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APPENDIX

Appendix A Gas Chromatograph's Calibration Curves

Table A1 Gas chromatograph's calibration curves for hydrogen (H_2)

Volume of Hydrogen (mL)	Peak Area
0.02	16,313
0.04	58,770
0.06	131,648
0.08	180,674
0.1	226,743
0.2	427,198
0.3	610,005
0.4	778,509

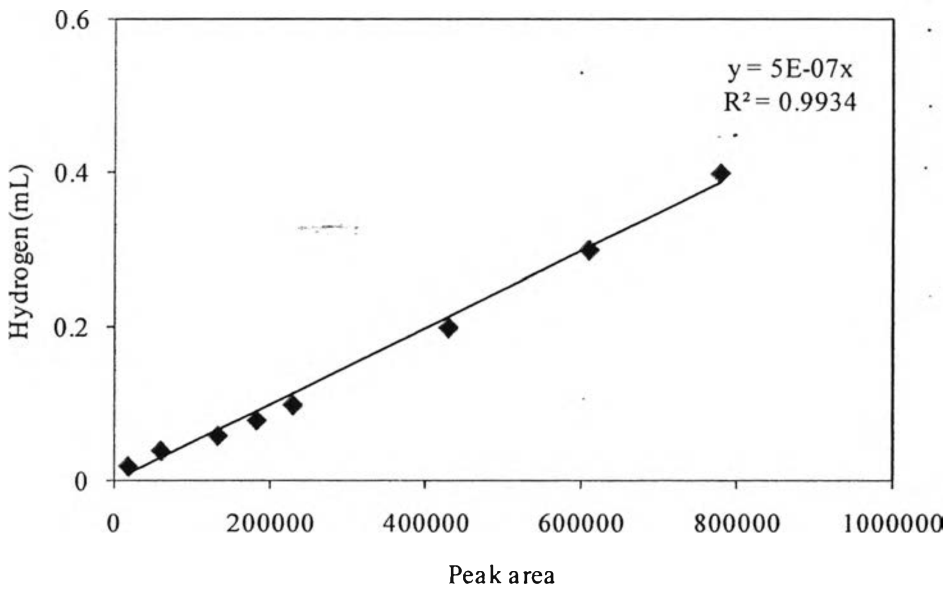


Figure A1 The relationship between amount of hydrogen (H_2) and peak area

Equation

$$\text{Amount of hydrogen} = 5\text{x}10^{-7} \text{ x Peak area}$$

Table A2 Gas chromatograph's calibration curves for air

Volume of Air (mL)	Peak Area
0.02	1,432
0.04	7,707
0.06	13,669
0.08	18,452
0.1	22,099
0.2	46,709
0.3	68,207
0.4	89,088

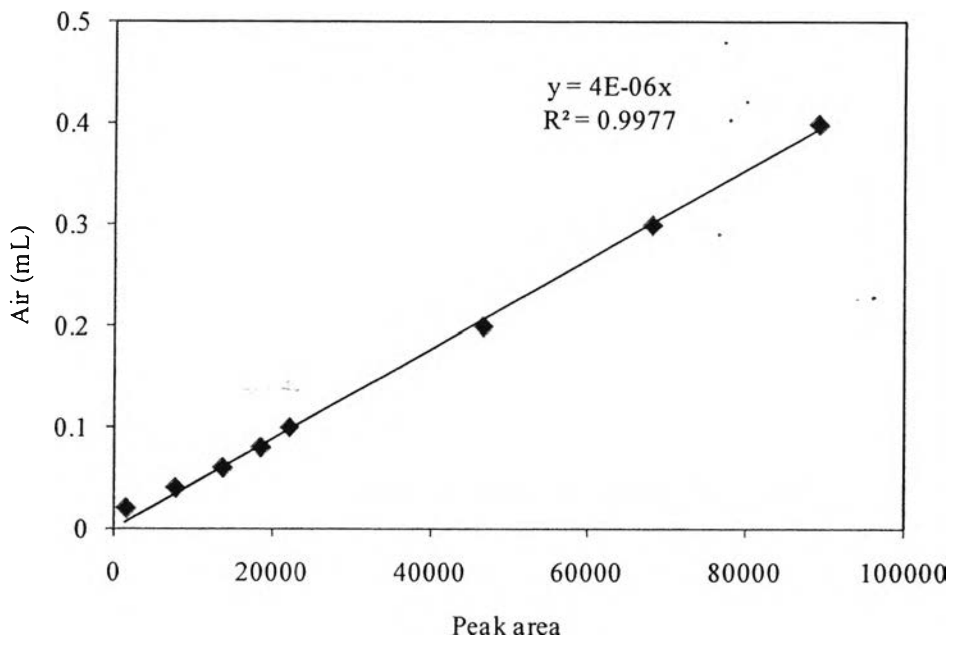


Figure A2 The relationship between amount of air and peak area

Equation

$$\text{Amount of air} = 4 \times 10^{-6} \times \text{Peak area}$$

Table A3 Gas chromatograph's calibration curves for methane (CH_4)

Volume of Methane (mL)	Peak Area
0.02	3,054
0.04	15,913
0.06	34,947
0.08	48,603
0.1	61,353
0.2	122,735
0.3	175,667
0.4	222,837

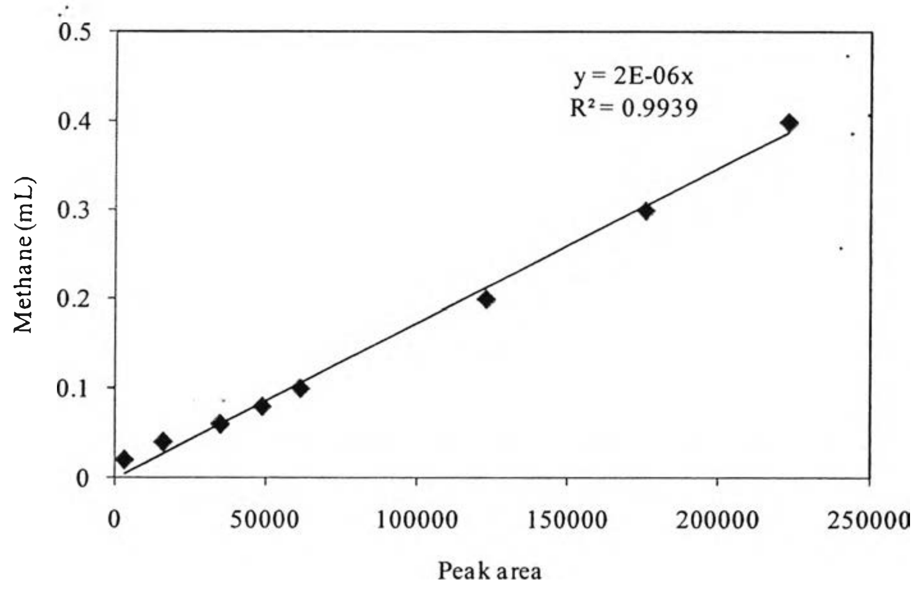


Figure A3 The relationship between amount of methane (CH_4) and peak area

Equation

$$\text{Amount of methane} = 2\times10^{-6} \times \text{Peak area}$$

Table A4 Gas chromatograph's calibration curves for carbon dioxide (CO_2)

Volume of Carbon Dioxide (mL)	Peak Area
0.02	1,184
0.04	5,078
0.06	9,486
0.08	13,382
0.1	17,500
0.2	37,803
0.3	55,725
0.4	72,322

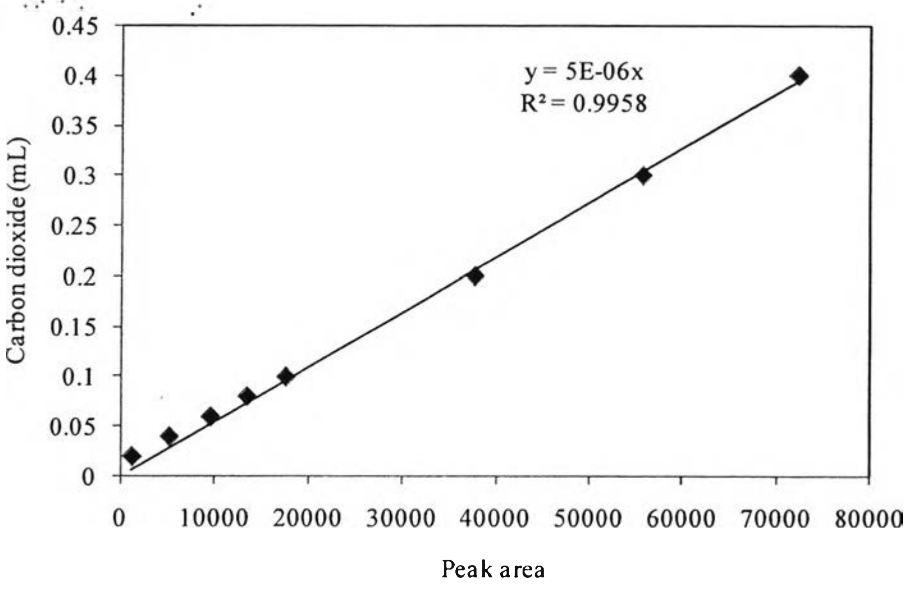


Figure A4 The relationship between amount of carbon dioxide (CO_2) and peak area

Equation

Amount of carbon dioxide = 5×10^{-6} x Peak area

Table A5 Gas chromatograph's calibration curves for acetic acid

Concentration (ppm)	Peak area of acetic acid / Peak area of n-propanol
800	0.870
1,600	1.716
2,400	2.817
3,200	3.622

