkalinity and pH value, which was out of the range that a robust AD reactor requires (**Table 3.2**). This may be the reason for the lowest biogas yields from 10 % feed.

The toxic and inhibitory inorganic compound of ammonia-nitrogen (NH_4-N) concerned for anaerobic process is within the moderate range up to 1500~3000 mg/L (Tchobanoglous *et al.,* 2003). From Table 3.2, it can be seen that all reactors gave rather low ammonia contents, which was suitable for the AD process. Table 3.2 also indicates that the lower biogas yield reactor with 10 % (weight %) feed had a lower NH_4-N value compared to the high biogas yield reactor with 2 % (weight %) feed. This implies that the low biogas yield reactor with 10 % (weight %) feed had no NH_4-N inhibition problems.

The results of “food to microorganism ratio” (F/M ratio) are shown in Table 3.3. In this study, the F/M ratio was defined as the rate of sCOD applied per unit biomass sCOD/g VSS*d.

Table 3.3 F/M ratio of the AD batch reactors in 15 experimental days and with the inoculum pretreated by sieve filter (500 µm pore size)

Apple Juice Content (weight %)	Substrate (Food) [g sCOD/L]	Biomass (Microorganisms) [g VSS/L]	Batch experimental days	F/M ratio [sCOD/VSS*d]
1	1.23	2.2	15	0.04
2	2.46	2.2	15	0.07
5	6.15	2.2	15	0.18
10	12.3	2.2	15	0.4

The F/M ratio is the indictor of the specific substrate loading rate. Typical F/M ratio of batch experiment is within the range of 0.04~0.1 (WEF, 1998; Crites and Tchobanoglous, 1998). Table 3.3 shows that the initial feed content of 10 % had a high F/M ratio, which was out of the typical range. This high F/M value can be considered as a possible factor causing substrate inhibition, which may explain the results that the high feed content (i.e. 10 % content) gave the low biogas yields. (**Figure 3.1 and Figure 3.2**).

The experimental data of the accumulated biogas yield within 15 days were plotted in Figure 3.2.

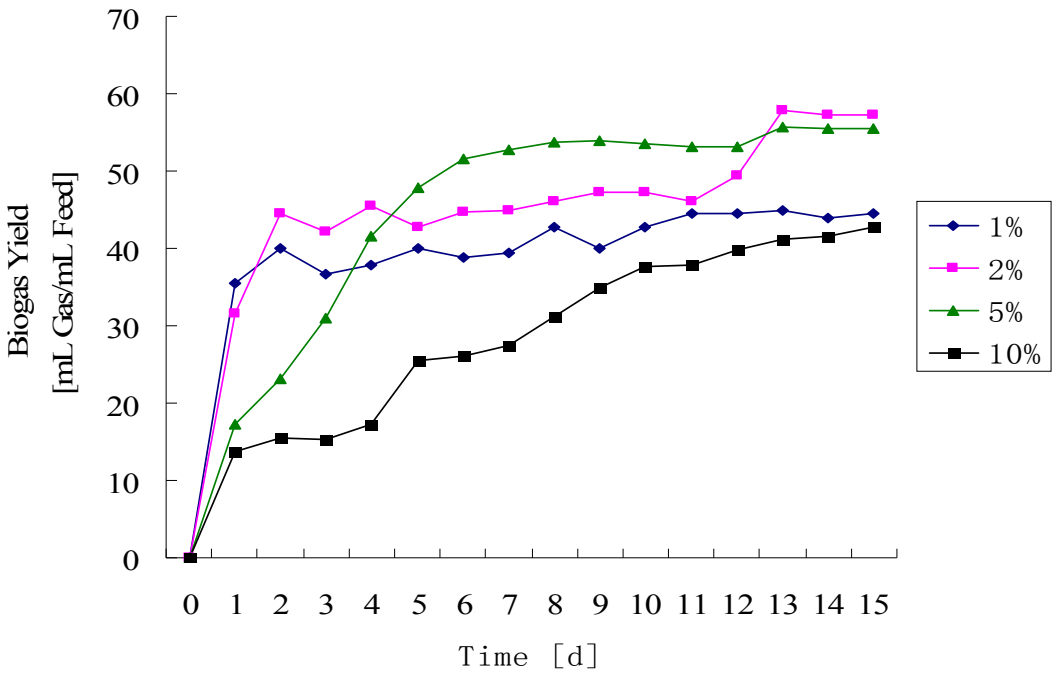


Figure 3.2 Accumulated biogas yield in 15 experimental days with different organic feed contents and with the inoculum pretreated by the sieve filters (pore size: 500µm)

Figure 3.2 shows that at the beginning of the experimental days, the 1 % and 2 %

gave a higher biogas production rate comparing to 5 % and 10 % feed content, while at the end, the 2 % and 5 % gave a higher biogas yield. Initial feed content of 1 % case gave a lower biogas yield due to the low organic content for the methanogenesis bacteria to grow, while 10 % case also gave low biogas yield due to the high pH value which was out of the idea range for the methanogenesis bacteria to grow efficiently (**Table 3.2**) or due to some unknown substrate inhibition, which is a complex issues related to the effect of long chain fatty acid (LCFAs) forming (Cirne, *et al.,* 2006), F/M ratio (Tchobanoglous ,1998), antibiotic substances produced by cellulolytic bacteria (Hobson & Wheatley, 1993), and enzyme activity.

3.2.2 Effect of Inoculum Pretreatment on Biogas Yield

Three different inoculum pretreatment methods were used in this study (**Chapter 2.1 and Table 2.1**). The biogas yield results in different cases are shown in Table 3.4 and Figure 3.3 bellow.

Table 3.4 Accumulated 15 days biogas yields from batch experiment by using different inoculum pretreatment methods (variation of the data is analyzed at the significance level of 0.05)

Reactor	Inoculum Pretreatment	Apple Juice Content (weight%)	Biogas Yield [mL gas/mL feed]
R3	Leachate from sieve filter (pore size: 500 μm)	2	57±2
R4		5	55±3
R6	Leachate from belt filter after the AD reactor	2	45±1
R7		5	23±3
R8	Slurry from outlet of AD reactor (unfiltered)	2	50±6
R9		5	49±3

Using 2 % (weight %) apple juice as the initial feed content, the biogas yields with different inoculum pretreatments were within the similar numerical range (**Table 3.4 and Figure 3.3**).

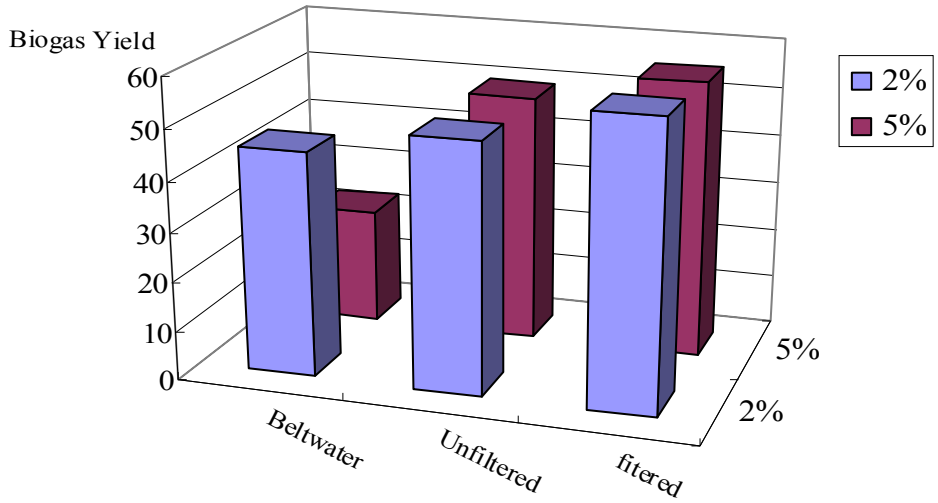


Figure 3.3 Accumulated biogas yields from batch experiment by using different inoculums Pretreatment methods; 2 % represents using 2 % (weight %) apple juice as feed; 5 % represents using 5 % (weight %) apple juice as feed)

Using 5 % (weight %) apple juice as initial feed content, the inoculum prepared from the belt filter gave a significantly lower biogas yield, where most of particulate mass from the AD reactor had been removed (**Figure 3.3 and Table 3.5**).

Table 3.5 Constituents of the inoculums pretreated with different methods

Inoculum Pretreatment	TS [g/L]	TSS [g/L]	VS [g/L]	VSS [g/L]
Leachate from sieve filter (pore size: 500 µm)	5.5±0.07	4.5±0.3	2.9±0.04	2.2±0.06
Leachate from belt filter after the AD reactor	8.4±0.2	0.75±0.04	1.7±0.2	0.57±0.02
Slurry from outlet of AD reactor (unfiltered)	19±0.3	18.5±0.1	8.5±0.3	10.8±1.6

The constituents found in the inoculum prepared from the sieve filter and unfiltered slurry were quite similar (Table 3.5). The samples of the leachate from belt filter were not fresh (kept for more than one month). Therefore, the constituent results analyzed from those samples were probably not representative, but assumed to give a useful indication.

The experimental data of the accumulated biogas yield influenced by using different feed pretreatment methods are plotted in Figure 3.4.

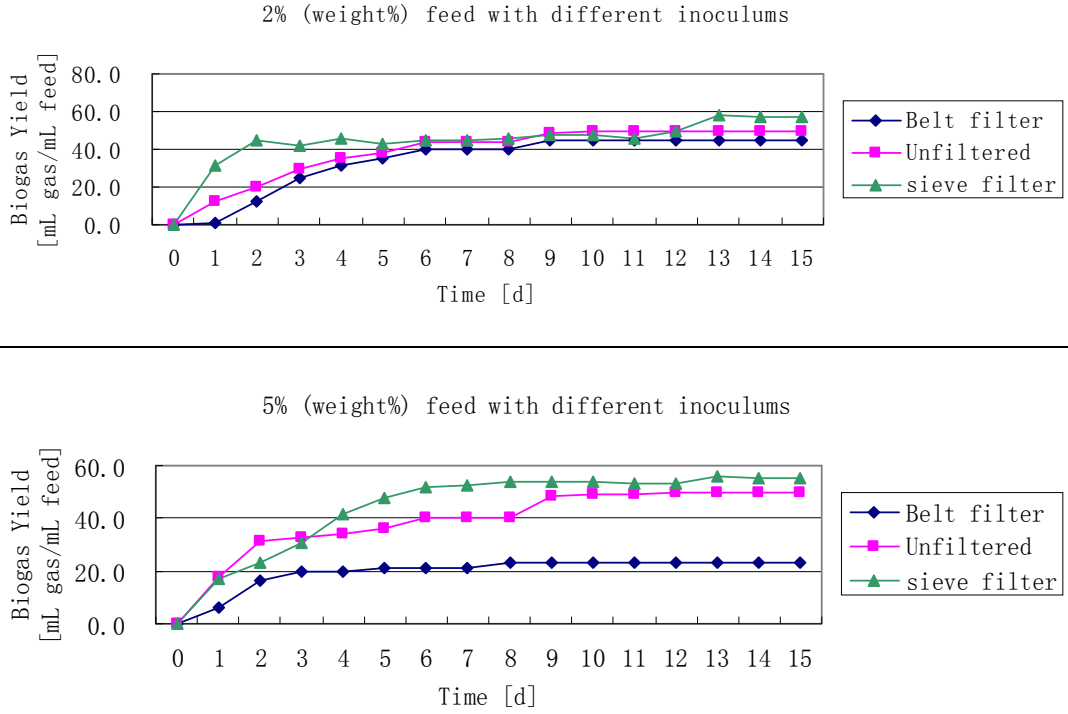


Figure 3.4 Biogas yields influenced by different inoculum pretreatment methods

Figure 3.4 illustrates that in the case of using 2 % (weight%) feed, the biogas yield using belt filtered inoculum was slightly lower than the others due to its lower VSS value, which is the indicator of the biomass in the inoculum (**Table 3.5**). In the case of 5 % (weight %) feed, the biogas yield using belt filtered inoculum was significantly lower than the others. This may be explained by the low biomass constituents in the inoculum and substrate inhibition problems due to the high initial F/M ratio.

3.2.3 Effect of Micro-aeration on Biogas Yield

The methane yields at the end of 15 experimental days of the AD bioreactors with micro-aeration conditions are presented in Table 3.6.

Table 3.6 Accumulated methane yield from AD bioreactors with micro-aeration conditions at the end of 15 experimental days

Apple Juice Content (weight %)	Head Space of Air [mL]	Biogas Yield [mL gas/mL feed]	CH_4 content (%)	CH_4 Yield [mL gas/mL feed]
2	0	57±2	70	40±1
2	5	85±3	53	46±2
5	0	55±3	67	37±2
5	10	50±2	47	23±1

The methane yields with micro-aeration conditions were also plotted in Figure 3.5.

