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APPENDICES

Appendix A Calibration Curves

Table A1 Calibration curve for hydrogen (H_2)

Volume of hydrogen (ml)	Peak area
0.02	16,313
0.04	58,770
0.08	180,674
0.1	226,743
0.2	427,198
0.4	778,509

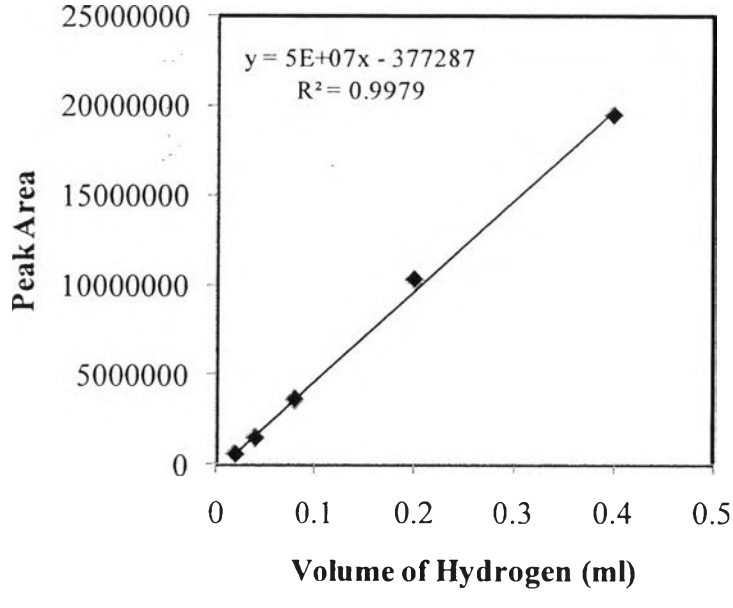

Figure A1 The relationship between volume of hydrogen (H_2) and peak area.

Equation

$$\text{Amount of hydrogen} = \frac{\text{Peak area} + 377287}{5 \times 10^7}$$

Table A2 Calibration curve for nitrogen

Volume of nitrogen (ml)	Peak area
0.02	34,210
0.04	128,767
0.08	305,287
0.1	393,916
0.2	809,433
0.4	1,602,475

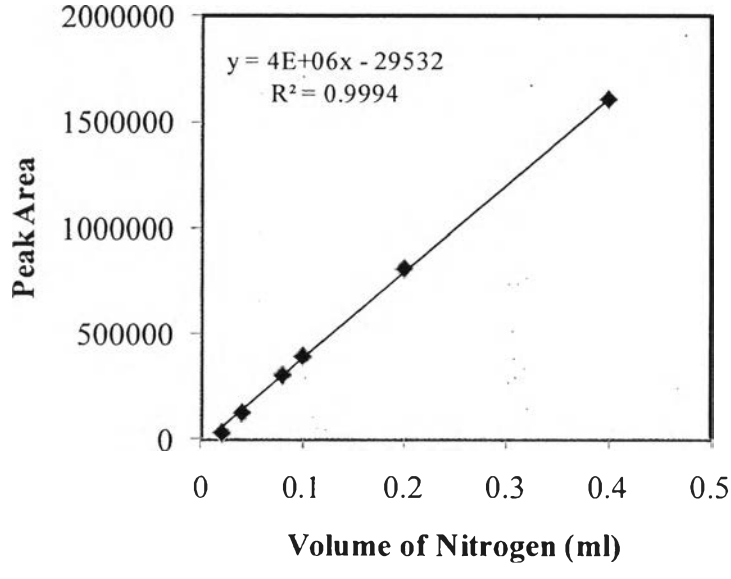

Figure A2 The relationship between volume of nitrogen (N_2) and peak area.