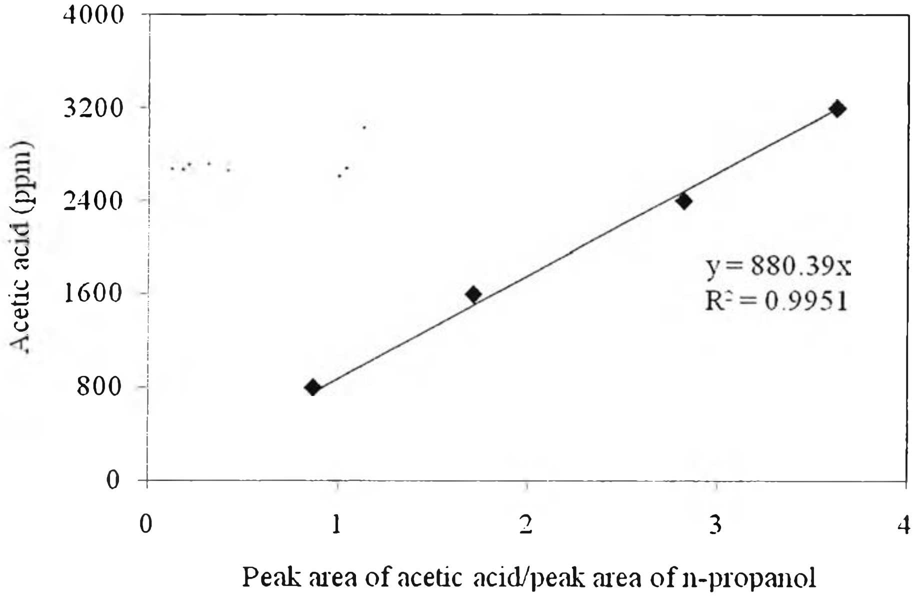


Figure A5 The relationship between concentration of acetic acid and ratio of peak area

Equation

Concentration of acetic acid (ppm) = 880.39 x Peak area ratio

Table A6 Gas chromatograph's calibration curves for valeric acid

Concentration (ppm)	$\frac{\text{Peak area of valeric acid}}{\text{Peak area of n-propanol}}$
800	1.389
1,600	2.631
2,400	4.306
3,200	5.563

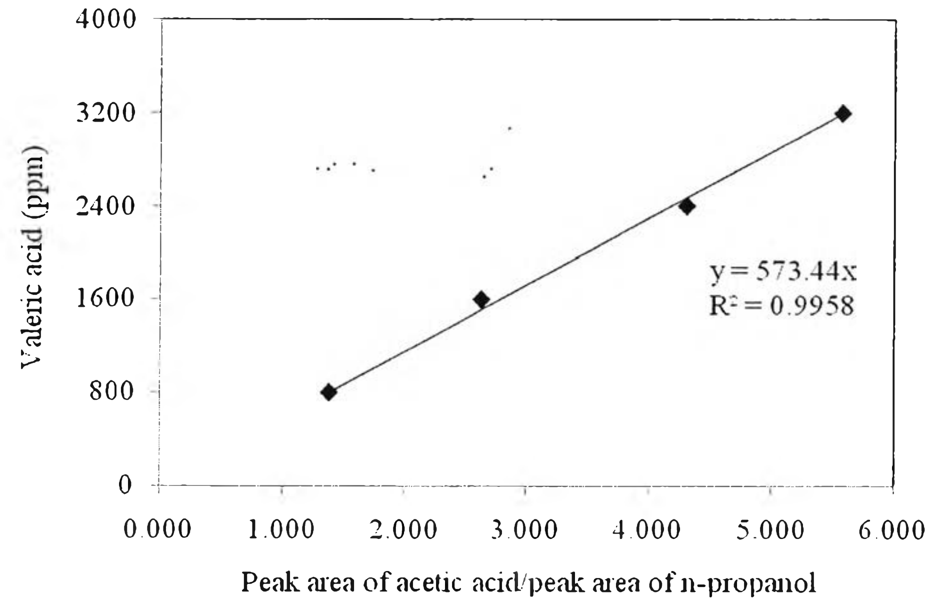


Figure A6 The relationship between concentration of valeric acid and ratio of peak area

Equation

Concentration of valeric acid (ppm) = 573.44 x Peak area ratio

Table A7 Gas chromatograph's calibration curves for propionic acid

Concentration (ppm)	Peak area of propionic acid / Peak area of n-propanol
800	1.781
1,600	3.203
2,400	5.096
3,200	6.725

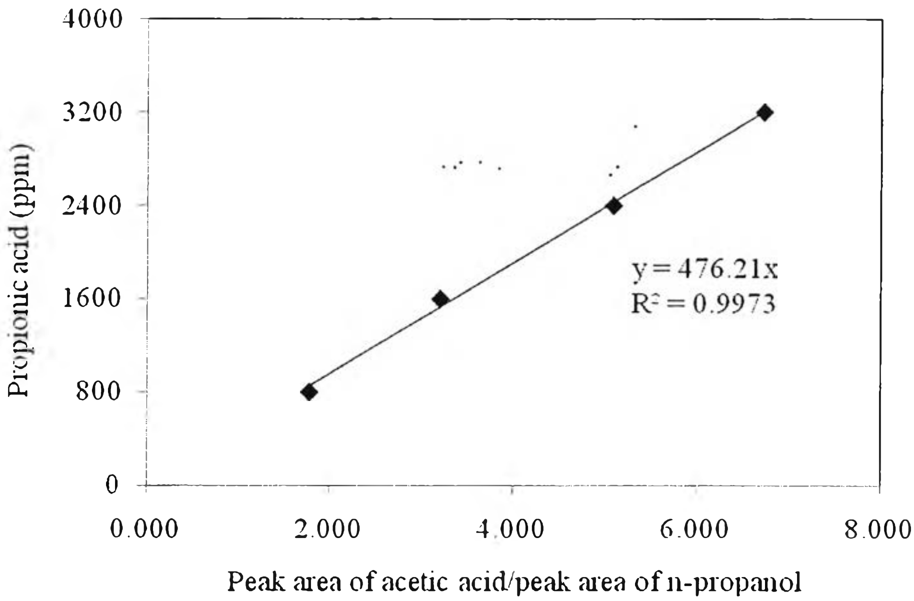


Figure A7 The relationship between concentration of propionic acid and ratio of peak area

Equation

Concentration of propionic acid (ppm) = 476.21 x Peak area ratio

Table A8 Gas chromatograph's calibration curves for butyric acid

Concentration (ppm)	Peak area of butyric acid / Peak area of n-propanol
800	1.860
1,600	3.507
2,400	5.700
3,200	7.364

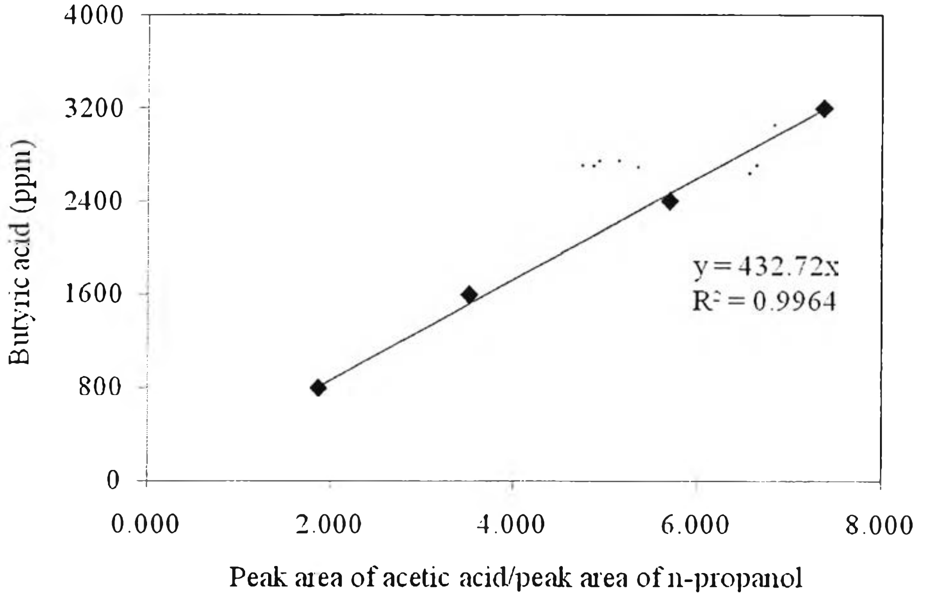


Figure A8 The relationship between concentration of butyric acid and ratio of peak area

Equation

Concentration of butyric acid (ppm) = 432.72 x Peak area ratio

Table A9 Gas chromatograph's calibration curves for ethanol

Concentration (ppm)	$\frac{\text{Peak area of ethanol}}{\text{Peak area of n-propanol}}$
800	0.986
1,600	1.928
2,400	2.926
3,200	3.947

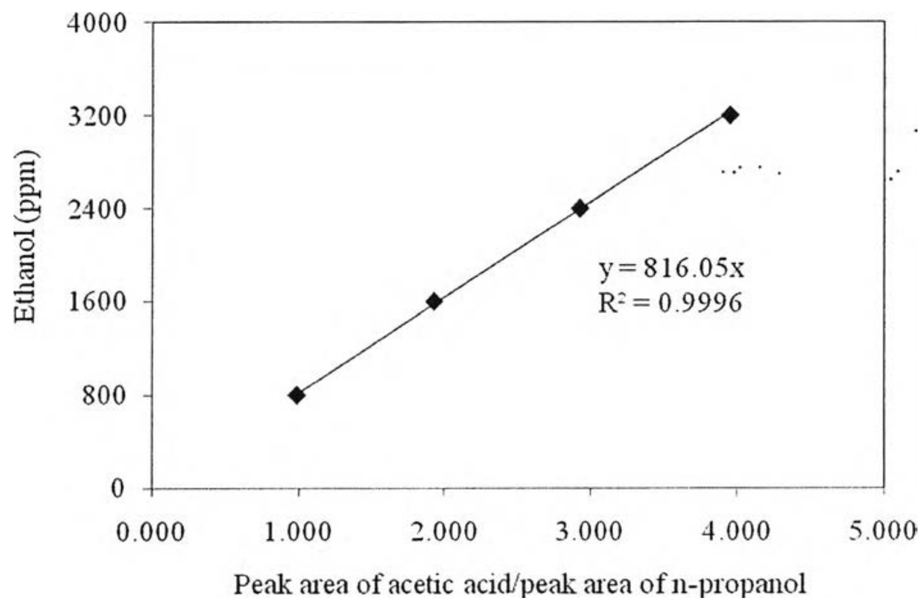


Figure A9 The relationship between concentration of ethanol and ratio of peak area