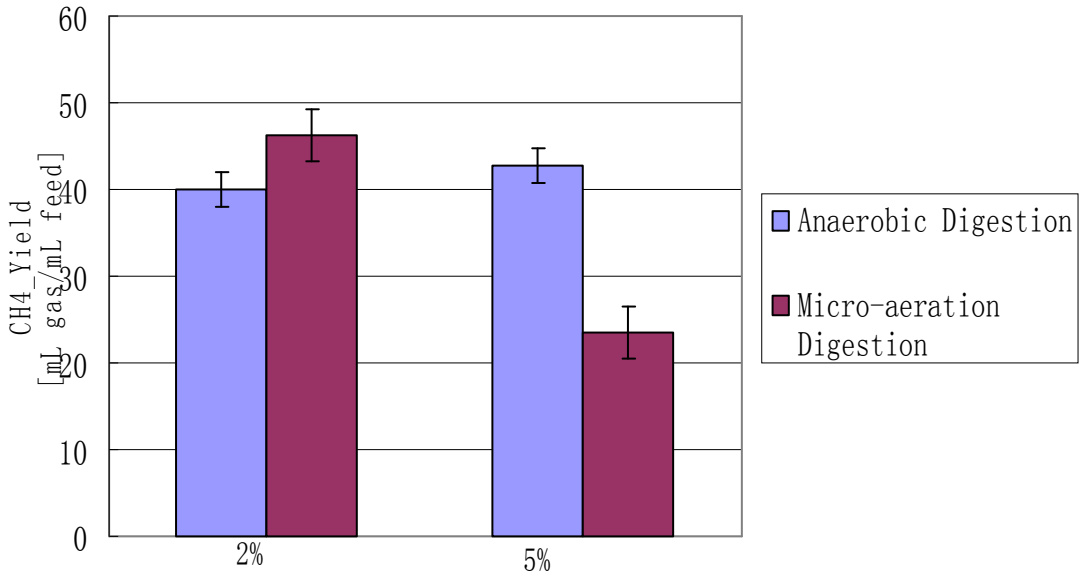


Figure 3.5 Methane yield at the end of 15 experimental days in Micro-aeration Conditions; 2 % represents using 2 % (weight %) apple juice as feed; 5 % represents using 5 % (weight %) apple juice as feed.

Both Table 3.6 and Figure 3.5 show that micro-aeration condition had a slightly positive effect on methane yield when using 2 % (weight %) feed and with 5mL air in headspace. This may be explained by the advantages of using micro-aeration in AD processes, such as: micro-aeration condition can enhance the rate of hydrolysis process, and reduced the H_2S toxicity in the AD reactors. Micro-aeration had a significant negative effects on methane yield in the case of using 5 % (weight %) feed and with 10 mL air in headspace. This may be explained by the fact that large amount of oxygen will cause oxygen toxicity to the bacteria cells in the AD reactor (Tango & Ghaly, 1999). These results show that suitable amount of air (oxygen) are required for the micro-aeration treatment in AD process.

3.3 Estimation of Substrate Compositions in ADM1 Simulations

The experimental data from batch experiments together with the simulation results are shown in Figure 3.6.

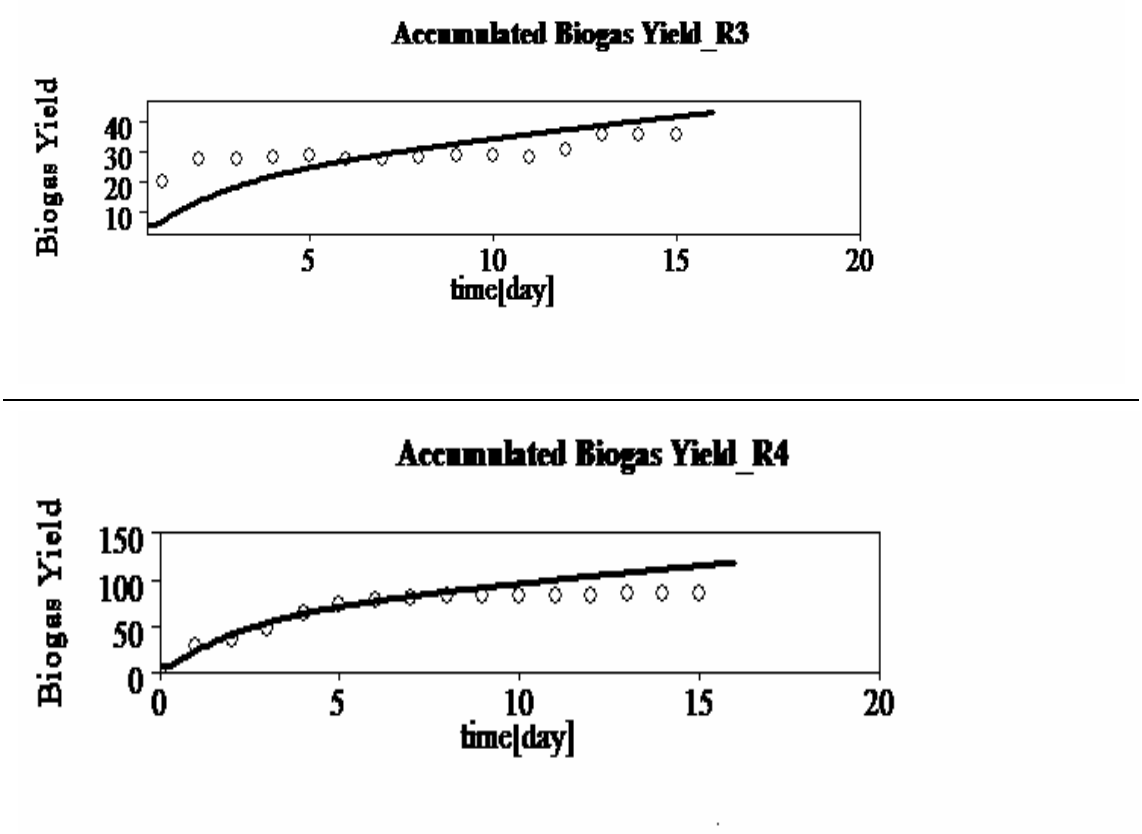


Figure 3.6 Comparison of the experimental data (circles) with the simulation curve on the accumulated biogas yield. R3 represents the bioreactor with 2 % (weight %) apple juice as feed; R4 is the bioreactor with 5 % (weight %) feed; the biogas yields are determined as the volume of biogas produced per volume of feed consumed.

Figure 3.6 illustrates that the simulations of batch biogas production gave the best fit to the experimental biogas production data when the initial conditions implied that the apple juice consisted mainly of sugar and some organic acids (**Table 3.7**) , similar to the measured apple juice composition. (**Table 3.7 and Table 3.1 in chapter 3.1**)

Table 3.7 Apple juice composition results from Lab analysis and estimations in ADM1 (under the assumptions that the total substrate COD sCOD =S_su+S_aa+S_ac+S_pro+S_bu+S_va+S_fa+S_ch4+S_h2+S_I , where the values of carbon content of methane(S_ch4), elemental hydrogen(S_h2), soluble inert COD(S_I) are assumed as zeros, S_aa are considered equal to protein)

Compositions Of 100% apple juice	Estimation from 2% Feed in ADM1 [g COD/L]	Estimation from 5% Feed in ADM1 [g COD/L]	Average results of Estimation in ADM1 [g COD/L]	Lab analysis results [g COD/L]
Sugar (S_su)	120	111	115	113
Amino Acid (S_aa)	1	1.38	1.19	1.5
Acetic Acid (S_ac)	0.12	0.09	0.1	0.13
Propionic Acid (S_pro)	0.33	0.19	0.26	0.21
Butyric Acid (S_bu)	0.17	0.17	0.17	0.15
Valeric Acid (S_va)	0.43	0.09	0.26	0.48
Fatty Acid (S_fa)	3.05	3.46	3.26	2.91
sCOD=S_su+S_aa+S_ac+S_pro +S_bu+S_va+S_fa	125	116	121	118

Table 3.7 shows that the sCOD was estimated to be 121 g COD/L, based on the most suitable initial conditions in the simulation (**Figure 3.6**). This was close to the measured value 118 g COD/L.

The estimated compositions of apple juice together with the laboratory analyzed results in Table 3.7 are also plotted in Figure 3.7.

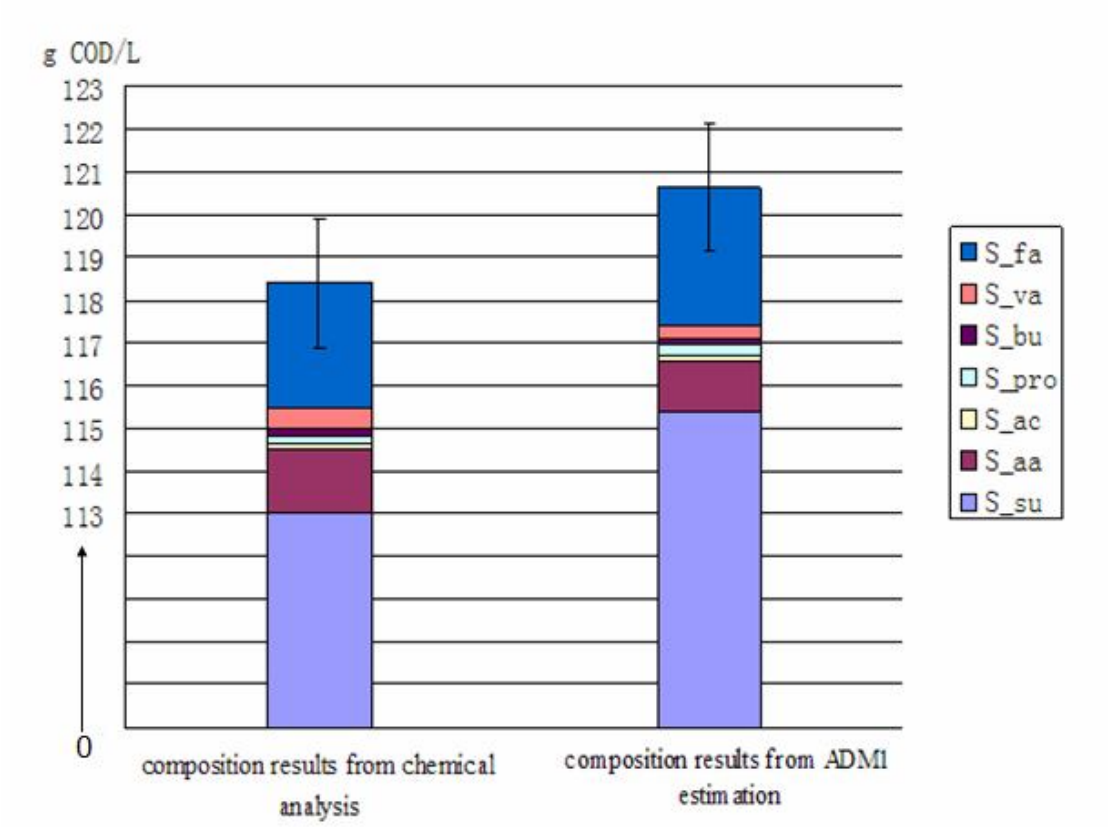


Figure 3.7 Comparison of the feed composition results from chemical analysis with ADM1 estimations

Figure 3.7 illustrates that the estimation results of the composition of apple juice were close to the real values analyzed from the Lab. This implies that ADM1 can be used as the soft sensor method to estimate the biogas substrate composition quite accurately. Both results show that sugar, some protein and fat are the main constituents of apple juice, which were also consistent with results from the literature (Jihong *et al.,* 2007).

3.4 Continuous Flow Bioreactor for Acidogenesis

This subchapter describes the results from a continuous flow bioreactor (CFB) with H_2 production, operated to study process steps prior to methanogenesis. Three main results will be shown as follows: the pH effect on H_2 yields and consumptions; pH effect on VFA distributions in the process; fate of substrate COD distributions in the process.

3.4.1 pH effect on H_2 yields and consumptions

Measured H_2 production rates with apple juice as substrates, with and without buffer solutions in continuous flow bioreactor (CFB), are shown in Figure 3.8.

The results of measured H_2 yields from CFB and the theoretical maximum H_2 yields

calculated from the molar basis of VFAs (mainly acetate and butyrate) values are shown in Table 3.8 and Figure 3.9. Observed yields are much lower than theoretical yields. The reason may be that the H_2 produced were consumed by some group of bacteria, i.e. homoacetogenesis bacteria, which consume H_2 and CO_2 to form acetate (Siriwongrungson *et al.,* 2007). The following Figure 3.11 shows that in the case of pH at 7.1 (high H_2 consumptions), the acetates were increased sharply.

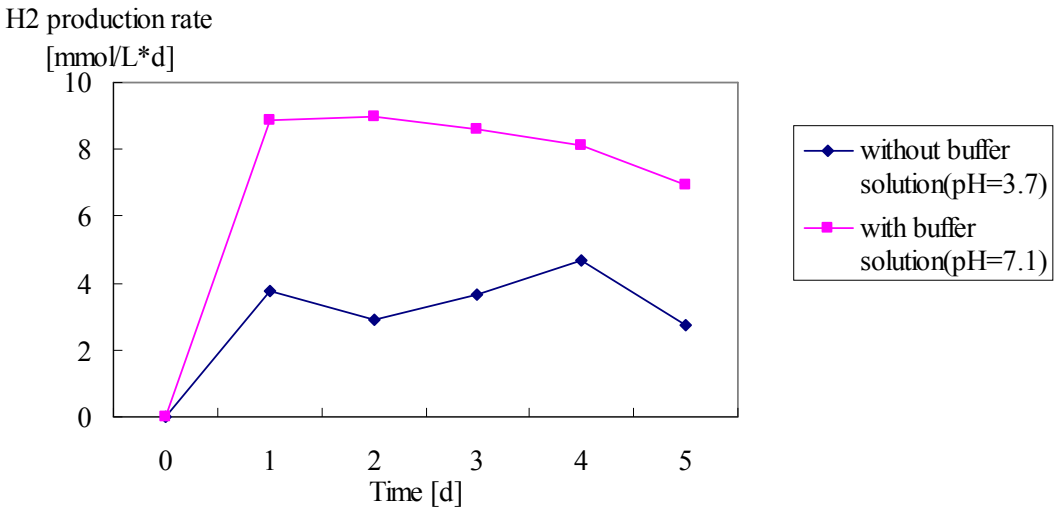


Figure 3.8 H_2 production rate in CFB with and without buffer solution conditions.