Equation

$$\text{Amount of nitrogen} = \frac{\text{Peak area} + 29532}{4 \times 10^6}$$

Table A3 Calibration curve for oxygen

Volume of oxygen (ml)	Peak area
0.02	12,286
0.04	43,995
0.08	104,342
0.1	135,546
0.2	280,220
0.4	562,001

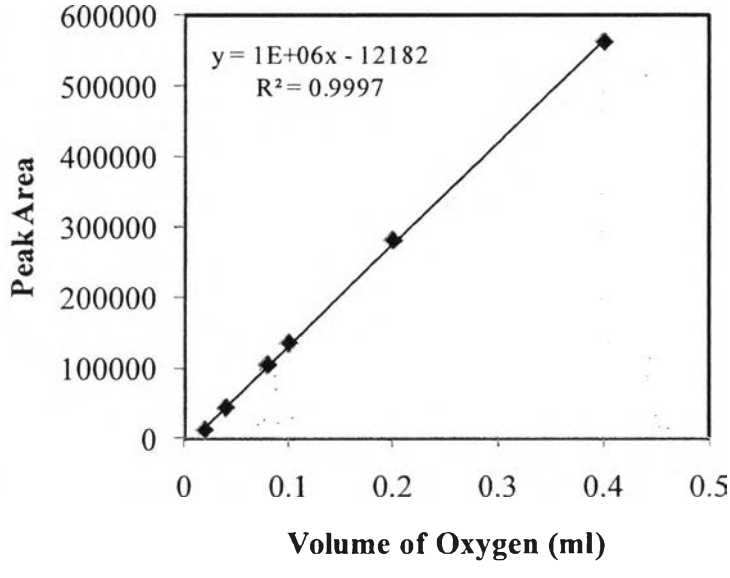

Figure A3 The relationship between volume of oxygen (O_2) and peak area.

Equation

$$\text{Amount of oxygen} = \frac{\text{Peak area} + 12182}{1 \times 10^6}$$

Table A4 Calibration curve for methane (CH_4)

Volume of methane (ml)	Peak area
0.02	92,517
0.04	381,106
0.1	1,293,552
0.2	2,674,654

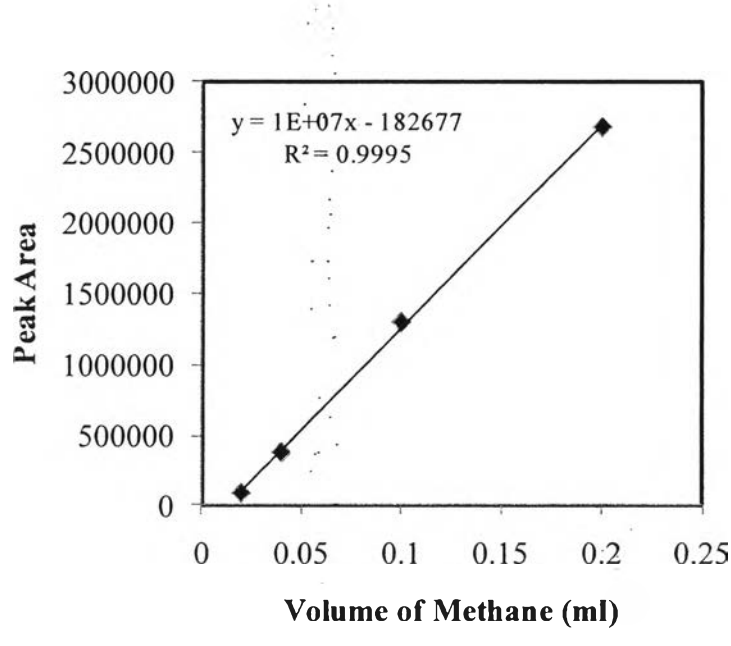

Figure A4 The relationship between volume of methane (CH_4) and peak area.

Equation

$$\text{Amount of methane} = \frac{\text{Peak area} + 182677}{1 \times 10^7}$$

Table A5 Calibration curve for carbon dioxide (CO_2)

Volume of carbon dioxide (ml)	Peak area
0.02	26,118
0.04	97,539
0.08	220,122
0.2	596,414
0.4	1,315,885