Equation

Concentration of ethanol (ppm) = 816.05 x Peak area ratio

Table A10 Gas chromatograph's calibration curves for butanol

Concentration (ppm)	Peak area of butanol / Peak area of n-propanol
800	1.474
1,600	2.954
2,400	4.429
3,200	6.083

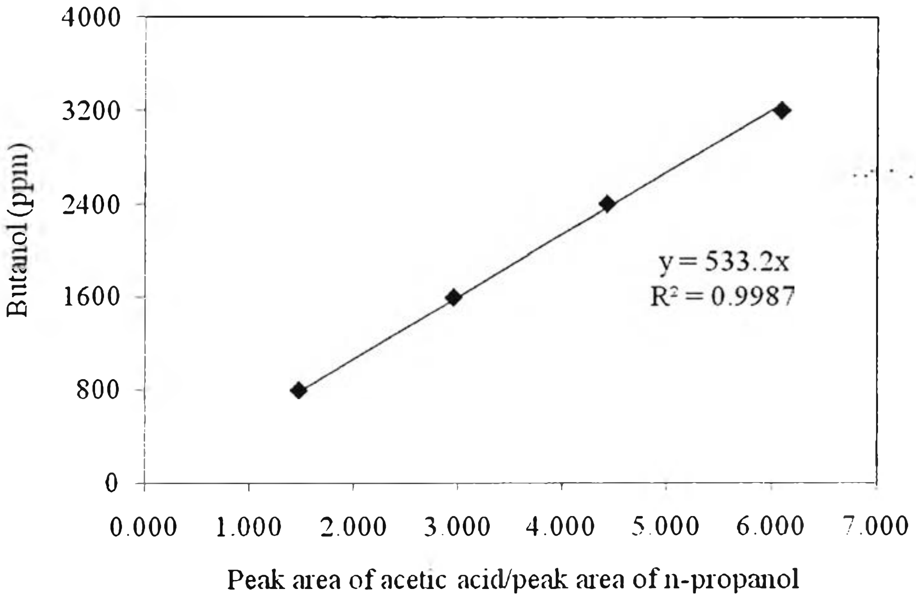


Figure A10 The relationship between concentration of butanol and ratio of peak area

Equation

Concentration of butanol (ppm) = 533.2 x Peak area ratio

Appendix B Feedstock and Seed Sludge Preparation

B 1. Feedstock Preparation

Example: prepare feedstock at COD loading of 40 kg m^{-3} d^{-1} or 40 g L^{-1} d^{-1}

	$C_6H_{12}O_6$	+	$6O_2$	→	$6CO_2$	+	$6H_2O$
(MW)	180		6(32)				
(Mass)	180 g		192 g				

For COD loading of 40 g L^{-1}

(180/192) x 40	(192/192) x 40
37.5 g	40 g

37.5 g of glucose is required to prepare feedstock at COD loading of 40 kg m^{-3} d^{-1}

Amount of glucose used for 4L of operation volume = 37.5 x 4 = 150 g / 4 L of water

B 2. Seed Sludge Preparation

Determine the amount of total suspended solid in seed sludge

From Equation 3.2

$$\frac{\text{mg total suspended solid}}{\text{L}} = \frac{(A - B) \times 100}{\text{sample volume, cm}^3}$$

Sample volume	= 5	mL
Weight of filter disk (B)	= 120.9	mg
Weight of filter disk + dried residual at 105°C (A)	= 299.0	mg

$$TSS = \frac{(299.0 - 120.9) \times 100}{5}$$

$$TSS = 35{,}622 \text{ mg L}^{-1}$$

Determine the volume of start-up seed sludge

For preparation of 0.5% (Weight/Volume) of start-up seed sludge

0.5% (Weight/Volume) = 0.5 g/100 mL

Volume of start-up seed sludge required for 4 L of operation volume

$$N_1V_1 = N_2V_2$$

$$0.035622 \times V1 = 0.005 \times 4$$

$$V1 = 0.561 \text{ L}$$

Appendix C Preparation of NaOH and H_2SO_4 Solution for pH Control System

C 1. Preparation of NaOH 1M

Concentration of NaOH (solid) = 99%

Molecular weight of NaOH = 40

Preparation of NaOH at concentration of 1 M

$$= \frac{1 \text{ mol}}{1 \text{ L}} \times \frac{40 \text{ g}}{1 \text{ mol}} \times \frac{100}{99} = 40.40 \text{ g}$$

C 2. Preparation of H_2SO_4 1M

Concentration of H_2SO_4 = 98%

Density of H_2SO_4 = 1.84 g mL^{-1}

Molecular weight of H_2SO_4 = 98

Determination of fresh HCl concentration in term of molar

$$= \frac{0.98 \text{ L of } H_2SO_4}{1 \text{ L of solution}} \times \frac{1.84 \text{ g of } H_2SO_4}{1 \text{ mL of } H_2SO_4} \times \frac{1 \text{ mol of } H_2SO_4}{98 \text{ g of } H_2SO_4} = 18.40 \text{ M}$$

Preparation of H_2SO_4 at concentration of 1 M

$$N_1V_1 = N_2V_2$$

$$V_1 = (1 \times 1)/18.40$$

$$= 0.05435 \text{ L}$$

$$= 54.35 \text{ mL}$$

Appendix D Volatile Fatty Acids (VFA) Quantification by Distillation Method

D 1. Acetic Acids Stock Solution Preparation for Recovery Factor (f) Determination

Concentration of fresh acetic acid (liquid)	=	99.7%
Density of acetic acid	=	1.07 g mL^{-1}
Molecular weight of acetic acid	=	60

Determination of fresh acetic acids concentration in term of molar

$$= \frac{0.997 \text{ L of acetic acid}}{\text{L of solution}} \times \frac{1.07 \text{ g of acetic acid}}{\text{mL of acetic acid}} \times \frac{1 \text{ mol of acetic acid}}{60 \text{ g of acetic acid}}$$

= 17.78 M

Preparation of acetic acid at concentration of 2,000 mg L^{-1}

$$= 2{,}000 \frac{\text{mg of acetic acid}}{\text{L of solution}} \times \frac{1 \text{ mole of acetic acid}}{60 \text{ g of acetic acid}}$$

= 0.0333 M

- Dilution of acetic acid

$$N_1V_1 = N_2V_2$$

$$V_1 = N_2V_2/N_1$$

$$= (0.0333x1)/17.78$$

$$= 1.873x10^{-3} \quad L$$

D 2. Standard Sodium Hydroxide (0.1) Preparation

Concentration of fresh NaOH (solid)	=	99%
Molecular weight of acetic acid	=	40

Preparation of acetic acid at concentration of 0.1 M

$$= \frac{0.1 \text{ mol}}{1 \text{ L}} \times \frac{40 \text{ g}}{1 \text{ mol}} \times \frac{100}{99}$$

= 4.04 g

D 3. Recovery Factor (f) Determination

Distill 150 mL of 0.0333 M of acetic acid in distillation apparatus

Calculate the recovery factor

$$f = \frac{a}{b}$$

where

a = volatile acid concentration recovered in distillate, mg L^{-1}

b = volatile acid concentration in standard solution used, mg L^{-1}

Find volatile acid concentration recovered in distillate by titration with 0.1 M of NaOH (MW of acetic acid = 60.5)

1) Distillate 50 mL NaOH 11.7 mL

Used NaOH	=	$11.7 \times 10^{-3} \times 0.1$	
	=	1.17×10^{-3}	mol
Acetic acid in distillate	=	1.17×10^{-3}	mol
	=	$1.17 \times 10^{-3} \times 60.5$	
	=	0.07	g

Concentration of acetic acid in distillate

	=	0.07/50	
	=	1.405×10^{-3}	g mL^{-1}
	=	1,405	mg L^{-1}

2) Distillate 25 mL NaOH 5.7 mL

Used NaOH	=	$5.7 \times 10^{-3} \times 0.1$	
	=	5.7×10^{-4} mol	
Acetic acid in distillate	=	5.7×10^{-4} mol	
	=	$5.7 \times 10^{-4} \times 60.5$	
	=	0.034	g

Concentration of acetic acid in distillate

	=	0.034/25	
	=	1.368×10^{-3}	g mL^{-1}
	=	1,368	mg L^{-1}
Average	=	1,387	mg L^{-1}