Figure 3.8 illustrates that H_2 production fluctuated with pH change. Adding buffer solution to the substrates, the H_2 production rate of CFB (with pH =7.3) was higher than the one without adding buffer solutions (pH =3.7) in CFB. This indicates that the low pH circumstance influenced the H_2 consumptions. The pH production rate had a decreasing trend within experimental days in the buffer case.

Table 3.8 Accumulated 5-experimental-day measured H_2 yields and theoretical H_2 yields in the cases of using substrates with and without buffer solutions; The theoretical values were calculated from the molar basis of VFAs (mainly acetate and butyrate) in the CFB

	H_2 yields with pH =3.7 (without buffer solutions) [mmol/L]	H_2 yields with pH =7.1 (with buffer solutions) [mmol/L]
Measured	18	42
Theoretical	106	147

Table 3.8 shows that in both cases, there was a significant difference between the measured H_2 production value and theoretical value. This means the H_2 produced were mostly consumed in the CFB. The similar results can be also found from the batch reactors, where H_2 were produced at the first few experimental days and then consumed or converted to CH_4 (**Appendix A**).

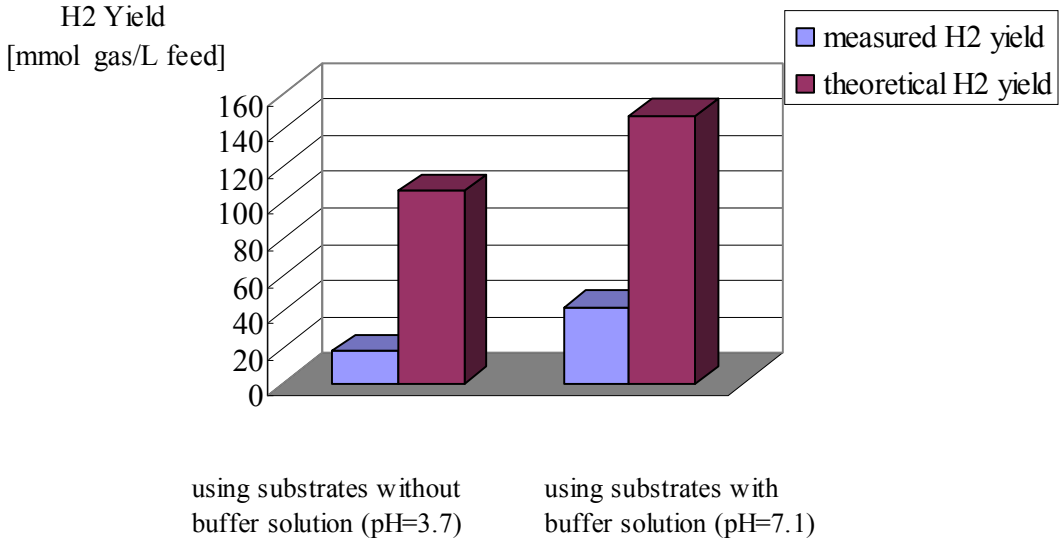
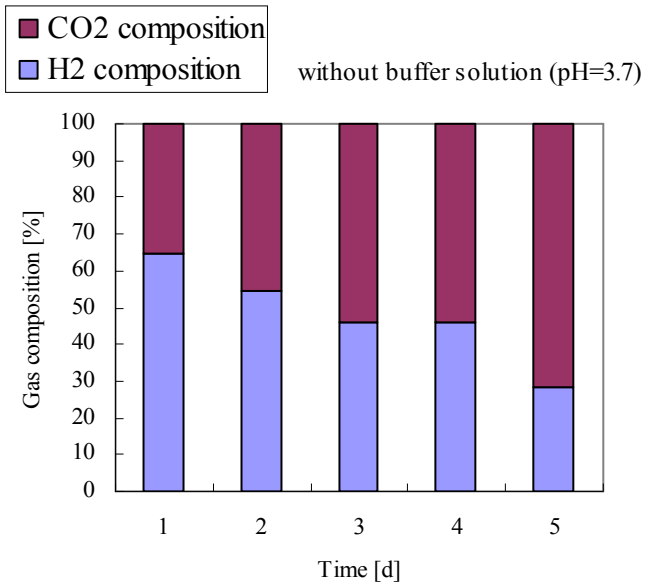


Figure 3.9 Accumulated 5-experimental-day measured H_2 yields in CFB and theoretical H_2 yields calculated from the molar basis of VFAs (mainly acetate and butyrate) in CFB in the cases of using substrates with and without buffer solutions

The big difference between the measured H_2 yields and theoretical H_2 yields (**Figure 3.9**) indicates that the H_2 produced were consumed and converted to other fermentation end products. One of the H_2 consumers is the homoacetogenesis bacteria in CFB. Since the methanogens in the inoculum were inactivated by the "heat shock" method (**Table 2.1 in chapter 2.1**), in the absence of competition from methanogens, the H_2 produced reacted immediately with CO_2 to generate acetate by the dominated homoacetogenesis bacteria (Siriwongrungson *et al.,* 2007).

Figure 3.9 also illustrates that the H_2 yields were higher with high pH circumstance in CFB. Similar results were observed by other researchers (Liu *et al.,* 2006) that H_2 production decreased when pH dropped to 4.8 due to the accumulation of the butyrate as the end product.

The H_2 consumptions can be implied by the deceasing H_2 compositions of the biogas produced within the experimental days (**Figure 3.10**).



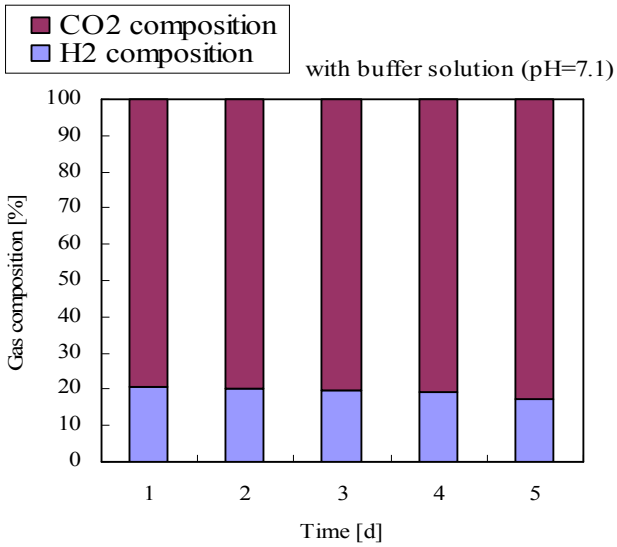


Figure 3.10 H_2 composition of the biogas in different pH circumstances

Figure 3.10 illustrates that with pH at 3.7, the H_2 compositions were high (around 40-60 %), but with a decreasing trend. With pH at 7.1, the H_2 compositions were low (around 20 %) and almost with constant values. Large amount of CO_2 were found at pH =7.1, this was due to the buffer solution HCO_3^- added to the substrate. It also implies that the H_2 consumptions were higher at pH =7.1. Because H_2 produced reacted immediately with CO_2 to generate acetate by homoacetogenesis bacteria (Siriwongrungson *et al.,* 2007). These results indicate that both H_2 yields (**Table 3.8 and Figure 3.9**) and consumptions (**Figure 3.10**) were higher with pH at 7.1. It implies that the substrates with buffer solutions (pH =7.1) were suitable for the acidogenesis and acteogenesis bacteria to grow in the CFB for H_2 production.

3.4.2 pH effect on VFAs distributions in CFB

The biological H_2 production yields depend stoichiometrically on the range of fermentation VFA products formed (Rodríguez *et al.,* 2006). The metabolic pathways of H_2 production bacteria are regulated by environmental factors such as pH, temperature and H_2 partial pressure (Liu *et al.*, 2006). In this study, only the influence of pH was tested to study the relationship between the end products VFA distributions and the H_2 yields/consumptions in different pH circumstances (**Figure 3.11**).

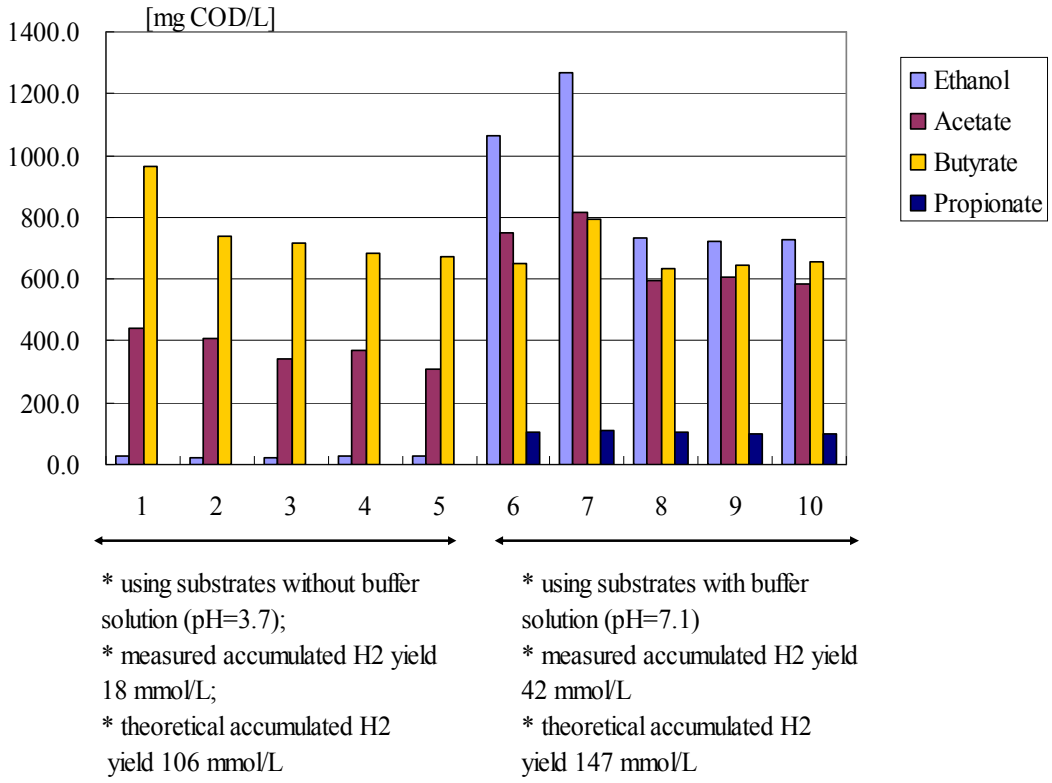


Figure 3.11 VFAs distributions development with different pH circumstances in CFB

Figure 3.11 illustrates that the distributions of VFAs were strongly influenced by pH. When pH was at 3.7, lower H_2 production was found together with butyrate as the main products. Little ethanol and no propionate were detected at low pH. When pH was increased to 7.1, acetate accumulated while butyrate remained high. Large amount of ethanol and small amount of propionate were also detected at pH at 7.1. The similar results have been found by other researchers (Liu *et al.,* 20006 and Kim *et al.,* 2004). A significant transient response was also observed when the buffer was introduced causing elevated levels of ethanol, acetate and butyrate for about two days, compared to the stable (semi steady state) levels afterwards (**Figure 3.11**).

Figure 3.11 can be used to explain why there was a big difference between the theoretical accumulated H_2 yields and measured H_2 yields. The ethanol, lactate and

other organic acids are the end products from the metabolism pathway of glucose fermentation in H_2 production process. Those products are also the "electron sink" to stop the formation of H_2. (**Figure 1.2 in the introduction chapter**). At pH 7.1, there were a large amount of ethanol produced (**Figure 3.11**), which gained the proton and reduced the formation of H_2. At pH 3.7, although almost no ethanol production was detected, the H_2 produced may be consumed to lactate and other organic acids which had not been analyzed yet in this study. Similar results from other researchers (Liu *et al.,* 2006) shows that the metabolism pathway in a H_2 production process shifted from the formation of H_2 to formation of acetic and butyric acids with pH range of 4.8 to 5.2.

3.4.3 Fate of substrate COD

Previous results of this study shows that the H_2 produced were gradually consumed which led to the difference between the theoretical H_2 yields and measure H_2 yields. It is also of interest to analyze the distribution of substrate COD converted into products. These results are shown by the ratio of the COD in the products to the COD in the substrate (Figure 3.12).