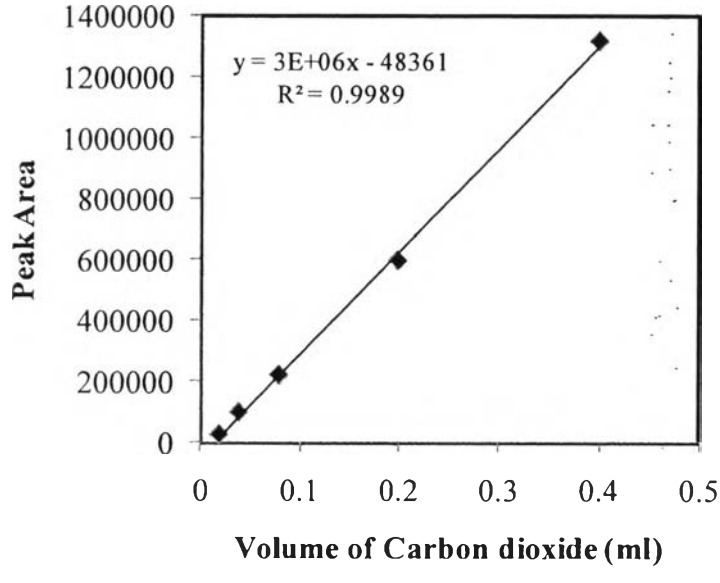

Figure A5 The relationship between volume of carbon dioxide (CO_2) and peak area.

Equation

$$\text{Amount of carbon dioxide} = \frac{\text{Peak area} + 48361}{3 \times 10^6}$$

Appendix B Preparation of 5 wt./vol.% NaOH Solution for pH-controlled System

Preparation of NaOH at concentration of 5 wt./vol.%

$$= \frac{5}{100}\frac{g}{ml} = 50\frac{g}{l}$$

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

C 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
Molecular weight of acetic acid	=	60

Determination of fresh acetic acids concentration in term of molar

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

= 17.78 M

Preparation of acetic acid at concentration of 2,000 mg/L

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

= 0.0333 M

Dilution of acetic acid

N_1V_1	=	N_2V_2	
V_1	=	N_2V_2/N_1	
	=	(0.0333x1)/17.78	
	=	1.873×10^{-3}	L

C 2. Standard Sodium Hydroxide (0.1 M) Preparation

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

Preparation of acetic acid at concentration of 0.1 M

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

$$= 4.04\ \text{g}$$

C 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

b = volatile acid concentration in standard solution used, mg/L

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	=	$11.7\text{x}10^{-3}\text{x}0.1$
	=	$1.17\text{x}10^{-3}$ mol
Acetic acid in distillate	=	$1.17\text{x}10^{-3}$ mol
	=	$1.17\text{x}10^{-3}$ x 60.5
	=	0.07 g
Concentration of acetic acid in distillate		
	=	0.07/50
	=	$1.405\text{x}10^{-3}$ g/ml
	=	1,405 mg/l

2) Distillate 25 ml NaOH 5.7 ml

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

Concentration of acetic acid in distillate

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

Recovery factor (f)	=	1,387/2,000	
	=	0.6935	

Appendix D Raw Data of Effect of COD Loading Rate

D 1. COD loading rate = 10 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.132	0.381	0.048	0.561	23.60	67.82	8.59
2	0.107	0.349	0.113	0.569	18.76	61.36	19.87
3	0.056	0.334	0..190	0.580	9.72	57.53	32.75
4	0.041	0.333	0.225	0.598	6.85	55.63	37.52
5	0.018	0.328	0.248	0.594	3.00	55.29	41.71
6	0.043	0.303	0.257	0.604	7.19	50.25	42.55
7	0.047	0.350	0.218	0.615	7.69	56.84	35.47
8	0.040	0.370	0.206	0.615	6.49	60.09	33.42
9	0.046	0.335	0.211	0.592	7.76	56.53	35.71
10	0.080	0.365	0.135	0.580	13.78	62.91	23.31
11	0.073	0.374	0.127	0.574	12.72	65.12	22.16
12	0.092	0.326	0.126	0.544	16.97	59.91	23.12
13	0.099	0.319	0.124	0.543	18.28	58.82	22.90
14	0.093	0.369	0.109	0.570	16.24	64.68	19.08
15	0.101	0.353	0.107	0.561	18.08	62.91	19.00
16	0.102	0.334	0.102	0.562	18.96	62.12	18.92
Avg.	0.099	0.352	0.106	0.564	17.76	63.24	19.00