Recovery factor (f) = 1,387/2,000

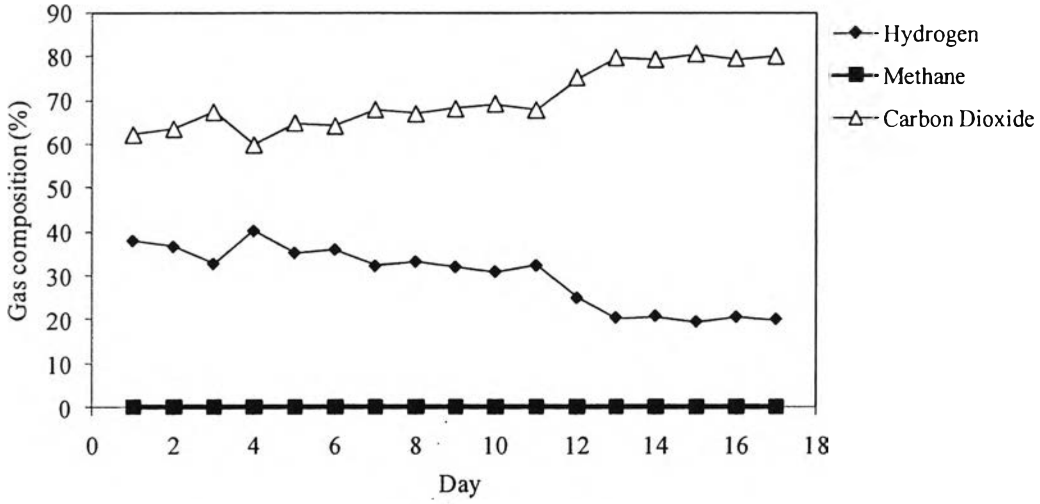
= 0.6935

Appendix E Raw Data of The Effect of COD Loading Rate under System with pH Control

COD loading rate 10 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0360	-	0.0589	0.0949	37.93	-	62.07
2	0.0342	-	0.0593	0.0935	36.58	-	63.42
3	0.0315	-	0.0648	0.0963	32.71	-	67.29
4	0.0409	-	0.0609	0.1018	40.18	-	59.82
5	0.0352	-	0.0648	0.1000	35.20	-	64.80
6	0.0361	-	0.0645	0.1006	35.88	-	64.12
7	0.0309	-	0.0652	0.0961	32.15	-	67.85
8	0.0314	-	0.0634	0.0948	33.12	-	66.88
9	0.0306	-	0.0651	0.0957	31.97	-	68.03
10	0.0294	-	0.0659	0.0953	30.85	-	69.15
11	0.0308	-	0.0646	0.0954	32.29	-	67.71
12	0.0241	-	0.0729	0.0970	24.85	-	75.15
13	0.0220	-	0.0862	0.1082	20.33	-	79.67
14	0.0228	-	0.0871	0.1099	20.75	-	79.25
15	0.0206	-	0.0855	0.1061	19.42	-	80.58
16	0.0218	-	0.0846	0.1064	20.49	-	79.51
17	0.0212	-	0.0848	0.1060	20.00		80.00



Gas production rate	=	0.41	$L\ h^{-1}$
Hydrogen production rate	=	0.08	$L\ h^{-1}$
Specific hydrogen production rate	=	0.48	L H_2/L d
Production of VFA	=	5,287	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.386	mol H_2/mol glucose consumed
% Glucose removal	=	98.86	%
% COD removal	=	66.28	%
VSS	=	848	mg/L

Volume of injection = 910 μl

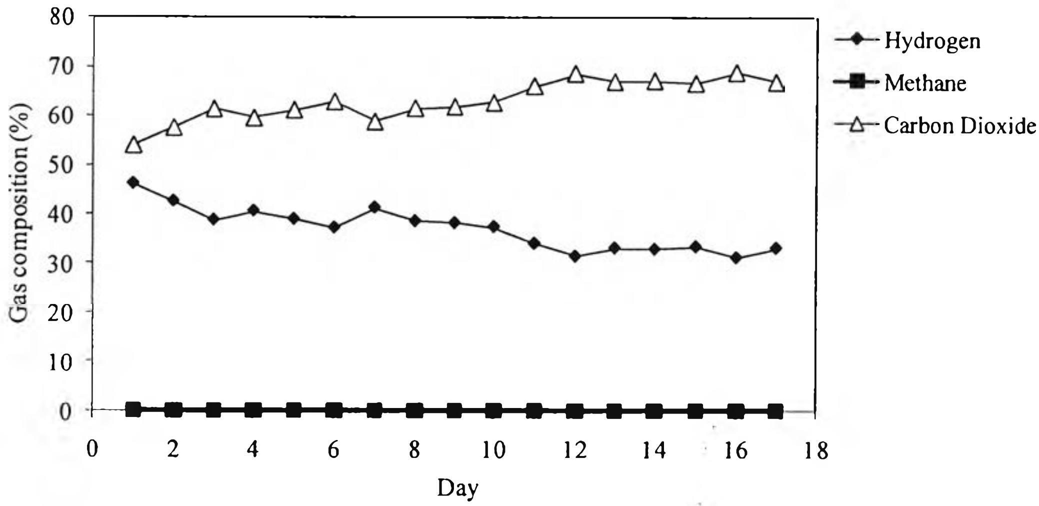
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	842.54	4.53
Butanol	0.00	0.00
Acetic acid	2,246.24	42.80
Propionic acid	397.13	5.25
Butyric acid	2,228.97	41.38
Valeric acid	776.72	6.04

COD loading rate 20 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0475	-	0.0549	0.1024	46.11	-	53.89
2	0.0442	-	0.0593	0.1035	42.59	-	57.41
3	0.0375	-	0.0598	0.0973	38.82	-	61.18
4	0.0409	-	0.0609	0.1018	40.65	-	59.35
5	0.0352	-	0.0648	0.1000	39.02	-	60.98
6	0.0361	-	0.0645	0.1006	37.30	-	62.70
7	0.0309	-	0.0652	0.0961	41.34	-	58.66
8	0.0314	-	0.0634	0.0948	38.61	-	61.39
9	0.0306	-	0.0651	0.0957	38.26	-	61.74
10	0.0294	-	0.0659	0.0953	37.48	-	62.52
11	0.0308	-	0.0646	0.0954	34.14	-	65.86
12	0.0241	-	0.0600	0.0968	31.61	-	68.39
13	0.0220	-	0.0862	0.1082	33.25	-	66.75
14	0.0228	-	0.0871	0.1099	33.04	-	66.96
15	0.0206	-	0.0855	0.1061	33.55	-	66.45
16	0.0218	-	0.0846	0.1064	31.37	-	68.63
17	0.0212	-	0.0848	0.1060	33.29		66.71



Gas production rate	=	1.22	L h^{-1}
Hydrogen production rate	=	0.41	L h^{-1}
Specific hydrogen production rate	=	2.46	L H_2/L d
Production of VFA	=	10,555	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.957	mol H_2/mol glucose consumed
% Glucose removal	=	98.55	%
% COD removal	=	74.46	%
VSS	=	855	mg/L

Volume of injection = 942 μl

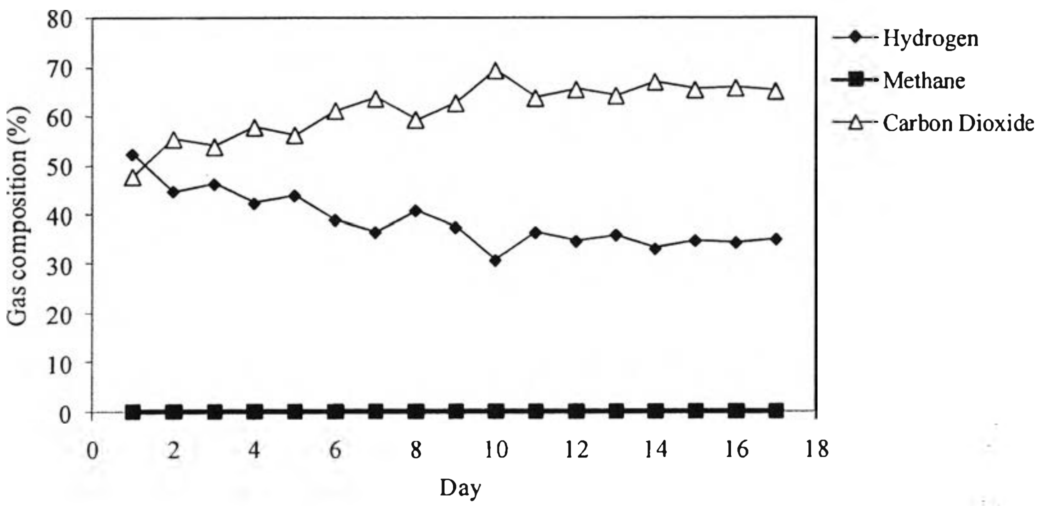
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	655.11	9.62
Butanol	0.00	0.00
Acetic acid	2,826.13	39.87
Propionic acid	395.17	4.83
Butyric acid	2,993.59	38.80
Valeric acid	669.26	6.89

COD loading rate 30 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0360	-	0.0589	0.0949	52.27	-	47.73
2	0.0342	-	0.0593	0.0935	44.67	-	55.33
3	0.0315	-	0.0648	0.0963	46.18	-	53.82
4	0.0409	-	0.0609	0.1018	42.24	-	57.76
5	0.0352	-	0.0648	0.1000	43.82	-	56.18
6	0.0361	-	0.0645	0.1006	38.91	-	61.09
7	0.0309	-	0.0652	0.0961	36.45	-	63.55
8	0.0314	-	0.0634	0.0948	40.72	-	59.28
9	0.0306	-	0.0651	0.0957	37.37	-	62.63
10	0.0294	-	0.0659	0.0953	30.74	-	69.26
11	0.0308	-	0.0646	0.0954	36.36	-	63.64
12	0.0241	-	0.0600	0.0968	34.61	-	65.39
13	0.0220	-	0.0862	0.1082	35.85	-	64.15
14	0.0228	-	0.0871	0.1099	33.07	-	66.93
15	0.0206	-	0.0855	0.1061	34.69	-	65.31
16	0.0218	-	0.0846	0.1064	34.31	-	65.69
17	0.0212	-	0.0848	0.1060	34.98		65.02