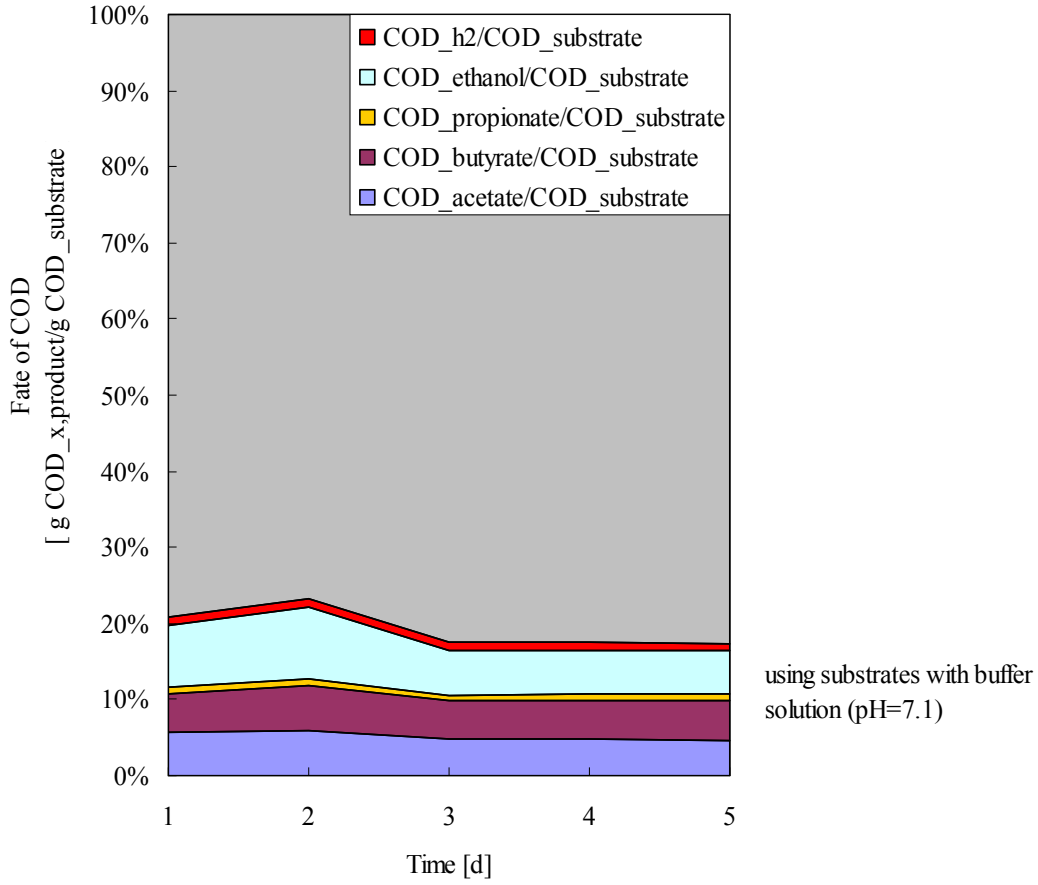


Figure 3.12 Fate of substrate COD [g COD_x, product/ g COD_substrate]

Figure 3.12 shows the fate of substrate COD distributions in the products at pH =7.1.

Similar results were also found at pH =3.7 (**Appendix H**).

Significant COD imbalance can be seen from Figure 3.12, implying that only about 20 % of all substrate degraded is account for in the products measured. This imbalance indicates that the product of ethanol, acetate, butyrate, propionate and H_2 accounted for small amount of contents in the consumption of substrate COD. From the metabolic pathway of glucose fermentation in H_2 production process (**Figure 1.2 in Introduction chapter**), it can be assumed that the consumed substrate COD may also be distributed in the products of sugar, lactate, other organic acids and biomass which were not analyzed in this study. This assumption needs to be tested in future work.

4 Discussions

This chapter discusses the results of the hypothesis tests (**Chapter 1 Introduction**) where the main findings are summarized in Table 4.1.

*Table 4.1 Hypothesis Tests Based on the Experimental Results (**Chapter 3**)*

Hypothesis	Testing Results	Comments
1. Apple juice can be used as the model substrate for the study of biogas production process.	Supported	See chapter 3.1 and the following chapter 4.1
2. Analyze the F/M ratios to identify the substrate overload issues or the substrate inhibition problems.	Supported	See chapter 3.2.1 and the following chapter 4.2
3. Leachate from the belt filter process after the AD reactor can be used as the inoculum for the study of biogas production process.	Supported	See chapter 3.2.2 and the following chapter 4.3
4. Biogas substrate composition can be estimated from simple batch testes and ADM1 simulations.	Supported	See chapter 3.3 and the following chapter 4.4
5. pH influences the metabolic pathway selection of glucose fermentation.	Supported	See chapter 3.4 and the following chapter 4.5

The detailed information on the hypothesis tests are discussed as follows.

4.1 Model Substrate

For the AD process, the methanogenic bacteria are crucial to the anaerobic stabilization of a variety of substrate, which can be domestic wastewater, wastes from food industry and so on. However, known methanogens utilize only a narrow array of relatively simple substrates for growth and metabolism (Malina & Pohland, 1992). The apple juice used as the substrate in this study can be regarded as a good model substrate to test biogas reactions from the liquid fraction of the food industry wastes. The advantages are as follows:

(1) The apple juice can be used as the substrate to mimic the liquid phase that is extracted from the fruit or juice industry WOSW for the biogas research. The sCOD 125 g/L was close to the leachate from apple waste of which the sCOD was 160 g/L, estimated from the methane production (Song *et al.,* 2007).

(2) The apple juice has a relatively stable chemical composition, i.e. the sCOD, TOC,

VFA and etc. It is convenient to be able to repeat the experiments with the same feed compositions.

(3) The chemical compositions of apple juice include mainly sugar, fat and some organic acids. These components are good reactants for the acidogens and acetogens to grow and produce feed for the methanogens. The C:N ratio is about 250:3 (**Table 3.1 in Chapter 3.1** and under the assumption that the carbon source was from tCOD, and nitrogen source is from protein). This value is in the suitable range (400:7~1000:7) for the metabolism of the methanogens (Henze *et al.,* 1983 and van den Berg *et al.,* 1978). There are also no heavy metals or other toxicity components in the substrate, which is good for AD process.

(4) The value of sCOD 123 g /L and tCOD 125 g/L are close. This implies that most substrate COD is soluble, which is a benefit for the hydrolysis step in the AD process.

4.2 Analyze the F/M ratio to Identify the Substrate Overload Issues

The substrate overload issues always cause big problems for the AD process. It will not only yield the low biogas production, but also shut down the treatment plants for several weeks (Xing *et al.,* 1997). Normally, such overload issues lead to pH drop, i.e. pH value below 6.2, which is not thermodynamically favorable for the methanogens to grow (Murnleitner *et al.,* 2002). However, in this study, it was found that even within the suitable pH range, the high substrate content with 10 % (weight %) gave a lower biogas yield (**Table 3.2 and Figure 3.1 in Chapter 3.1**). This can be explained by the substrate inhibition caused by high F/M ratio (**Table 3.3 in Chapter 3.1**), which means the "food" (substrate) was overloaded to the unit of biomass in the AD reactor. Those "extra food" may cause the forming of LCFAs, decreasing the enzyme activities of the cells and other substrate inhibition problems.

Therefore, in addition to the indicator from the pH drop in the AD reactor, the substrate overload issues can also be found by analyzing the F/M ratio when planning batch reactor studies.

The results from this study (**Table 3.1 and Table 3.3 in Chapter 3.2.1**) shows that the F/M ratio is suitable for the AD process when using 2-5 % (weight %) apple juice as substrates.

4.3 Inoculum Pretreatment

As described in Table 4.1, the third hypothesis that leachate from belt filter treatment after AD process can be used as the inoculum for biogas production, was supported by the results from Table 3.4 and Figure 3.3 (**Chapter 3.2.2**).

The advantages of using the leachate from belt filter process as inoculum are as

follows:

(1) Using the filtered leachate as inoculum gave a similar biogas yield comparing to using unfiltered inoculum in the case of using 2 % (weight %) feed (**Table 3.4**). Therefore, using the belt filtered inoculum for the AD process had no negative influence on biogas yield.

(2) In this study, it was found that using the inoculum pretreated from belt filter inoculums did not cause blockage problem in the syringes (AD reactors) that unfiltered inoculums can cause. This is due to the removal of large particles, i.e. fibers. Using the unfiltered inoculum normally block the syringes (AD reactors), which cause troubles for moving the piston to release the accumulated biogas in the AD reactor (**Chapter 2.2.1 sampling methods**).

(3) From an industrial point of view, it is necessary to pretreat the inoculum to remove the large degradable or undegradable particles; otherwise, they would destroy or block the pump, pipes and internal structures of the digester. Using inoculum pretreated from belt filter will not cause such problems.

(4) From the biology point of view, the bacteria attached on solid particles are from the surface. The bigger the surface area per unit weight, i.e. the smaller particle, in general the faster and more complete will be the bacterial degradation in AD process. (Hobson & Wheatley, 1993). The inoculums pretreated by belt filter are qualified for the bacterial degradation due to the removal of large particles and retain of the smaller particles.

4.4 Substrate Composition Estimation in ADM1

Based on the results that the estimated substrate composition was similar to the analytical values from the lab (**Table 3.6, Figure 3.6 and 3.7 in Chapter 3.3**), the hypothesis that ADM1 can be used as a soft sensor method to estimate the biogas substrate compositions, was supported.

Feed composition is the key parameters for the biogas production plant, but it is both time and economical consuming to measure the composition directly. This study presents a technique that the feed composition can be estimated based on the simple experimental measurements combined with simulations in ADM1.

For future work, it is also possible to test the hypothesis that the biomass composition can also similarly be estimated in ADM1.

4.5 pH Effect on H_2 Yields and Consumptions

The pH effect on H_2 yields and consumptions was correlated to the metabolic pathways of glucose fermentation. Those metabolic pathways are influenced by the transportation of acidic species through the cell membrane, where the proton transportation activities and ATP energy consumptions are involved. Different pH circumstance influences the concentration gradient of the protons. This gradient ultimately has an effect on the metabolic product formations (Rodríguez, *et al.,* 2005).

Results from Chapter 3.4 show that the pH factor influenced the VFA distributions in the H_2 production process. The end fermentation products vary with the pH circumstance. The ethanol, lactate and other organic acids are the end products from the metabolism pathway of glucose fermentation. Those products are also the "electron sink" to stop the formation of H_2 (**Figure 1.2 in the introduction chapter**). Therefore, it can be concluded that the metabolic pathway of H_2 producing microorganisms are regulated by environmental factors, i.e. pH.

The composition of lactate, glucose, and other organic acids need to be analyzed in the future work to further test the assumption that the COD imbalance found in Figure 3.12 (**chapter 3.4.3**) are due to the consumed substrate COD distributed in those components.

For future work, it is also of interest to do the simulations in ADM1 with non-methanogenesis system to study the process of H_2 production.

5 Conclusions

Five main conclusions were drawn in this study: (1) Apple juice can be used as the model substrate for the study of fermentation and biogas production; (2) Food to microorganism (F/M) ratios can be used to identify the substrate overload issues or the substrate inhibition problems; (3) Leachate from the belt filter process after the AD reactor can be used as the inoculum for the study of biogas production processes; (4) Biogas substrate composition can be estimated from simple batch tests and ADM1 simulations; (5) pH influences the metabolic pathway selection of glucose fermentation.

Some sub-conclusions are presented as follows:

- Apple juice can be used as the substrate to mimic the liquid phase extracted from the WOSW from food industry. The sCOD of apple juice was 123 g COD/L, which was close to the tCOD 125 g COD/L, implying that the organic matter is dissolved. The chemical compositions of apple juice included mainly sugar, some fat and some organic acids.

- The initial feed content of 2 % and 5 % (weight %) gave the high biogas yields 55 (mL biogas per mL feed consumed), compared to the 1 % and 10 %, which gave the biogas yields of 42 (mL biogas per mL feed consumed).

- 1 % feed was too low to get precise reading while 10 % feed case resulted in an overload case where intermediate production formation caused inhibition of biogas production reaction.

- The biogas yields from 2 % and 5 % were used for simulations in ADM1 to estimate the substrate compositions. The soluble chemical oxygen demand (sCOD) was estimated to be 121 g COD/L, based on the most suitable initial conditions in the simulation. This is close to the measured value of 118 g COD/L. This implies that ADM1 can be used as the soft sensor method to estimate the biogas substrate composition quite accurately.

- Except for the reactor with feed content of 10 %, all the other reactors (1 %-5 % feed) were within the suitable AD alkalinity range of 2000~5000 mg/L as $CaCO_3$ (Tchobanoglous *et al.,* 2003).

- The toxic and inhibitory inorganic compound of ammonia-nitrogen (NH_4-N) concerned for anaerobic process were low in all the batch reactors with the feed content from 1 % to 10 % (weight %). There were no NH_4-N inhibition problems.

- The feed content of 2 % and 5 % had a suitable F/M ratio within the 0.04-0.1 range (WEF, 1998; Crites and Tchobanoglous, 1998) for microorganism to grow.

The initial feed content of 10 % had high F/M ratio which was out of the suitable range. This may explain why 10 % feed gave lower biogas yields.

- Using belt filter leachate as inoculum gave a similar biogas yield 45 [mL gas per mL feed consumed] comparing to using unfiltered inoculum in the case of using the 2 % (weight %) feed. Therefore, it had no negative influence on biogas yields.