Gas production rate	=	4.73	l/d
Hydrogen production rate	=	0.83	l/d
Specific hydrogen production rate	=	4.15	ml H_2/g MLVSS d
VFA Concentration	=	13,038	mg/l as acetic acid
Hydrogen yield	=	9.29	ml H_2/g COD removed
COD removal efficiency	=	37.16	%

MLVSS	=	8,317	mg/l
TSS	=	1,556	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	991
Acetic acid	371
Propionic acid	4,079
Butyric acid	797
Valeric acid	4,438

D 2. COD loading rate = 15 kg/ m^3d pH = 5.5 Temperature = 37 °C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.064	0.356	0.207	0.626	10.22	56.78	33.00
2	0.063	0.370	0.192	0.625	10.09	59.14	30.78
3	0.070	0.287	0.210	0.567	12.35	50.57	37.08
4	0.077	0.321	0.183	0.581	13.26	55.26	31.48
5	0.117	0.241	0.170	0.528	22.07	45.67	32.26
6	0.146	0.146	0.135	0.427	34.15	34.23	31.62
7	0.124	0.354	0.095	0.573	21.56	61.77	16.67
8	0.152	0.330	0.086	0.568	26.87	58.05	15.08
9	0.160	0.332	0.069	0.560	28.57	59.18	12.25
10	0.172	0.355	0.045	0.571	30.07	62.05	7.88
11	0.186	0.365	0.028	0.579	32.05	63.10	4.85
12	0.174	0.381	0.024	0.579	30.05	65.84	4.11
13	0.173	0.373	0.026	0.572	30.25	65.19	4.56
14	0.176	0.370	0.026	0.572	30.76	64.72	4.52
15	0.174	0.375	0.024	0.572	30.33	65.43	4.24
Avg.	0.174	0.373	0.025	0.572	30.45	65.11	4.44

Gas production rate	=	11.16	l/d
Hydrogen production rate	=	3.40	l/d
Specific hydrogen production rate	=	14.51	ml H_2/g MLVSS d
VFA concentration	=	12,848	mg/l as acetic acid
Hydrogen yield	=	29.66	ml H_2/g COD removed
COD removal efficiency	=	31.84	%
MLVSS	=	9,767	mg/l
TSS	=	1,848	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	1,915
Acetic acid	2,913
Propionic acid	1,532
Butyric acid	2,678
Valeric acid	2,202

D 3. COD loading rate = 20 kg/ m^3d pH = 5.5 Temperature = 37 °C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.280	0.200	0.021	0.500	55.97	39.91	4.12
2	0.254	0.253	0.020	0.528	48.16	47.99	3.85
3	0.260	0.270	0.020	0.550	47.19	49.14	3.67
4	0.242	0.270	0.021	0.528	45.85	50.20	3.95
5	0.230	0.286	0.020	0.536	42.97	53.23	3.80
6	0.241	0.273	0.020	0.534	45.16	51.05	3.79
7	0.259	0.340	0.024	0.623	41.54	54.56	3.90
8	0.215	0.326	0.019	0.560	38.44	58.18	3.38
9	0.203	0.304	0.019	0.526	38.69	57.78	3.54
10	0.236	0.372	0.023	0.631	37.47	58.94	3.59
Avg.	0.218	0.334	0.020	0.572	38.20	58.30	3.50

Gas production rate	=	14.98	l/d
Hydrogen production rate	=	5.72	l/d
Specific hydrogen production rate	=	21.57	ml H_2/g MLVSS d
VFA concentration	=	12,545	mg/l as acetic acid
Hydrogen yield	=	40.85	ml H_2/g COD removed
COD removal efficiency	=	29.17	%
MLVSS	=	11,050	mg/l
TSS	=	1,863	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	2,598
Acetic acid	3,985
Propionic acid	1,531
Butyric acid	2,731
Valeric acid	1,499

D4. COD loading rate = 25 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.279	0.278	0.036	0.594	46.97	46.88	6.14
2	0.257	0.337	0.025	0.618	41.51	54.53	3.96
3	0.214	0.402	0.020	0.635	33.68	63.22	3.10
4	0.207	0.428	0.020	0.656	31.63	65.30	3.07
5	0.197	0.422	0.020	0.639	30.83	66.03	3.14
6	0.191	0.432	0.020	0.642	29.73	67.16	3.12
7	0.201	0.420	0.019	0.641	31.45	65.51	3.04
Avg.	0.196	0.425	0.020	0.641	30.67	66.23	3.10