Gas production rate	=	2.15	$L\ h^{-1}$
Hydrogen production rate	=	0.75	$L\ h^{-1}$
Specific hydrogen production rate	=	4.50	$L\ H_2/L\ d$
Production of VFA	=	11,723	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	1.161	mol H_2/mol glucose consumed
% Glucose removal	=	98.80	%
% COD removal	=	75.09	%
VSS	=	869	mg/L

Volume of injection = 922 μl

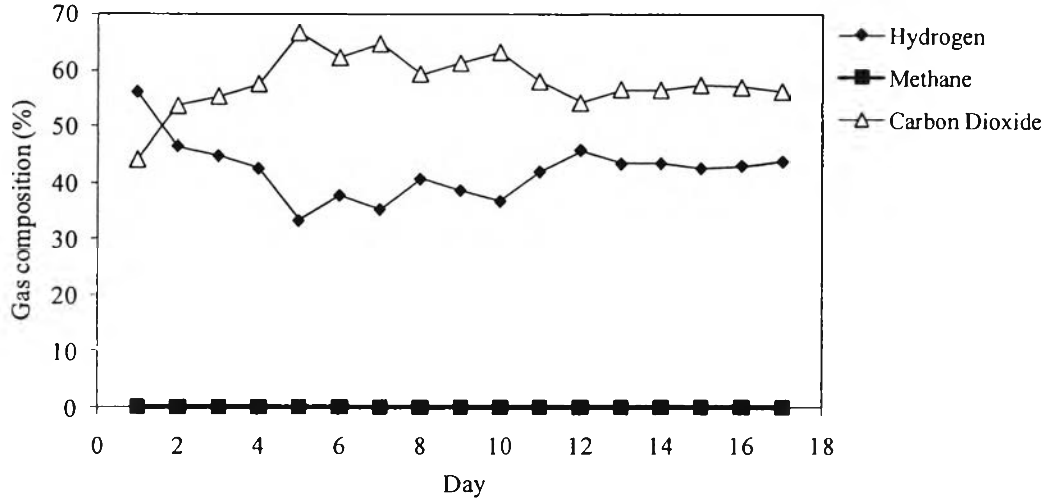
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	719.83	7.68
Butanol	0.00	0.00
Acetic acid	2896.00	39.46
Propionic acid	370.19	4.99
Butyric acid	3176.16	40.77
Valeric acid	526.99	7.10

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0622	-	0.0490	0.1112	55.94	-	44.06
2	0.0679	-	0.0783	0.1462	46.44	-	53.56
3	0.0711	-	0.0878	0.1589	44.74	-	55.26
4	0.0623	-	0.0842	0.1465	42.53	-	57.47
5	0.0452	-	0.0903	0.1355	33.36	-	66.64
6	0.0443	-	0.0729	0.1172	37.80	-	62.20
7	0.0469	-	0.0858	0.1327	35.34	-	64.66
8	0.0703	-	0.1024	0.1727	40.71	-	59.29
9	0.0694	-	0.1098	0.1792	38.73	-	61.27
10	0.0463	-	0.0795	0.1258	36.80	-	63.20
11	0.0707	-	0.0975	0.1682	42.03	-	57.97
12	0.0600	-	0.0709	0.1309	45.84	-	54.16
13	0.0464	-	0.0604	0.1068	43.45	-	56.55
14	0.0524	-	0.0680	0.1204	43.52	-	56.48
15	0.0493	-	0.0663	0.1156	42.65	-	57.35
16	0.0569	-	0.0752	0.1321	43.07	-	56.93
17	0.0507	-	0.0650	0.1157	43.82		56.18



Gas production rate	=	2.88	L h^{-1}
Hydrogen production rate	=	1.24	L h^{-1}
Specific hydrogen production rate	=	7.44	L H_2/L d
Production of VFA	=	17,304	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	1.46	mol H_2/mol glucose consumed
% Glucose removal	=	99.66	%
% COD removal	=	80.24	%
VSS	=	860	mg/L

Volume of injection = 986 μl

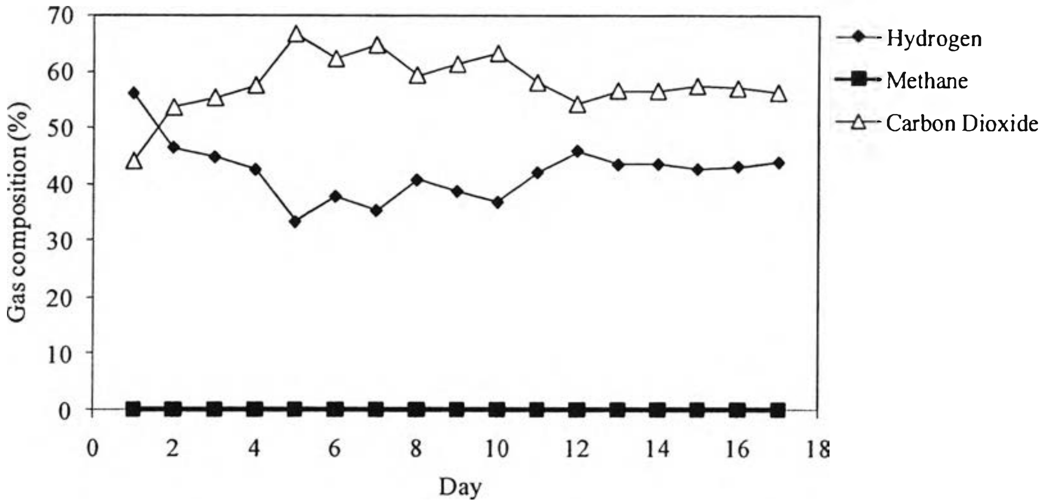
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	606.50	5.80
Butanol	85.79	1.22
Acetic acid	3,030.17	39.64
Propionic acid	360.55	5.15
Butyric acid	3,460.43	41.47
Valeric acid	470.13	6.71

COD loading rate 50 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0631	-	0.0917	0.1548	40.75	-	59.25
2	0.0573	-	0.1111	0.1684	34.02	-	65.98
3	0.054	-	0.1100	0.1640	32.93	-	67.07
4	0.0462	-	0.1388	0.1850	24.98	-	75.02
5	0.0469	-	0.1481	0.1950	24.05	-	75.95
6	0.0809	-	0.1263	0.2072	39.05	-	60.95
7	0.0633	-	0.1313	0.1946	32.52	-	67.48
8	0.0556	-	0.1262	0.1818	30.58	-	69.42
9	0.0126	-	0.0950	0.1076	11.71	-	88.29
10	0.0186	-	0.1269	0.1455	12.78	-	87.22
11	0.0425	-	0.1203	0.1628	26.11	-	73.89
12	0.0506	-	0.1084	0.1590	31.82	-	68.18
13	0.0622	-	0.1394	0.2016	30.84	-	69.16
14	0.0676	-	0.1425	0.2101	32.17	-	67.83
15	0.0507	-	0.1200	0.1707	29.70	-	70.30
16	0.0525	-	0.1225	0.1750	30.00	-	70.00
17	0.0512	-	0.1175	0.1687	30.35		69.65



Gas production rate	=	1.33	L h^{-1}
Hydrogen production rate	=	0.41	L h^{-1}
Specific hydrogen production rate	=	2.46	L H_2/L d
Production of VFA	=	5,768	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.406	mol H_2/mol glucose consumed
% Glucose removal	=	81.462	%
% COD removal	=	42.06	%
VSS	=	818	mg/L

Volume of injection = 936 μl

Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	807.81	6.25
Butanol	122.74	0.17
Acetic acid	2769.89	42.13
Propionic acid	406.25	4.55
Butyric acid	2842.76	40.26
Valeric acid	473.02	6.64

Appendix F Comparative Results between System without and with pH Control for the Effect of COD Loading Rate

Comparison of gas production rate between systems without and with pH control

COD loading rate	Gas production rate ($L\ h^{-1}$)	
($kg\ m^{-3}\ d^{-1}$)	Without pH control	pH = 5.5
10	0.27	0.41
20	1.09	1.22
30	2.09	2.15
40	2.1	2.88
50	1.88	1.33

Comparison of hydrogen production rate between systems without and with pH control

COD loading rate	H_2 production rate ($L\ h^{-1}$)	
($kg\ m^{-3}\ d^{-1}$)	Without pH control	pH = 5.5
10	0.05	0.08
20	0.35	0.40
30	0.73	0.75
40	0.81	1.24
50	0.68	0.40

Comparison of specific hydrogen production rate between systems without and with pH control

COD loading rate	**Specific H_2 production rate ($L\ h^{-1}$)**	
($kg\ m^{-3}\ d^{-1}$)	**Without pH control**	**pH = 5.5**
10	0.30	0.48
20	2.10	2.40
30	4.38	4.50
40	4.86	7.44
50	4.08	2.40

Comparison of yield of hydrogen production between systems without and with pH control

COD loading rate	**Yield of hydrogen production (mol H_2/mol glucose consumed)**	
($kg\ m^{-3}\ d^{-1}$)	**Without pH control**	**pH = 5.5**
10	0.259	0.386
20	0.829	0.957
30	1.156	1.161
40	0.968	1.460
50	0.705	0.406

Comparison of glucose removal percentage between systems without and with pH control

COD loading rate	**Glucose removal (%)**	
(kg m^{-3} d^{-1})	**Without pH control**	**pH = 5.5**
10	99.582	98.860
20	99.233	98.555
30	99.173	98.803
40	99.087	99.645
50	84.987	81.462

Comparison of COD removal percentage between systems without and with pH control

COD loading rate	**COD removal (%)**	
(kg m^{-3} d^{-1})	**Without pH control**	**pH = 5.5**
10	54.38	66.28
20	69.23	74.46
30	68.44	75.09
40	48.58	80.24
50	34.74	42.06