- The belt filtered inoculum did not cause blockage problems in syringe (AD reactors) that unfiltered inoculums can cause due to the removal of large particles, i.e. fibers. This character of belt filtered inoculum made it good for the biogas process study.

- Micro-aeration condition had a slightly positive effect on methane yield when using 2 % (weight %) feed and with 5mL air in headspace. Micro-aeration had a significant negative effects on methane yield in the case of using 5 % (weight %) feed and with 10 mL air in headspace. These results show that suitable amount of air (oxygen) are required for good micro-aeration treatment in AD process.

- H_2 production fluctuated with pH change. Adding buffer solution to the substrates, the H_2 production rate of CFB (with pH =7.3) was 8 mmol/L per day, which was higher than the one without adding buffer solutions (pH =3.7) with H_2 yields of 4 mmol/L per day.

- There was a significant difference between the measured H_2 production value and theoretical value. This means the H_2 produced were mostly consumed in the CFB.

- The big difference between the measured H_2 yields and theoretical H_2 yields indicates that the H_2 produced were consumed and led to other fermentation end products, such as consumed by the homoacetogenesis bacteria in CFB to form acetate.

- Biogas H_2 content at pH 3.7 was around 40 %-60 %, but with a decreasing trend. Biogas H_2 content at pH 7.1 was lower (around 20 %) and almost constant.

- Large amount of biogas CO_2 was found at pH =7.1 due to the buffer solution HCO_3^- added to the substrate. It also implies that the H_2 consumptions were higher at pH =7.1, because H_2 can be consumed with CO_2 to form acetate via homoacetogenesis bacteria.

- Both H_2 yields and consumptions were higher with pH at 7.1. It implies that the substrates with buffer solutions (pH =7.1) were suitable for the acidogenesis and acteogenesis bacteria to grow in the CFB for H_2 production.

- The distributions of VFAs were influenced by pH. When pH was at 3.7, lower H_2

production was found together with the accumulated butyrate as the main products. Little ethanol and no propionate were detected at pH =3.7. When pH was increased to 7.1, acetate and ethanol also accumulated.

- The ethanol, lactate and other organic acids were the end products from the metabolism pathway of glucose fermentation studied. Those products can be the "electron sink" to stop the formation of H_2. This may explain the big difference between the measured H_2 yields and theoretical H_2 yields.

- Significant COD imbalance implies that the product of ethanol, acetate, butyrate, propionate and H_2 accounted for small amount of contents in the consumption of substrate COD. It can be assumed that the consumed substrate COD may also be distributed in the products of sugar, lactate, other organic acids and biomass which were not analyzed in this study. This assumption needs to be tested in future work.

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7 Appendixes

Appendix A

Batch Experiments for the Study of Acidogenesis

- Inoculum pretreatment by "Heat shock" methods (Siriwongrungson *et al.,* 2007)

(1) sieve filtered with the effluents from AD reactor (sieve pore size: 500 μm)
(2) "heat shock" treatment to kill the methanogenesis bacteria:
$40^0C \rightarrow 60^0C \rightarrow 80^0C \rightarrow 104^0C \xrightarrow{overnight} 35^0C$ time difference between each heating is ~2 h
(3) adding 1 g coffee granules (instant coffee) to form the biofilms

- Substrates: Substrate used in this experiment is the apple juice (Brand: TINE) made from Meierienes Juice factory in Norway

Table Quantitative dosing of apple juice, inoculum

Reactor	Apple juice Content (weight%)	Parallels	Inoculum [mL]	Apple Juice [mL]
6	0	3	30	0
7	2	3	30	0.6
8	5	3	30	1.5

- Experimental setup: the same as the batch experiments setup in chapter 2.2.1.
- Results:

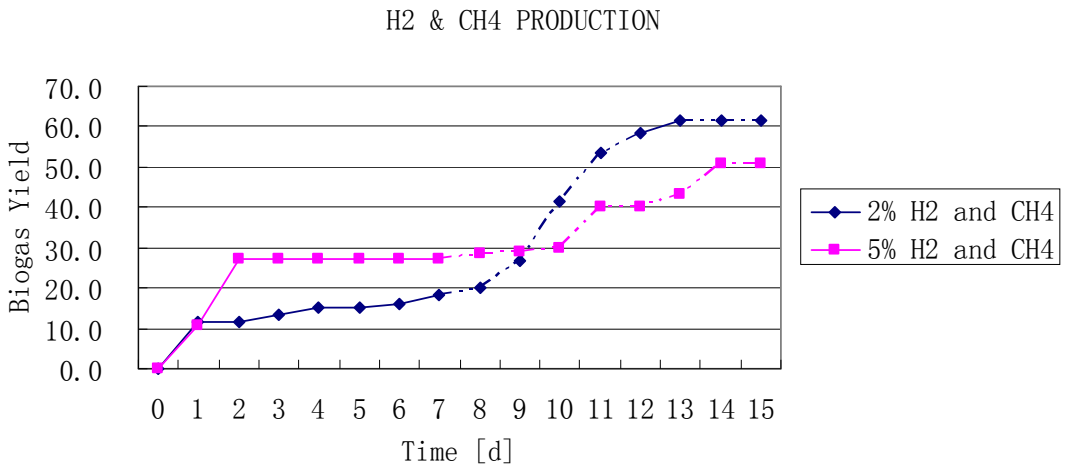

Figure 1 Biogas yields using inoculum pretreated by "Heat shock" methods ((Oh et al., 2003; Okamoto et al., 2000); The biogas yield is determined as the volume of biogas produced per volume of substrate (apple juice) consumed. The dash line presents the methane yields.

Appendix B

Estimation of the Amount of Air as Headspace for Micro-aeration in AD

- Introduction

The conventional anaerobic digestion (AD) process includes three main steps: hydrolysis, acidogenesis and methanogenesis. The most time-consuming step is the hydrolysis which makes the macromolecules in the WOSW converted into smaller dissolved molecules suitable for methane production. The introduction of micro-aerobic treatment into the first stage of AD process can reduce the hydrolysis time in the biogas production process.

- Calculation Methods

Since the micro-aerobic treatment is introduced to the hydrolysis step, the main calculation is focused on Chemical Oxygen Demand (COD) for the hydrolysis step. The two different calculation methods are discussed below.

➢ **Method 1 Based on general COD mass balance**

Assumption:

$$tCOD = sCOD + pCOD + nbCOD \quad (1)$$

Where tCOD is total COD , sCOD is soluble COD (readily biodegradable) , pCOD is particulate COD(slowly biodegradable), nbCOD is non-biodegradable COD

In this case, the feed we will use is the apple juice, which has a high content of different Volatile Fatty Acids (VFA) according to the results of VFA analysis. This indicates that most of the macromolecules can be hydrolyzed in the apple juice. Based on this point, it can be assumed also that nbCOD is zero. Then, the equation 1 will be simplified as following equation 2.

$$tCOD = sCOD + pCOD \quad (2)$$

Known information of apple juice (TINE):

tCOD [mg/L]	125000
sCOD [mg/L]	123000

Based on equ. 2, the pCOD value is 2000 mg/L.

Calculation on Oxygen Demand for micro-aeration:

We assumed that the oxygen demand for micro-aeration is the amount of oxygen needed for pCOD in the hydrolysis steps. That is 2000 mg O_2/L.

In the case of 2% (weight percentage) of apple juice, the apple juice is 0.6 mL of the total 30 mL volume.

Then the Oxygen demand for 2% (weight percentage) of apple juice is as follows:

$$0.6mL \times \frac{2000mg}{1000mL} = 1.2 \text{ mg } O_2$$

$$\frac{1.2mg}{30mL} = 0.04 \text{ mg } O_2\text{/mL=40 mg/L} > 7 \text{ mg/L}$$

According to literature, the maximum dissolved oxygen (DO) is 7 mg/L at temperature of 35℃. This indicates that it is impossible to achieve using air aeration.

So supplying air in the headspace is needed in the syringe (small bioreactor) for the micro-aeration purpose. Then the volume of initial headspace in the reactor is calculated as follows:
Amount of air moles required to supply 1.2 mg O_2

$$\frac{\left(\frac{1.2mg}{1000mg \times 32g/mol}\right)}{0.21} = 1.78 \times 10^{-4} \text{ mol air}$$

Assume that air behaves like idea gas at T= 35℃,using idea gas law PV=nRT

$$V = \frac{nRT}{P} = \frac{1.78 \times 10^{-4} \times 8.314 \times 308}{1 \times 10^5} = 0.45 \times 10^{-5} m^3 = 4.5mL \approx 5 \text{ mL air}$$

(Note: assume that the initial DO value in the reactor is consider zero for this calculation)

Similarly, the requirement of air for micro-aeration of the substrate content of 5% (weight %) apple juice can be calculated in the same way. The results are as follows:

Juice content (weight %)	**Air requirement for micro-aeration**
2%	5 mL
5%	10 mL

Appendix C

Calculation of COD Equivalent Coefficient of Different Components

- The COD equivalent coefficient of different components can be calculated by the following formula:

COD equivalent coefficient = $\frac{8 \bullet \lambda \bullet C}{M}$

Where 8 is the molecular weigh of the electron of oxygen ($\frac{32[g/mol]}{4eletron}$=8 g/mol per electron)

λ is the numbers of electron per carbon of component
C is the numbers of carbon in the component
M is the molecular weight of component

- For example:

Component	λ	C	M	COD equivalent coefficient
Glucose ($C_6H_{12}O_6$)	4 g/mol per electron	6	180 g/mol	1.07

- COD equivalent coefficient used in this study

Component	COD equivalent coefficient
Acetic Acid	1.06
Propionic Acid	1.51
Isobutyric Acid	1.82
Butyric Acid	1.82
Isovaleric Acid	2.8
Valeric Acid	2.8
Isocaproic Acid	2.21
Caproic Acid	2.21
* Protein	1.5
Sugar (glucose)	1.07
* Fat (lipids)	2.91
Note: Components mark with "*"are the values from the literature (Zeeman and Gerbans, 2002) The others are calculated based on the above formula.	

Appendix D

Batch Experimental Procedures

- **Equipments:**

60 ml syringes (reactors), needles, pipette graduated cylinder, 50 ml, beaker 100ml
magnetic stirrer, stirring bars,
rubbery stoppers
laboratory shaker, incubator

- **Reagents**:

Apple juice(TINE)
Inoculum (after pressing belt)

- Procedures

Do the preparation in an exhaust arrangement. Mix the inoculum well , place the solution on a magnetic stirrer. Do the following steps:

1. Transfer 650 mL of inoculum into a 800 mL beaker.
2. Measure the pH of the inoculum.
3. Purge the inoculum solution with Argon gas.
4. Connect a needle on each syringe.
5. Measure the inoculum volume in a 50 mL graduated cylinder. (measure 30 mL for each syringe)
6. Transfer the inoculum a 50 mL beaker
7. Use an automatic pipette to measure the volume of apple juice
8 Measure the pH of the mixture (inoculum and apple juice)
9 Place the rubber stopper at the needle point.
10 Transfer the mixture (inoculum, apple juice) to the syringe
11 Remove the stopper, release the air in the syringe by pressing it through the needle.
12 Place the stopper at the needle point.
13 Place the syringes on a test tube rack and place it in the incubator.
14 Keep the temperature at 35 ± 1.0°C.

Tips & hints

- Press and pull the piston (and check if it goes back to the start position) before taking a biogas reading.
- Release the gas if the gas volume is larger than 10 mL and meanwhile collecting gas samples for some reactors

Appendix E

Gas Sample Pretreatment

- The gas samples were collected by the syringes connected to the batch AD bioreactor. (Figure 1).

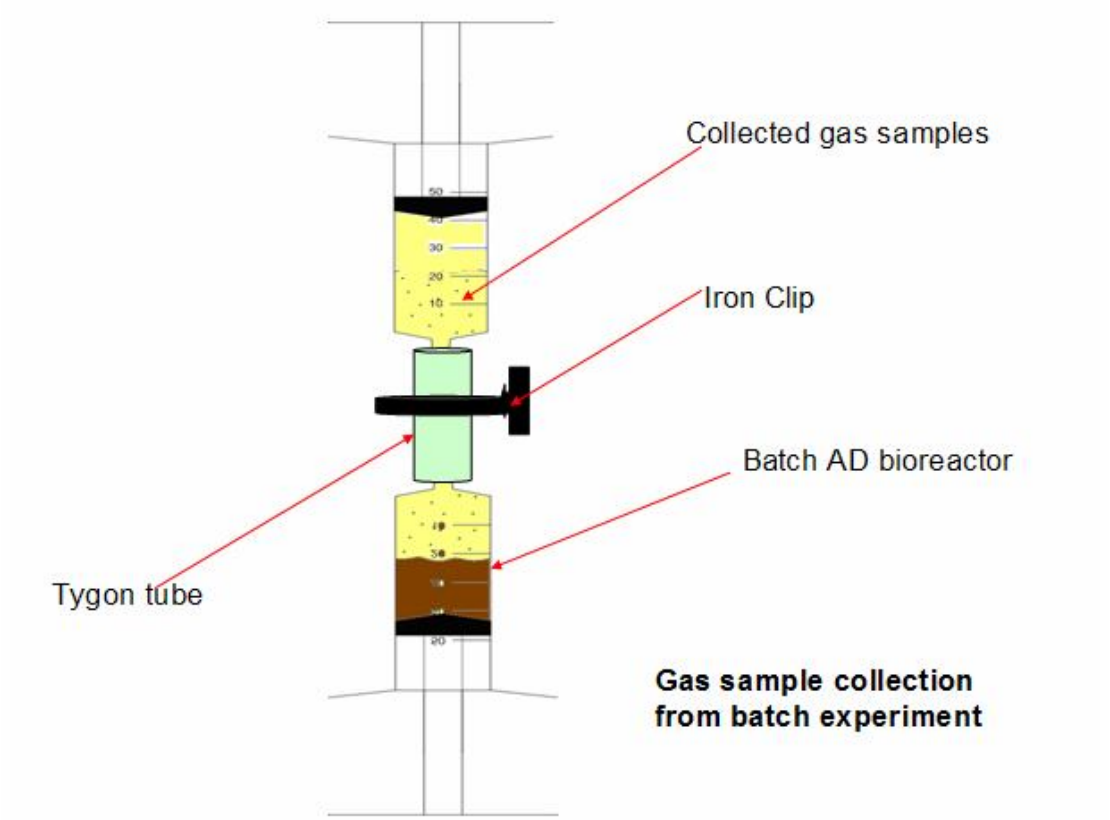

Figure 1 gas sample transfer from AD bioreactor (down) to the syringe (up)

- Before the gas composition analysis, the collected gas samples are put in the refrigerator to freeze the water content in the gas sample.

Appendix F

Preparations of stock solution A ,B, vitamins , minerals and buffer for the substrates used in Continuous Flow Bioreactor (CFB)

- Stock Solution A

Component	Concentration [g/L]
NH_4Cl	100
$MgCl_2*6H_2O$	10
$CaCl_2*2H_2O$	5
NaCl	10

- Stock Solution B

Component	Concentration [g/L]
K_2HPO_4	150

- Mineral Solution

Component	Concentration [g/L]
$MnSO_4*H_2O$	0.04
$FeSO_4*7H_2O$	2.8
$CuSO_4*5H_2O$	0.06
$NiCl*6H_2O$	0.092
$ZnSO_4*7H_2O$	0.09
$CoCl*6H_2O$	0.05
H_3BO_3	0.05
$(NH_4)_6Mo_7O_{24}*4H_2O$	0.05
$AlCl_3$	0.05
$Na_2SeO_3*5H_2O$	0.1
EDTA	0.5
HCl (37%)	1 mL

- Vitamin Solution

A 10 times concentrated vitamin solution as described by Wolin et al., 1963.

- Buffer Solution

Component	Concentration [g/L]
Na_2HCO_3	3.5

- Organic Source

Apple juice (Brand: TINE) made from Meierienes Juice factory in Norway.
Diluted for 10 g COD/L

Appendix G

Raw Experimental Data

- Biogas Yield from batch experiments

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate content of 0% (blank) Using inoculum pretreated by size filter (sieve pore size: 500 µm)	26.02.08	0	0	0	0
	27.02.08	1	2	2	2
	28.02.08	2	5	6	5
	29.02.08	3	8	8	7
	01.03.08	4	9	9	8
	02.03.08	5	11	11	9
	04.03.08	6	11	14	10
	05.03.08	7	12	14	10
	06.03.08	8	13	14	10
	07.03.08	9	14	14	11.5
	08.03.08	10	15	14	12
	09.03.08	11	17	14	12
	10.03.08	12	17	14	12
	11.03.08	13	17	14	12
	12.03.08	14	17	14	13
	13.03.08	15	17	14	13
AD batch reactor with substrate (apple juice) content of 1% (weight %) Using inoculum pretreated by size filter (sieve pore size: 500 µm)	26.02.08	0	0	0	0
	27.02.08	1	13	12	13
	28.02.08	2	18	16	18
	29.02.08	3	19	17	20
	01.03.08	4	19	18	23
	02.03.08	5	23	20	24
	04.03.08	6	23.5	22	24.5
	05.03.08	7	23.5	23	25
	06.03.08	8	25	24.5	26
	07.03.08	9	25	24.5	26
	08.03.08	10	26	27.5	26
	09.03.08	11	28.5	28	26.5
	10.03.08	12	28.5	28	26.5
	11.03.08	13	29	28	26.5
	12.03.08	14	29	28	26.5
	13.03.08	15	29.5	28	26.5

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate content of 2% Using inoculum pretreated by size filter (sieve pore size: 500 μm)	26.02.08	0	0	0	0
	27.02.08	1	23	21	19
	28.02.08	2	35	32	29
	29.02.08	3	39	33	27
	01.03.08	4	41.5	36	30.5
	02.03.08	5	44.5	36	27.5
	04.03.08	6	48	38.5	29
	05.03.08	7	48	39	30
	06.03.08	8	48.5	40	31.5
	07.03.08	9	48.5	41.5	34.5
	08.03.08	10	48.5	42	35.5
	09.03.08	11	50.5	44	31.5
	10.03.08	12	50.5	45	36.5
	11.03.08	13	50.5	45	51.5
	12.03.08	14	50.5	45	51.5
	13.03.08	15	50.5	47	49.5
AD batch reactor with substrate (apple juice) content of 5% (weight %) Using inoculum pretreated by size filter (sieve pore size: 500 μm)	26.02.08	0	0	0	0
	27.02.08	1	28	32	24
	28.02.08	2	40	43	37
	29.02.08	3	54	57	51
	01.03.08	4	71	71	71
	02.03.08	5	82	83	81
	04.03.08	6	89	93	85
	05.03.08	7	91	95	87
	06.03.08	8	93	95	91
	07.03.08	9	94	97	91
	08.03.08	10	94	99	89
	09.03.08	11	94	100	88
	10.03.08	12	94.5	100.5	87
	11.03.08	13	94.5	101.5	98
	12.03.08	14	94.5	102	97.5
	13.03.08	15	94.5	102	97.5

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate (apple juice) content of 10% (weight %) Using inoculum pretreated by size filter (sieve pore size: 500 μm)	26.02.08	0	0	0	0
	27.02.08	1	43	45	42
	28.02.08	2	50.5	52	53
	29.02.08	3	53	54	54
	01.03.08	4	59.5	60	61
	02.03.08	5	62	60	62
	04.03.08	6	64.5	64	63
	05.03.08	7	67	68	65.5
	06.03.08	8	73.5	75	76
	07.03.08	9	82.5	83	84
	08.03.08	10	90	89	88
	09.03.08	11	92	89	88.5
	10.03.08	12	95	95	92
	11.03.08	13	97.5	98	95
	12.03.08	14	98	98	97.5
	13.03.08	15	100	101	99
AD batch reactor with substrate (apple juice) content of 2% (weight %) Micro-aeration Condition Using inoculum pretreated by size filter (sieve pore size: 500 μm)	26.02.08	0	5 (headspace)	5 (headspace)	5 (headspace)
	27.02.08	1	26	28	30
	28.02.08	2	46	49	46
	29.02.08	3	57	61	57
	01.03.08	4	67	64.5	68
	02.03.08	5	67	64.5	69
	04.03.08	6	68	65.5	70
	05.03.08	7	68	67	70
	06.03.08	8	69	67.5	70
	07.03.08	9	70	68.5	70.5
	08.03.08	10	71	69	70.5
	09.03.08	11	72	69	64
	10.03.08	12	72	71	66
	11.03.08	13	72	71	66
	12.03.08	14	72	72	67
	13.03.08	15	72	73	68

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate (apple juice) content of 2% (weight %) Micro-aeration Condition Using inoculum pretreated by size filter (sieve pore size: 500 μm)	26.02.08	0	10 (headspace)	10 (headspace)	10 (headspace)
	27.02.08	1	33	34	34
	28.02.08	2	51.5	49	50
	29.02.08	3	66.5	61	64
	01.03.08	4	78.5	74	76
	02.03.08	5	79.5	86	85
	04.03.08	6	89	98	95
	05.03.08	7	92	98.5	97
	06.03.08	8	95	99	98
	07.03.08	9	95	99	99
	08.03.08	10	95	99.5	99
	09.03.08	11	98	99.5	101
	10.03.08	12	98	99.5	101
	11.03.08	13	98	99.5	101
	12.03.08	14	98	99.5	101
	13.03.08	15	98	99.5	101
AD batch reactor with substrate (apple juice) content of 2% (weight %) Using inoculum pretreated by belt filter	26.02.08	0	0	These experimental data were provided by the 1st –year master students	
	27.02.08	1	0.3		
	28.02.08	2	7.6		
	29.02.08	3	14.6		
	01.03.08	4	19.		
	02.03.08	5	21.		
	04.03.08	6	24		
	05.03.08	7	24		
	06.03.08	8	24		
	07.03.08	9	27		
	08.03.08	10	27		
	09.03.08	11	27		
	10.03.08	12	27		
	11.03.08	13	27		
	12.03.08	14	27		
	13.03.08	15	27		

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate (apple juice) content of 5% (weight %) Using inoculum pretreated by belt filter	26.02.08	0	0	These experimental data were provided by the 1st –year master students	
	27.02.08	1	9		
	28.02.08	2	25		
	29.02.08	3	30		
	01.03.08	4	30		
	02.03.08	5	30		
	04.03.08	6	31		
	05.03.08	7	31		
	06.03.08	8	31		
	07.03.08	9	35		
	08.03.08	10	35		
	09.03.08	11	35		
	10.03.08	12	35		
	11.03.08	13	35		
	12.03.08	14	35		
	13.03.08	15	35		
AD batch reactor with substrate (apple juice) content of 2% (weight %) Using inoculum (unfilted)	26.02.08	0	0	These experimental data were provided by the 1st –year master students	
	27.02.08	1	7		
	28.02.08	2	12		
	29.02.08	3	18		
	01.03.08	4	21		
	02.03.08	5	23		
	04.03.08	6	26		
	05.03.08	7	26		
	06.03.08	8	26		
	07.03.08	9	29		
	08.03.08	10	30		
	09.03.08	11	30		
	10.03.08	12	30		
	11.03.08	13	30		
	12.03.08	14	30		
	13.03.08	15	30		

Reactor	Date	Accumulated days	Accumulated biogas yields from 3 parallels AD bioreactors		
			A	B	C
AD batch reactor with substrate (apple juice) content of 5% (weight %) Using inoculum pretreated by belt filter	26.02.08	0	0	These experimental data were provided by the 1st –year master students	
	27.02.08	1	27		
	28.02.08	2	47		
	29.02.08	3	49		
	01.03.08	4	51		
	02.03.08	5	55		
	04.03.08	6	60		
	05.03.08	7	60		
	06.03.08	8	60		
	07.03.08	9	73		
	08.03.08	10	74		
	09.03.08	11	74		
	10.03.08	12	74		
	11.03.08	13	75		
	12.03.08	14	75		
	13.03.08	15	75		

- **TS, VS, TSS, VSS of the inoculum used for the batch experiments**

- TS, VS

Sample	Parallel	Sample size [mL]	Dish [g]	Dish sample After 105°C [g]	Dish sample After 550°C [g]
Slurry from outlet of AD (unfiltered)	A	20	21.54	21.92	21.75
	B	20	20.13	20.52	20.34
Leachate from sieve filter (pore size :500 µm)	A	20	21.52	21.70	21.66
	B	20	21.50	21.66	21.63
Leachate from belt filter after AD reactor	A	20	22.08	22.19	22.13
	B	20	21.94	22.05	21.99

- TSS, VSS

Sample	Parallel	Sample size [mL]	Dish +filter paper [g]	Dish+paper with sample After 105°C [g]	Dish+paper with sample After 550°C [g]
Leachate from belt filter after AD reactor	A	50	105.81	106.05	105.94
	B	50	97.29	97.50	97.39
Sample	Parallel	Sample size [mL]	Filter paper [g]	Filter paper with sample After 105 [g]	Filter paper wth sample After 550 [g]
Slurry from outlet of AD (unfiltered)	A	50	0.50	0.96	0.69
	B	50	0.49	0.95	0.72
Leachate from sieve filter (pore size :500 µm)	A	50	0.49	0.53	0.50
	B	50	0.49	0.53	0.50
Note	Filter paper pore size: 1.25 µm				

- **Raw Data from Continuous Flow Bioreactor (CFB)**

- Raw Data from CFB using substrates without buffer solution
- COD_feed [10 g COD/L] , pH_feed=3.7

Date	Day	pH	HRT [h]	Liquid Flow [mL/h]	Gas Flow [mL/h]
10.03.08	1	3.8	8.6	31.4	4.5
11.03.08	2	3.8	8.8	30.7	4
12.03.08	3	3.8	8.7	31	6
13.03.08	4	3.7	8.9	30.3	7.5
14.03.08	5	3.8	7.9	34.2	8