Comparison of total VFA between systems without and with pH control

COD loading rate	**Production of VFA (mg as acetic acid L^{-1})**	
(kg m^{-3} d^{-1})	**Without pH control**	**pH = 5.5**
10	7,611	5,278
20	12,286	10,555
30	32,877	11,723
40	34,607	17,304
50	16,222	5768

Comparison of volatile suspended solid between systems without and with pH control

COD loading rate	**VSS (mg L^{-1})**	
(kg m^{-3} d^{-1})	**Without pH control**	**pH = 5.5**
10	725	803
20	773	845
30	797	869
40	780	890
50	727	818

Comparison between specific hydrogen production rate and final pH of system under systems without pH control

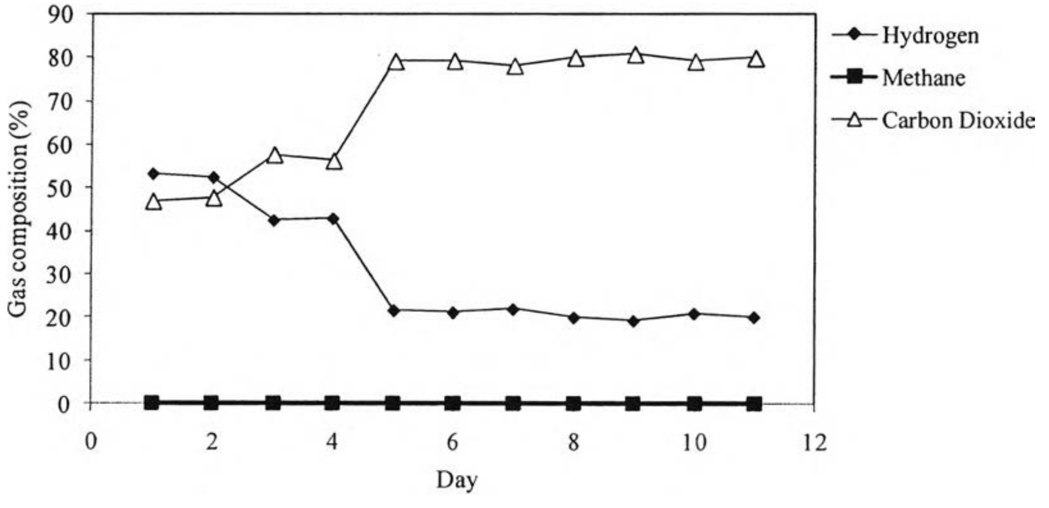
COD loading rate	**Without pH control**	
(kg m^{-3} d^{-1})	**Specific H_2 production rate (L h^{-1})**	**Final pH**
10	0.30	6.67
20	2.10	5.59
30	4.38	5.23
40	4.86	5.23
50	4.08	4.98

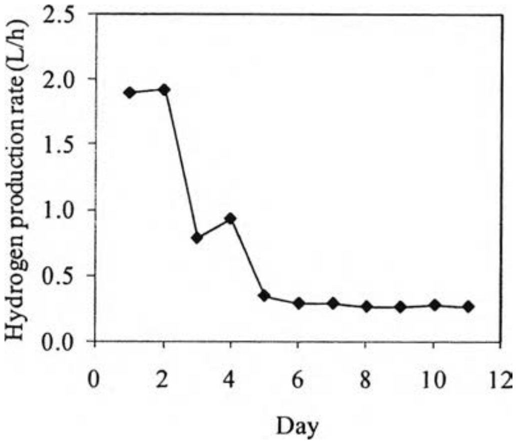
Appendix G Raw Data of The Effect of COD:N ratio under System without and with pH Control

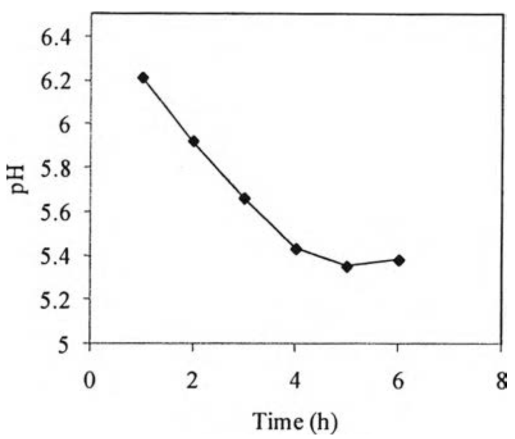
COD loading rate 40 kg m^{-3} d^{-1} without pH control Temperature = 37°C

COD:N:P ratio = 100 : 1.4 : 0.78

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0536	-	0.0474	0.1010	53.11	-	46.89
2	0.0560	-	0.0511	0.1071	52.30	-	47.70
3	0.0400	-	0.0544	0.0944	42.41	-	57.59
4	0.0448	-	0.0600	0.1048	42.80	-	56.17
5	0.0204	-	0.0736	0.0940	21.70	-	79.05
6	0.0221	-	0.0821	0.1042	21.23	-	79.13
7	0.0228	-	0.0810	0.1038	21.99	-	78.01
8	0.0200	-	0.0795	0.0996	20.11	-	79.89
9	0.0200	-	0.0835	0.1035	19.34	-	80.66
10	0.0207	-	0.0780	0.0987	21.02	-	78.98
11	0.0212	-	0.0835	0.1046	20.22	-	79.78







Gas production rate	=	1.28	$L\ h^{-1}$
Hydrogen production rate	=	0.26	$L\ h^{-1}$
Specific hydrogen production rate	=	1.56	$L\ H_2/L\ d$
Production of VFA	=	22,872	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.31	mol H_2/mol glucose consumed
% Glucose removal	=	98.18	%
% COD removal	=	31.27	%
MLSS	=		Mg/L
VSS	=	749	mg/L
MLVSS	=	9,428	mg/L

Volume of injection = 800 μl

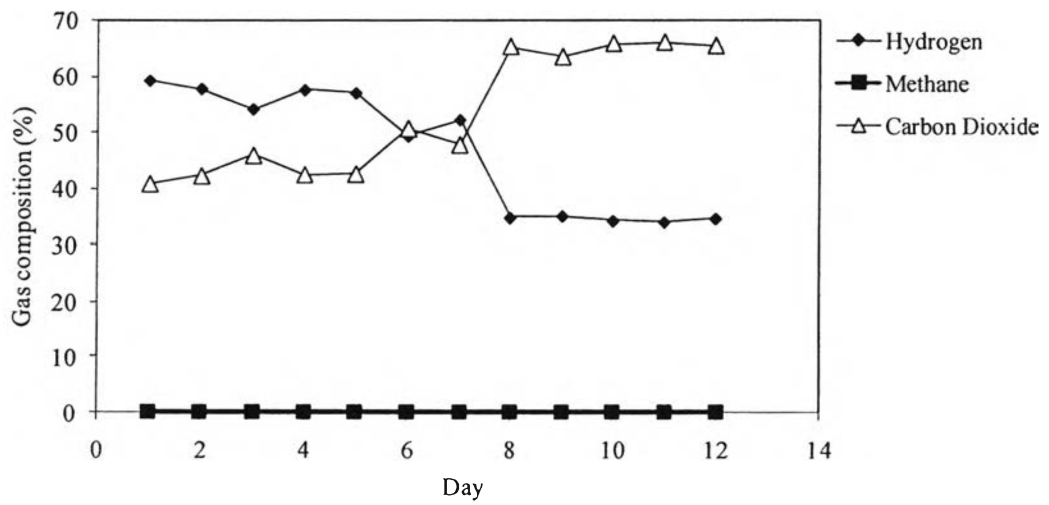
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

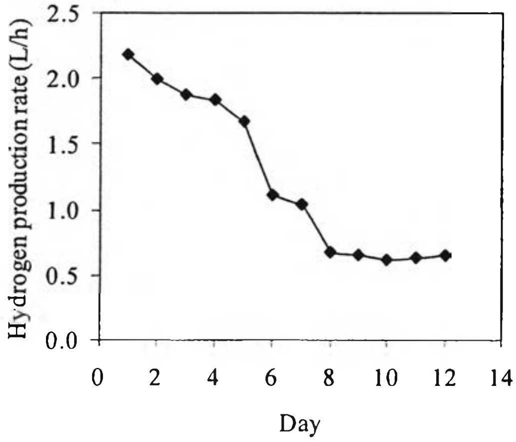
VFA	concentration (ppm)	%
Ethanol	101.21	0.54
Butanol	0.00	0.00
Acetic acid	4482.41	23.74
Propionic acid	11303.00	59.87
Butyric acid	1027.11	5.44
Valeric acid	1966.43	10.42

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 1.4 : 0.78

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0551	-	0.0379	0.0930	59.21	-	40.79
2	0.0539	-	0.0394	0.0933	57.76	-	42.24
3	0.0592	-	0.0502	0.1093	54.13	-	45.87
4	0.0534	-	0.0394	0.0928	57.53	-	42.47
5	0.0000	-	0.0000	0.0000	57.00	-	42.56
6	0.0501	-	0.0514	0.1016	49.35	-	50.65
7	0.0578	-	0.0529	0.1107	52.22	-	47.78
8	0.0322	-	0.0604	0.0925	34.76	-	65.24
9	0.0000	-	0.0000	0.0000	35.05	-	63.42
10	0.0318	-	0.0611	0.0929	34.21	-	65.79
11	0.0316	-	0.0615	0.0931	33.98	-	66.02
12	0.0318		0.0601	0.0918	34.61		65.39





Gas production rate	=	1.86	$L\ h^{-1}$
Hydrogen production rate	=	0.64	$L\ h^{-1}$
Specific hydrogen production rate	=	3.84	L H_2/L d
Production of VFA	=	15,689	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.76	mol H_2/mol glucose consumed
% Glucose removal	=	99.24	%
% COD removal	=	55.28	%
MLSS	=		Mg/L
VSS	=	770	mg/L
MLVSS	=	9,788	mg/L