Date	Day	H_2%	CO_2%	H_2 [mmol/L]	CO_2 [mmol/L]	H_2 [mgCOD/L]
10.03.08	1	64.5	35.5	3.8	2.1	66
11.03.08	2	54.8	45.2	2.9	2.4	51
12.03.08	3	45.9	54.1	3.6	4.3	63.4
13.03.08	4	46.1	53.9	4.7	5.5	81.4
14.03.08	5	28.4	71.6	2.7	6.9	47.5
Note 1	Convert H_2 [%] into H_2 [mmol/L] : $H_2\ [\text{mmol/L}] = \frac{gas\_flow\left[\frac{mL}{h}\right] \bullet H_2[\%] \bullet \frac{1}{24.4}\left[\frac{mmol}{mL}\right] \bullet \frac{1000}{1}\left[\frac{mL}{L}\right]}{liquid\_flow\left[\frac{mL}{h}\right]}$ Assumed that H_2 behaves as idea gas , using PV=nRT (T=35, p=1 atm)					
Note 2	Convert H_2 [%] into H_2 [mgCOD/L] : Using COD equivalent coefficient as 8 (Appendix 3)					

Date	Day	Acetate [mg/L]	Butyrate [mg/L]	Propionate [mg/L]	Ethanol [mg/L]
10.03.08	1	413.9	531.5	0	11.9
11.03.08	2	381.1	405.9	0	10.6
12.03.08	3	321.7	395.4	0	11.0
13.03.08	4	347.7	377.3	0	14.5
14.03.08	5	289.7	371.1	0	13

- **Raw Data from Continuous Flow Bioreactor (CFB)**

- Raw Data from CFB using substrates with buffer solution
- COD_feed [10 g COD/L] , pH_feed=8.2

Date	Day	pH	HRT [h]	Liquid Flow [mL/h]	Gas Flow [mL/h]
21.03.08	1	7.3	7.9	34.2	36.1
22.03.08	2	7.3	7.7	35.1	38
23.03.08	3	7.3	7.5	36.	38
24.03.08	4	7.1	7.5	36.	37.5
25.03.08	5	7	7.6	35.5	35

Date	Day	H_2%	CO_2%	H_2 [mmol/L]	CO_2 [mmol/L]	H_2 [mgCOD/L]
21.03.08	1	20.5	79.5	8.9	34.4	142.
22.03.08	2	20.2	79.8	9.	35.4	143.5
23.03.08	3	19.9	80.1	8.6	34.7	137.7
24.03.08	4	19	81	8.1	34.6	129.8
25.03.08	5	17.2	82.8	6.9	33.4	111.1
Note 1	Convert H_2 [%] into H_2 [mmol/L] : $H_2\ [\text{mmol/L}] = \dfrac{gas\_flow\left[\frac{mL}{h}\right] \bullet H_2[\%] \bullet \frac{1}{24.4}\left[\frac{mmol}{mL}\right] \bullet \frac{1000}{1}\left[\frac{mL}{L}\right]}{liquid\_flow\left[\frac{mL}{h}\right]}$ Assumed that H_2 behaves as idea gas , using PV=nRT (T=35, p=1 atm)					
Note 2	Convert H_2 [%] into H_2 [mgCOD/L] : Using COD equivalent coefficient as 8 (Appendix 3)					

Date	Day	Acetate [mg/L]	Butyrate [mg/L]	Propionate [mg/L]	Ethanol [mg/L]
21.03.08	1	704.5	358.1	70.4	509.7
22.03.08	2	762.2	437.3	72.2	607.5
23.03.08	3	560	349	68	352
24.03.08	4	568	356	64	345
25.03.08	5	548	361	64	348

Appendix H

Fate of Substrate COD with pH =3.7

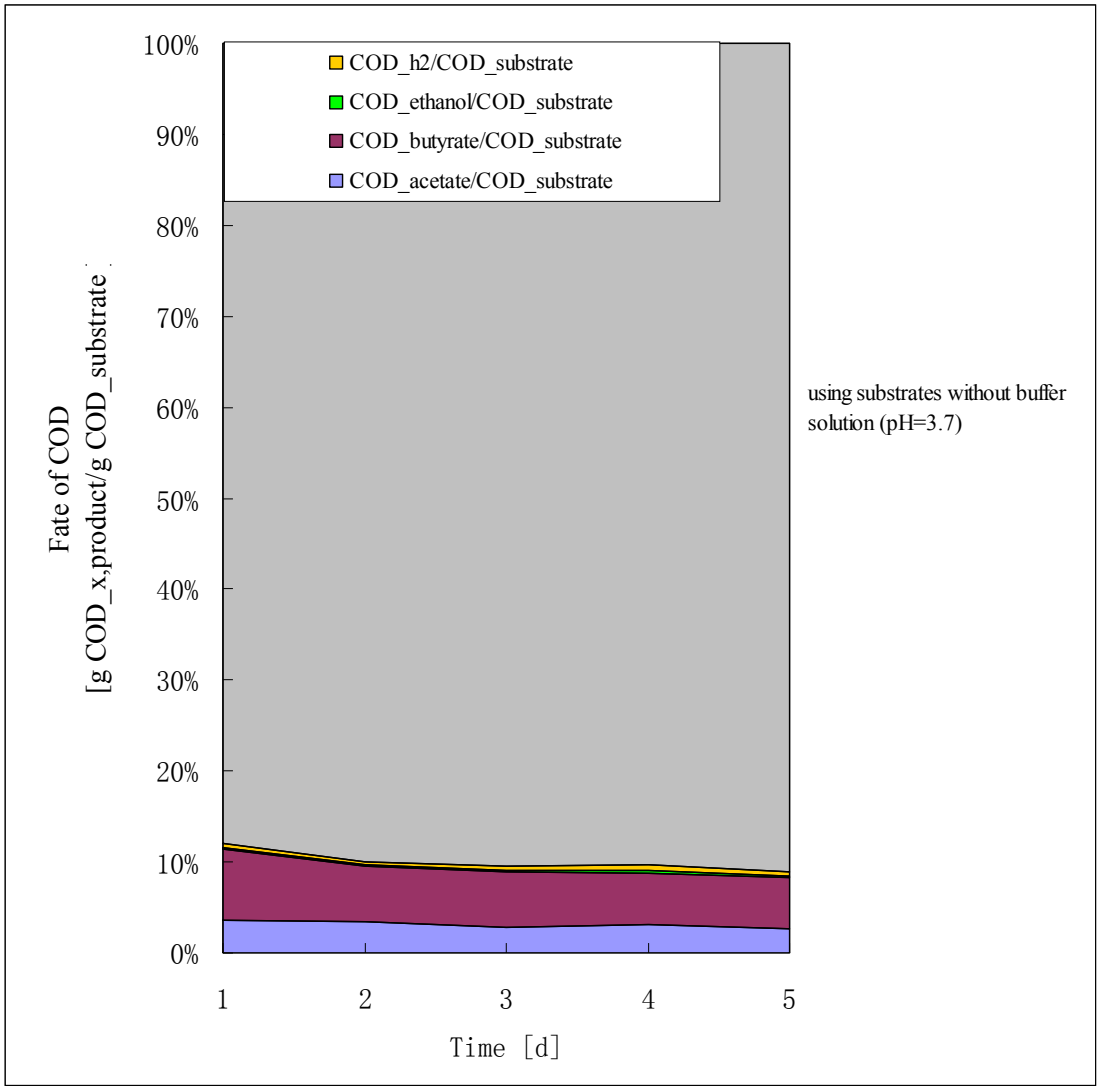

Figure Fate of substrate COD (pH =3.7)

Appendix I

Simulation Data in ADM1

AQUASIM Version 2.1d (win/mfc) - Listing of System Definition

Variables

****

COD_S: S_aa+S_ac+S_bu+S_ch4+S_fa+S_h2+S_I+S_pro+S_su+S_va

COD_Tot: COD_S+COD_X

COD_X:

X_aa+X_ac+X_c+X_c4+X_ch+X_fa+X_h2+X_I+X_li+X_pr+X_pro+X_su

C_aa: 0.03

C_ac: 2/64

C_biom: 5/160

C_bu: 4/160

C_ch4: 1/64

C_fa: 0.0217

C_li: 0.022

C_pro: 3/112

C_SI: 0.03

C_su: 6/192

C_va: 5/208

C_Xc: 0.0279

C_XI: 0.03

deltaH0_Ka_co2:7646

deltaH0_Ka_h2o:55900

deltaH0_Ka_nh4:51965

deltaH0_KH_ch4:-14240

deltaH0_KH_co2:-19410

deltaH0_KH_h2:-4180

exp_2_GAS: Real List Variable (t)

exp_5_GAS: Real List Variable (t)

exp_COD_S: Real List Variable (t)

exp_COD_tot: Real List Variable (t)

exp_gasflow: Real List Variable (t)

exp_gas_acc_1: Real List Variable (t)

exp_gas_acc_2: Real List Variable (t)

exp_gas_acc_3: Real List Variable (t)

exp_gas_acc_4: Real List Variable (t)

exp_gas_acc_5: Real List Variable (t)

exp_pH: Real List Variable (t)

exp_produced_gas_1:

Real List Variable (t)

exp_produced_gas_Blank:

Real List Variable (t)

exp_p_ch4: Real List Variable (t)

exp_p_co2: Real List Variable (t)

exp_p_h2: Real List Variable (t)

exp_S_ac: Real List Variable (t)

exp_S_hco3: Real List Variable (t)

exp_S_IN: Real List Variable (t)

exp_S_pro: Real List Variable (t)

exp_yield_1: Real List Variable (t)

exp_yield_2: Real List Variable (t)

exp_yield_3: Real List Variable (t)

exp_yield_4: Real List Variable (t)

fe: 0.5

ff: 1

f_ac_aa: 0.4

f_ac_su: 0.67*nue_1_su+0.22*nue_2_su

f_bu_aa: 0.26

f_bu_su: 0.83*nue_3_su

f_ch_xc: 0.2

f_fa_li: 0.95

f_h2_aa: 0.06

f_h2_su: 0.33*nue_1_su+0.17*nue_3_su

f_li_xc: 1-f_ch_xc-f_pr_xc-f_SI_xc-f_XI_xc

f_pro_aa: 0.05

f_pro_su: 0.78*nue_2_su

f_pr_xc: 0.2

f_SI_xc: 0.1

f_va_aa: 0.23

f_XI_xc: 0.2

gasflow:

if P_headspace<P_atm then 0 else V*(P_headspace-P_atm)/P_atm*10000 endif

gasflow1: gasflow(headspace,Bulk Volume,0)

input_Qin: 0

input_Qin_dyn: Real List Variable (t)

input_Qin_ss: 1.5

input_S_aa_in: 4.2

input_S_fa_in: 6.3

input_S_IC_in: 0.005

input_S_IN_in: 0.0035714

input_S_I_in: 0.7

input_S_su_in: 2.8

input_X_c_in: 10

input_X_I_in: 18

I_h2_c4: 1/(S_h2/KI_h2_c4+1)

I_h2_fa: 1/(S_h2/KI_h2_fa+1)

I_h2_pro: 1/(S_h2/KI_h2_pro+1)

```
I_nh3_ac:       1/(S_nh3/KI_nh3_ac+1)
I_NH_limit:     if S_IN<0 then 0 else 1/(Ks_IN/S_IN+1) endif
I_ph_ac:        if pH<I_ph_ac_ul then exp(-3*((pH-I_ph_ac_ul)/(I_ph_ac_u
                l-I_ph_ac_ll))^2) else 1 endif
I_ph_ac_ll:     6
I_ph_ac_ul:     7
I_ph_bac:       if pH<I_ph_bac_ul then exp(-3*((pH-I_ph_bac_ul)/(I_ph_ba
                c_ul-I_ph_bac_ll))^2) else 1 endif
I_ph_bac_ll:    4
I_ph_bac_ul:    5.5
I_ph_h2:        if pH<I_ph_h2_ul then exp(-3*((pH-I_ph_h2_ul)/(I_ph_h2_u
                l-I_ph_h2_ll))^2) else 1 endif
I_ph_h2_ll:     5
I_ph_h2_ul:     6
kAB_co2:        1e+014
Ka_ac:          10^(-pKa_ac)
Ka_bu:          10^(-pKa_bu)
Ka_co2:         10^(-pKa_co2)*exp(deltaH0_Ka_co2/(R*100)*(1/298-1/T))
Ka_h2o:         10^(-pKa_h2o)*exp(deltaH0_Ka_h2o/(R*100)*(1/298-1/T))
Ka_nh4:         10^(-pKa_nh3)*exp(deltaH0_Ka_nh4/(R*100)*(1/298-1/T))
Ka_pro:         10^(-pKa_pro)
Ka_va:          10^(-pKa_va)
```

kdec_xaa: 0.02

kdec_xac: 0.02

kdec_xc4: 0.02

kdec_xfa: 0.02

kdec_xh2: 0.02

kdec_xpro: 0.02

kdec_xsu: 0.02

kdis: 0.5

khyd_ch: 10

khyd_li: 10

khyd_pr: 10

KH_ch4: 0.0014*R*T*exp(deltaH0_KH_ch4/(R*100)*(1/298-1/T))

KH_co2: 0.035*R*T*exp(deltaH0_KH_co2/(R*100)*(1/298-1/T))

KH_h2: 0.00078*R*T*exp(deltaH0_KH_h2/(R*100)*(1/298-1/T))

KI_h2_c4: 1e-005

KI_h2_fa: 5e-006

KI_h2_pro: 3.5e-006

KI_nh3_ac: 0.0018

KLa: V_reactor*kLa

kLa: 200

km_aa: 50

km_ac: 8

km_c4: 20

km_fa: 6

km_h2: 35

km_pro: 13

km_su: 30

Ks_aa: 0.3

Ks_ac: 0.15

Ks_c4: 0.2

Ks_fa: 0.4

Ks_h2: 7e-006

Ks_IN: 0.0001

Ks_pro: 0.1

Ks_su: 0.5

mue_X_aa: km_aa*X_aa*S_aa/(Ks_aa+S_aa)*I_ph_bac*I_NH_limit*Y_aa

mue_X_ac:

km_ac*X_ac*S_ac/(Ks_ac+S_ac)*I_ph_ac*I_nh3_ac*I_NH_limit*Y_ac

mue_X_c4:

km_c4*X_c4*S_bu/(Ks_c4+S_bu)*S_bu/(S_bu+S_va+0.1)*I_ph_bac*I_h2_c4*I_NH_limit*Y_c4+km_c4*X_c4*S_va/(Ks_c4+S_va)*S_va/(S_va+S_bu+0.1)*I_ph_bac*I_h2_c4*I_NH_limit*Y_c4

mue_X_fa: km_fa*X_fa*S_fa/(Ks_fa+S_fa)*I_ph_bac*I_h2_c4*Y_fa

mue_X_h2: km_h2*X_h2*S_h2/(Ks_h2+S_h2)*I_ph_h2*I_NH_limit*Y_h2

mue_X_pro:

km_pro*X_pro*S_pro/(Ks_pro+S_pro)*I_ph_bac*I_h2_pro*I_NH_limit*Y_h2

mue_X_su: km_su*X_su*S_su/(Ks_su+S_su)*I_ph_bac*I_NH_limit*Y_su

nue_1_su: 0.495

nue_2_su: 0.345

nue_3_su: 1-nue_1_su-nue_2_su

N_aa: 0.007

N_biom: 0.00625

N_SI: 0.002

N_Xc: 0.002

N_XI: 0.002

pH: -log10(S_h_ion)

pH_reactor: pH(reactor,Bulk Volume,0)

pKa_ac: 4.76

pKa_bu: 4.84

pKa_co2: 6.35

pKa_h2o: 14

pKa_nh3: 9.25

pKa_pro: 4.88

pKa_va: 4.8

P_atm: 1.013

p_ch4: S_ch4/64*R*T

p_ch4_adjust: p_ch4/P_headspace*100

p_co2: S_co2*R*T

p_co2_adjust: p_co2/P_headspace*100

p_h2: S_h2/16*R*T

p_h2o: 0.0313*exp(5290*(1/298-1/T))

p_h2_adjust: p_h2/P_headspace*100

P_headspace: p_co2+p_h2+p_ch4+p_h2o

Qout: Discharge

R: 0.08314

S_aa: Dyn. Volume State Var.

S_ac: Dyn. Volume State Var.

S_ac_ion: Eq. State Variable

S_an: Dyn. Volume State Var.

S_bu: Dyn. Volume State Var.

S_bu_ion: Eq. State Variable

S_cat: Dyn. Volume State Var.

S_ch4: Dyn. Volume State Var.

S_co2: Dyn. Volume State Var.

S_fa: Dyn. Volume State Var.

S_h2: Dyn. Volume State Var.

S_hco3_ion: Dyn. Volume State Var.

S_h_ion: Eq. State Variable

S_I:	Dyn. Volume State Var.
S_IN:	Dyn. Volume State Var.
S_nh3:	Eq. State Variable
S_nh4_ion:	Eq. State Variable
S_oh_ion:	Eq. State Variable
S_pro:	Dyn. Volume State Var.
S_pro_ion:	Eq. State Variable
S_su:	Dyn. Volume State Var.
S_va:	Dyn. Volume State Var.
S_va_ion:	Eq. State Variable
T:	308
t:	Time
tres_x:	0
V:	Reactor Volume
V2:	Bulk Volume
V_headspace:	V(headspace,Bulk Volume,0)
V_reactor:	V(reactor,Bulk Volume,0)
X_aa:	Dyn. Volume State Var.
X_ac:	Dyn. Volume State Var.
X_c:	Dyn. Volume State Var.
X_c4:	Dyn. Volume State Var.
X_ch:	Dyn. Volume State Var.

X_fa: Dyn. Volume State Var.

X_h2: Dyn. Volume State Var.

X_I: Dyn. Volume State Var.

X_li: Dyn. Volume State Var.

X_pr: Dyn. Volume State Var.

X_pro: Dyn. Volume State Var.

X_su: Dyn. Volume State Var.

Y_aa: 0.08

Y_ac: 0.05

Y_c4: 0.06

Y_fa: 0.06

Y_h2: 0.06

Y_pro: 0.04

Y_su: 0.1

****

Processes

****

decay_aa: kdec_xaa*X_aa

X_c : 1

X_aa : -1

S_co2 : C_biom-C_Xc

S_IN : N_biom-N_Xc

```
decay_ac:      X_ac*kdec_xac
                  X_ac : -1
                  X_c : 1
                  S_co2 : C_biom-C_Xc
                  S_IN : N_biom-N_Xc
decay_c4:      X_c4*kdec_xc4
                  X_c4 : -1
                  X_c : 1
                  S_co2 : C_biom-C_Xc
                  S_IN : N_biom-N_Xc
decay_fa:      X_fa*kdec_xfa
                  X_fa : -1
                  X_c : 1
                  S_co2 : C_biom-C_Xc
                  S_IN : N_biom-N_Xc
decay_h2:      X_h2*kdec_xh2
                  X_c : 1
                  X_h2 : -1
                  S_co2 : C_biom-C_Xc
                  S_IN : N_biom-N_Xc
decay_pro:     X_pro*kdec_xpro
                  X_pro : -1
```

X_c : 1

S_co2 : C_biom-C_Xc

S_IN : N_biom-N_Xc

decay_su: X_su*kdec_xsu

X_su : -1

X_c : 1

S_co2 : C_biom-C_Xc

S_IN : N_biom-N_Xc

disintegration:kdis*X_c

X_c : -1

X_ch : f_ch_xc

S_I : f_SI_xc

X_pr : f_pr_xc

X_I : f_XI_xc

X_li : f_li_xc

S_IN : N_Xc-f_XI_xc*N_XI-f_SI_xc*N_SI-f_pr_xc*N_aa

S_co2 :

C_Xc-f_XI_xc*C_XI-f_SI_xc*C_SI-f_pr_xc*C_aa-f_

ch_xc*C_su-f_li_xc*C_li

dyn_acid_base_co2:

kAB_co2*(S_hco3_ion*S_h_ion-Ka_co2*S_co2)

S_co2 : 1

```
                    S_hco3_ion : -1
equilib_ac:     S_ac_ion : 0 = Ka_ac*S_ac-(Ka_ac+S_h_ion)*S_ac_ion
equilib_bu:     S_bu_ion : 0 = Ka_bu*S_bu-(Ka_bu+S_h_ion)*S_bu_ion
equilib_charge:S_h_ion:
0=S_h_ion+S_cat-S_an-S_oh_ion-S_hco3_ion+S_nh4_ion-S_ac_ion/64-S_pro_
ion/112-S_bu_ion/160-S_va_ion/208
equilib_h2o:    S_oh_ion : 0 = S_oh_ion-Ka_h2o/S_h_ion
equilib_IN:     S_nh4_ion : 0 = S_IN*S_h_ion-(Ka_nh4+S_h_ion)*S_nh4_ion
equilib_IN_bal:S_nh3 : 0 = S_nh3+S_nh4_ion-S_IN
equilib_prop:   S_pro_ion : 0 = Ka_pro*S_pro-(Ka_pro+S_h_ion)*S_pro_ion
equilib_va:     S_va_ion : 0 = Ka_va*S_va-(Ka_va+S_h_ion)*S_va_ion
hyd_ch:         khyd_ch*X_ch
                    S_su : 1
                    X_ch : -1
hyd_li:         khyd_li*X_li
                    S_su : (1-f_fa_li)
                    S_fa : f_fa_li
                    X_li : -1
hyd_pr:         khyd_pr*X_pr
                    S_aa : 1
                    X_pr : -1
uptake_aa:      km_aa*X_aa*S_aa/(Ks_aa+S_aa)*I_ph_bac*I_NH_limit
```

S_h2 : (1-Y_aa)*f_h2_aa

S_co2 :

C_aa-(1-Y_aa)*f_ac_aa*C_ac-(1-Y_aa)*f_bu_aa*C_bu-(1-Y_aa)*f_pro_aa*C_

pro-(1-Y_aa)*f_va_aa*C_va-Y_aa*C_biom

S_ac : (1-Y_aa)*f_ac_aa

S_bu : (1-Y_aa)*f_bu_aa

S_aa : -1

S_pro : (1-Y_aa)*f_pro_aa

S_va : (1-Y_aa)*f_va_aa

S_IN : N_aa-Y_aa*N_biom

X_aa : Y_aa

uptake_ac:

km_ac*X_ac*S_ac/(Ks_ac+S_ac)*I_ph_ac*I_nh3_ac*I_NH_limit

S_ac : -1

X_ac : Y_ac

S_IN : -(N_biom)*Y_ac

S_ch4 : (1-Y_ac)

S_co2 : C_ac-Y_ac*C_biom-(1-Y_ac)*C_ch4

uptake_bu:

km_c4*X_c4*S_bu/(Ks_c4+S_bu)*1/(1+S_va/S_bu)*I_ph_bac*I_h2_c4*I_NH_limit

S_h2 : (1-Y_c4)*0.2

S_ac : (1-Y_c4)*0.8

X_c4 : Y_c4

S_IN : -(N_biom)*Y_c4

S_bu : -1

uptake_fa:

km_fa*X_fa*S_fa/(Ks_fa+S_fa)*I_ph_bac*I_h2_fa*I_NH_limit

S_h2 : (1-Y_fa)*0.3

S_ac : (1-Y_fa)*0.7

X_fa : Y_fa

S_IN : -(N_biom)*Y_fa

S_fa : -1

uptake_h2: km_h2*X_h2*S_h2/(Ks_h2+S_h2)*I_ph_h2*I_NH_limit

S_h2 : -1

X_h2 : Y_h2

S_IN : -(N_biom)*Y_h2

S_ch4 : (1-Y_h2)

S_co2 : -Y_h2*C_biom-(1-Y_h2)*C_ch4

uptake_pro:

km_pro*X_pro*S_pro/(Ks_pro+S_pro)*I_ph_bac*I_h2_pro*I_NH_limit

S_h2 : (1-Y_pro)*0.43

S_ac : (1-Y_pro)*0.57

X_pro : Y_pro

S_IN : -(N_biom)*Y_pro

S_pro : -1

S_co2 : C_pro-(1-Y_pro)*0.57*C_ac-Y_pro*C_biom

uptake_su: km_su*X_su*S_su/(Ks_su+S_su)*I_ph_bac*I_NH_limit

S_h2 : (1-Y_su)*(f_h2_su)

S_co2 :

C_su-(1-Y_su)*(f_ac_su)*C_ac-(1-Y_su)*f_pro_su

*C_pro-(1-Y_su)*f_bu_su*C_bu-Y_su*C_biom

S_ac : (1-Y_su)*(f_ac_su)

X_su : Y_su

S_IN : -(N_biom)*Y_su

S_su : -1

S_bu : (1-Y_su)*f_bu_su

S_pro : (1-Y_su)*f_pro_su

uptake_va:

km_c4*X_c4*S_va/(Ks_c4+S_va)*1/(1+S_bu/S_va)*I_ph_bac*I_h2_c4*I_NH_limit

S_h2 : (1-Y_c4)*0.15

S_ac : (1-Y_c4)*0.31

X_c4 : Y_c4

S_IN : -(N_biom)*Y_c4

S_va : -1

S_pro : (1-Y_c4)*0.54

Compartments

****

headspace: Mixed Reactor Compartment

Active Variables: S_ch4, S_co2, S_h2

Active Processes:

outlet: Mixed Reactor Compartment

Active Variables:

S_aa, S_ac, S_bu, S_ch4, S_co2, S_fa, S_h2, S_hco3_ion, S_I, S_IN, S_pro, S_su, S_va, X_aa, X_ac, X_c, X_c4, X_ch, X_fa, X_h2, X_I, X_li,X_ pr, X_pro, X_su

Active Processes:

reactor: Mixed Reactor Compartment

Active Variables:

S_ac_ion, S_bu_ion, S_cat, S_h_ion, S_nh3, S_nh4_ion, S_oh_ion, S_pro_ion, S_va_ion, S_aa, S_ac, S_bu, S_ch4, S_co2, S_fa, S_h2, S_hco3_ion,S_I, S_IN, S_pro, S_su, S_va, X_aa, X_ac, X_c, X_c4, X_ch, X_fa, X_h2, X_I, X_li, X_pr, X_pro, X_su, S_an

Active Processes:

dyn_acid_base_co2, decay_aa, decay_ac, decay_c4, decay_h2, decay_fa, decay_pro, decay_su, equilib_ac, equilib_bu, equilib_charge, equilib_h2o, equilib_IN, equilib_IN_bal, equilib_prop, equilib_va, hyd_ch, hyd_li, hyd_pr, uptake_aa, uptake_ac, uptake_bu, uptake_h2,

```
uptake_fa, uptake_pro, uptake_su, uptake_va, disintegration
*******************************************************************
Links
*******************************************************************
Effluent:       reactor -> outlet
                   recirculation: -> reactor
gas_trans:      headspace <-> reactor
```