Volume of injection = 918 µl

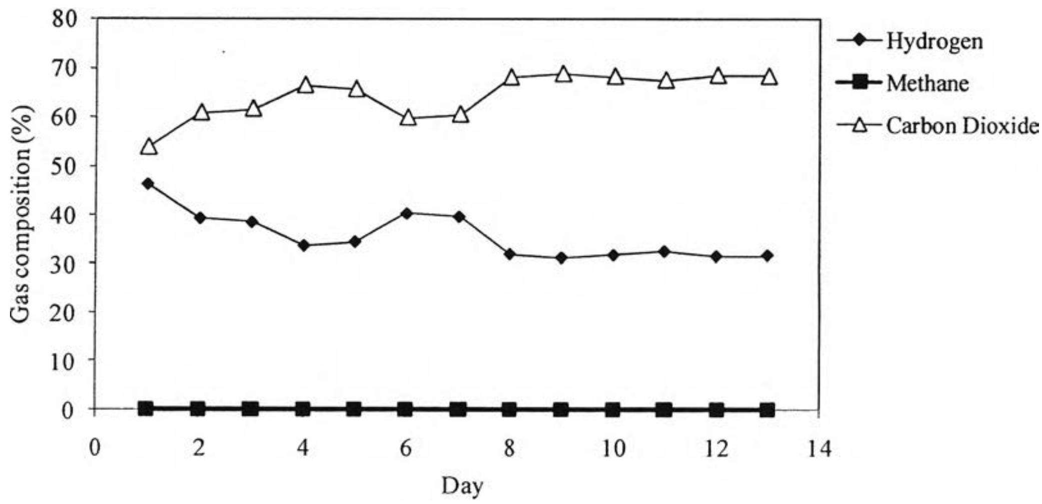
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

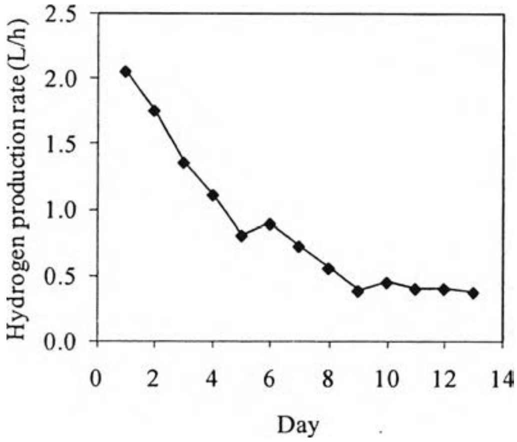
VFA	concentration (ppm)	%
Ethanol	115.54	1.68
Butanol	0	0
Acetic acid	2,485.78	36.04
Propionic acid	2,745.98	39.81
Butyric acid	735.49	10.66
Valeric acid	815.05	11.82

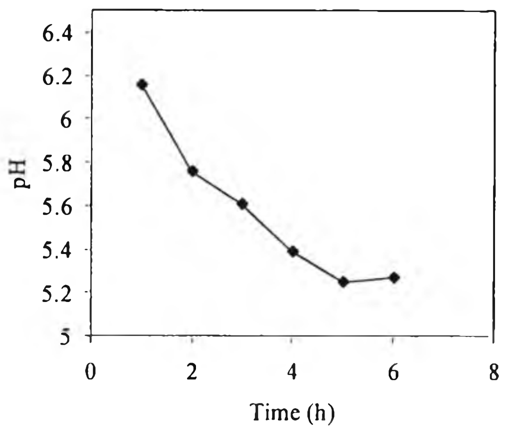
COD loading rate 40 kg m^{-3} d^{-1} without pH control Temperature = 37°C

COD:N:P ratio = 100 : 3.3 : 0.56

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0497	-	0.0579	0.1076	46.20	-	53.80
2	0.0490	-	0.0761	0.1251	39.19	-	60.81
3	0.0417	-	0.0665	0.1082	38.39	-	61.61
4	0.0353	-	0.0701	0.1055	33.62	-	66.38
5	0.0372	-	0.0714	0.1087	34.41	-	65.59
6	0.0380	-	0.0566	0.0946	40.17	-	59.83
7	0.0422	-	0.0646	0.1068	39.52	-	60.48
8	0.0366	-	0.0779	0.1145	31.95	-	68.05
9	0.0384	-	0.0844	0.1228	31.26	-	68.74
10	0.0363	-	0.0780	0.1143	31.79	-	68.21
11	0.0362	-	0.0750	0.1111	32.54	-	67.46
12	0.0360		0.0781	0.1141	31.54		68.46
13	0.0360		0.0773	0.1133	31.74		68.26







Gas production rate	=	1.10	$L\ h^{-1}$
Hydrogen production rate	=	0.37	$L\ h^{-1}$
Specific hydrogen production rate	=	2.22	L H_2/L d
Production of VFA	=	25,494	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.44	mol H_2/mol glucose consumed
% Glucose removal	=	99.09	%
% COD removal	=	42.73	%
MLSS	=		Mg/L
VSS	=	785	mg/L
MLVSS	=	9,990	mg/L

Volume of injection = 883 µl

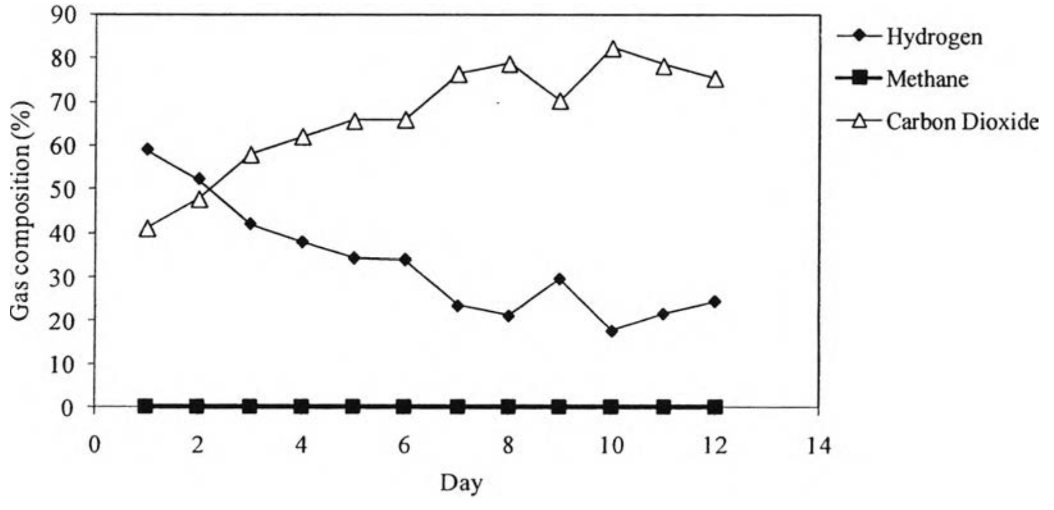
Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

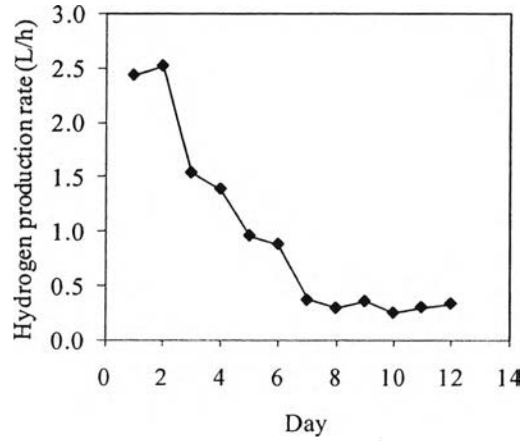
VFA	concentration (ppm)	%
Ethanol	42.19	0.19
Butanol	0	0
Acetic acid	6,301.85	27.86
Propionic acid	12,589.33	55.66
Butyric acid	1,671.25	7.39
Valeric acid	2,012.32	8.90

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 3.3 : 0.56

Day	Amount of each component			Total amount	Produced gas composition		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0653	-	0.0456	0.1109	58.88	-	41.12
2	0.0555	-	0.0507	0.1062	52.21	-	47.79
3	0.0437	-	0.0598	0.1036	42.06	-	57.94
4	0.0368	-	0.0599	0.0966	37.95	-	62.05
5	0.0323	-	0.0617	0.0940	34.40	-	65.60
6	0.0343	-	0.0668	0.1011	34.06	-	65.94
7	0.0252	-	0.0798	0.1050	23.66	-	76.34
8	0.0203	-	0.0752	0.0956	21.32	-	78.68
9	0.0136	-	0.0637	0.0772	29.74	-	70.26
10	0.0164	-	0.0750	0.0914	17.79	-	82.21
11	0.0200	-	0.0718	0.0919	21.81	-	78.19
12	0.0235		0.0713	0.0947	24.67		75.33





Gas production rate	=	1.33	$L\ h^{-1}$
Hydrogen production rate	=	0.33	$L\ h^{-1}$
Specific hydrogen production rate	=	1.98	$L\ H_2/L\ d$
Production of VFA	=	19,322	mg VFA as acetic acid L^{-1}
Yield of hydrogen production	=	0.39	mol H_2/mol glucose consumed
% Glucose removal	=	98.53	%
% COD removal	=	40.53	%
MLSS	=		Mg/L
VSS	=	790	mg/L
MLVSS	=	10,420	mg/L

Volume of injection = 900 μl

Distillated sample 2 mL + Internal standard (n-propanol 3,000 ppm) 0.5 mL

VFA	concentration (ppm)	%
Ethanol	238.77	2.33
Butanol	0	0
Acetic acid	3,507.87	34.29
Propionic acid	1,371.67	13.41
Butyric acid	1,655.16	16.18
Valeric acid	3,456.44	33.79

Appendix H Comparative Results between System without and with pH Control for the Effect of COD:N Ratio

Comparison of gas production rate between systems without and with pH control

Gas production rate (L h^{-1})			
COD loading rate ($kg\ m^{-3}\ d^{-1}$)	COD : N	pH	
		Without pH control	pH = 5.5
40	100:1.4	1.28	.1.86
	100:2.4	2.14	2.88
	100:3.3	1.10	1.33

Comparison of hydrogen production rate between systems without and with pH control

H_2 production rate (L h^{-1})			
COD loading rate ($kg\ m^{-3}\ d^{-1}$)	COD : N	pH	
		Without pH control	pH = 5.5
40	100:1.4	0.26	0.64
	100:2.4	0.81	1.24
	100:3.3	0.37	0.33

Comparison of specific hydrogen production rate between systems without and with pH control

Specific H_2 production rate (L h^{-1})			
COD loading rate ($kg\ m^{-3}\ d^{-1}$)	COD : N	pH	
		Without pH control	pH = 5.5
40	100:1.4	1.55	3.86
	100:2.4	4.86	7.44
	100:3.3	2.23	1.97

Comparison of yield of hydrogen production between systems without and with pH control

Yield of hydrogen production (mol H_2/mol glucose consumed)			
COD loading rate	COD : N	pH	
(kg m^{-3} d^{-1})		Without pH control	pH = 5.5
40	100:1.4	0.31	0.76
	100:2.4	0.97	1.46
	100:3.3	0.44	0.39

Comparison of glucose removal percentage between systems without and with pH control

Glucose removal (%)			
COD loading rate	COD : N	pH	
(kg m^{-3} d^{-1})		Without pH control	pH = 5.5
40	100:1.4	98.18	99.24
	100:2.4	99.09	99.66
	100:3.3	99.09	98.53

Comparison of COD removal percentage between systems without and with pH control

COD removal (%)			
COD loading rate	COD : N	pH	
(kg m^{-3} d^{-1})		Without pH control	pH = 5.5
40	100:1.4	31.27	55.28
	100:2.4	48.58	80.24
	100:3.3	42.73	40.53

Comparison of total VFA between systems without and with pH control

Production of VFA (mg as acetic acid L^{-1})			
COD loading rate	**COD : N**	**pH**	
(kg m^{-3} d^{-1})		**Without pH control**	**pH = 5.5**
40	100:1.4	22,872	15,689
	100:2.4	34,607	17,304
	100:3.3	25,494	19,322

Comparison of volatile suspended solid between systems without and with pH control

VSS (mg L^{-1})			
COD loading rate	**COD : N**	**pH**	
(kg m^{-3} d^{-1})		**Without pH control**	**pH = 5.5**
40	100:1.4	749	770
	100:2.4	780	890
	100:3.3	785	790

Comparison between specific hydrogen production rate and final pH of system under system without pH control

COD loading rate	**COD : N**	**Without pH control**	
(kg m^{-3} d^{-1})		**Specific H_2 production rate (L h^{-1})**	**Final pH**
40	100:1.4	1.55	5.38
	100:2.4	4.86	5.23
	100:3.3	2.23	5.27

Appendix I Example of Calculation

I 1. Volatile Suspended Solids (VSS) Determination

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Volume of solution	=	50 mL
Weight of filter paper	=	0.1231 g
Weight of residue + filter paper (100°C)	=	0.1676 g
Weight of residue + filter paper (500°C)	=	0.1246 g

VSS = (0.1676-0.1246)/50 = 8.60×10^{-4} g mL^{-1}

= 860 mg L^{-1}

COD loading rate (kg m^{-3} d^{-1})	COD:N ratio	pH	Volume of Solution (mL)	Weight of filter paper (g)	Weight (100°C) (g)	Weight (550°C) (g)
10	100:2.4	5.5	50	0.1133	0.1588	0.1164
20	100:2.4	5.5	50	0.1126	0.1597	0.1170
30	100:2.4	5.5	50	0.1142	0.1603	0.1169
40	100:2.4	5.5	50	0.1183	0.1676	0.1246
50	100:2.4	5.5	50	0.1171	0.1639	0.1230
40	100:1.4	w/o	40	0.1192	0.1512	0.1213
40	100:1.4	5.5	40	0.1172	0.1539	0.1231
40	100:3.4	w/o	40	0.1195	0.1548	0.1234
40	100:3.4	5.5	40	0.1157	0.1517	0.1201

I 2. Volatile Fatty Acids as Acetic Acid Determination by Distillation

Formula

$$\frac{\text{mg volitile acids as acetic acid}}{\text{L}} = \frac{\text{mL NaOH x N x 60,000}}{\text{mL sample x f}}$$

where

N = Normality of NaOH solution

f = recovery factor

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Distillate = 10 mL

NaOH 1 M = 2 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{2 \times 1 \times 60{,}000}{10 \times 0.6935}$$

$$= 17{,}303.53 \ \frac{\text{mg VFA as acetic acid}}{L}$$

COD loading rate (kg m^{-3} d^{-1})	COD:N ratio	pH	N	Volume of Distillate (mL)	Volume of NaOH (mL)
10	100:2.4	5.5	0.1	20	12.2
20	100:2.4	5.5	0.1	20	24.4
30	100:2.4	5.5	0.1	20	27.1
40	100:2.4	5.5	1	10	2
50	100:2.4	5.5	1	15	1
40	100:1.4	w/o	0.1	15	39.7
40	100:1.4	5.5	0.1	15	27.2
40	100:3.4	w/o	0.1	15	44.2
40	100:3.4	5.5	0.1	15	33.5

I 3. Glucose Removal Determination using UV Spectrophotometer

Formula

$$\frac{\text{mg glucose}}{\text{mL}} = \frac{(\Delta A) \times (TV) \times (F) \times (0.029)}{SV}$$

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

A(sample blank)	=	0.348
A(reagent blank)	=	0.026
A(total blank)	=	0.374
A(test)	=	0.4888
ΔA = A(test)-A(total blank)	=	0.4888-0.374 = 0.1148
mg glucose/mL	=	(0.1148x2x10x0.029)/2
	=	0.0333 g/L
% glucose removal	=	(9.375-0.0333)x100/9.375
	=	99.645%

COD loading rate (kg m^{-3} d^{-1})	COD:N ratio	pH	A (sample blank)	A (reagent blank)	A (test)
10	100:2.4	5.5	0.504	0.049	1.288
20	100:2.4	5.5	0.601	0.051	1.587
30	100:2.4	5.5	0.712	0.049	1.922
40	100:2.4	5.5	0.348	0.026	0.489
50	100:2.4	5.5	0.815	0.027	1.638
40	100:1.4	w/o	0.037	0.047	1.861
40	100:1.4	5.5	0.042	0.047	1.066
40	100:3.4	w/o	0.047	0.047	1.274
40	100:3.4	5.5	0.041	0.047	1.990

I 4. Yield of Hydrogen Determination

COD loading rate 40 kg m^{-3} d^{-1} pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Amount of used glucose	=	37.5-0.0333
	=	37.4667 g L^{-1}
(1 day)Amount of used glucose	=	37.467x4
	=	149.868 g
Mole of used glucose	=	149.868/180
	=	0.8326 mole
Volume of H_2 in 1 day	=	0.43x2.876x24
	=	29.68 L

Mole of H_2 produce in 1 day

$$n = \frac{PV}{RT} \quad ; R = 0.082 \frac{L \cdot atm}{mol \cdot K}$$

$$n = \frac{1x29.68}{0.082x(273+25)}$$

$$= 1.215 \text{ mole of } H_2$$

Yield of hydrogen production

$$= \frac{1.215}{0.8326} \frac{\text{mole of } H_2}{\text{mole of glucose}}$$

$$= 1.46 \frac{\text{mole of } H_2}{\text{mole of glucose}}$$

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	COD:N ratio	pH	Amount of used glucose ($g\ L^{-1}$)	Volume of H_2 ($L\ d^{-1}$)
10	100:2.4	5.5	9.16	1.92
20	100:2.4	5.5	18.48	9.84
30	100:2.4	5.5	27.79	18.00
40	100:2.4	5.5	37.47	29.68
50	100:2.4	5.5	43.40	9.56
40	100:1.4	w/o	36.99	6.24
40	100:1.4	5.5	37.22	15.36
40	100:3.4	w/o	37.15	8.88
40	100:3.4	5.5	36.95	7.92

I 5. COD Removal Determination

COD loading rate 40 $kg\ m^{-3}\ d^{-1}$ pH = 5.5 Temperature = 37°C

COD:N:P ratio = 100 : 2.4 : 0.69

Feed COD	=	41,500	$mg\ L^{-1}$
Product COD	=	8,200	$mg\ L^{-1}$
%COD removal	=	(41,500-8,200)/41,500	
	=	80.24%	

COD loading rate (kg m^{-3} d^{-1})	COD:N ratio	pH	Feed COD (mg L^{-1})	Product COD (mg L^{-1})
10	100:2.4	5.5	17,200	5,800
20	100:2.4	5.5	18,400	4,700
30	100:2.4	5.5	28,500	7,100
40	100:2.4	5.5	41,500	8,200
50	100:2.4	5.5	53,500	31,000
40	100:1.4	w/o	33,320	22,900
40	100:1.4	5.5	33,320	14,900
40	100:3.4	w/o	46,240	26,480
40	100:3.4	5.5	46,240	27,500

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1. Niyamapa, T., Neramitsuk, H., Sreethawong, T., Rangsunvigit, P., and Chavadej, S. (2007, November 21-24) Biohydrogen Production from Glucose-Containing Wastewater in Anaerobic Sequencing Batch Reactors: Effect of pH. Paper presented at The 5th Eco-Energy and Materials Science and Engineering Symposium, Pattaya, Thailand
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