Gas production rate	=	32.53	l/d
Hydrogen production rate	=	9.98	l/d
Specific hydrogen production rate	=	34.26	ml H_2/g MLVSS d
VFA concentration	=	12,476	mg/l as acetic acid
Hydrogen yield	=	68.82	ml H_2/g COD removed
COD removal efficiency	=	24.17	%
MLVSS	=	12,136	mg/l
TSS	=	2,023	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	2,959
Acetic acid	3,359
Propionic acid	1,957
Butyric acid	2,415
Valeric acid	1,788

D 5. COD loading rate = 30 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.104	0.398	0.126	0.628	16.57	63.34	20.09
2	0.084	0.386	0.142	0.613	13.78	62.98	23.24
3	0.090	0.377	0.144	0.611	14.74	61.65	23.61
4	0.084	0.392	0.149	0.625	13.43	62.67	23.89
5	0.094	0.349	0.155	0.598	15.74	58.35	25.91
6	0.072	0.402	0.142	0.616	11.74	65.27	22.98
7	0.063	0.438	0.139	0.640	9.86	68.37	21.77
8	0.069	0.395	0.146	0.611	11.35	64.74	23.91
9	0.067	0.471	0.110	0.648	10.31	72.66	17.03
10	0.102	0.473	0.081	0.656	15.52	72.10	12.38
11	0.152	0.435	0.053	0.641	23.73	67.90	8.38
12	0.196	0.399	0.037	0.632	31.06	63.15	5.79
13	0.217	0.392	0.025	0.633	34.22	61.89	3.89
14	0.185	0.421	0.024	0.630	29.32	66.88	3.80
15	0.188	0.418	0.023	0.628	29.85	66.52	3.63
16	0.192	0.416	0.024	0.632	30.35	65.80	3.85
Avg.	0.188	0.418	0.024	0.630	29.84	66.40	3.76

Gas production rate	=	44.71	l/d
Hydrogen production rate	=	13.34	l/d
Specific hydrogen production rate	=	41.36	ml H_2/l d
VFA concentration	=	12,529	mg/l as acetic acid
Hydrogen yield	=	93.93	ml H_2/g COD removed
COD removal efficiency	=	19.73	%
MLVSS	=	13,440	mg/l
TSS	=	3,720	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	2,990
Acetic acid	2,982
Propionic acid	2,159
Butyric acid	2,587
Valeric acid	1,821

D 6. COD loading rate = 35 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)			Total amount (ml)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.206	0.347	0.054	0.607	33.86	57.22	8.91
2	0.160	0.243	0.095	0.497	32.10	48.83	19.07
3	0.128	0.374	0.098	0.600	21.37	62.36	16.27
4	0.113	0.395	0.094	0.602	18.72	65.63	15.65
5	0.121	0.425	0.084	0.630	19.16	67.49	13.35
6	0.118	0.456	0.052	0.626	18.93	72.83	8.25
7	0.157	0.433	0.037	0.627	25.07	69.08	5.85
8	0.181	0.403	0.025	0.609	29.80	66.17	4.03
9	0.183	0.421	0.028	0.633	28.95	66.61	4.43
10	0.136	0.333	0.018	0.487	27.90	68.35	3.75
11	0.191	0.426	0.025	0.641	29.71	66.47	3.82
Avg.	0.170	0.393	0.024	0.587	28.85	67.14	4.00

Gas production rate	=	50.56	l/d
Hydrogen production rate	=	14.59	l/d
Specific hydrogen production rate	=	46.08	ml H_2/g MLVSS d
VFA concentration	=	12,734	mg/l as acetic acid
Hydrogen yield	=	114.49	ml H_2/g COD removed
COD removal efficiency	=	15.17	%
MLVSS	=	13,193	mg/l
TSS	=	4,375	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	3,143
Acetic acid	2,518
Propionic acid	2,007
Butyric acid	2,254
Valeric acid	1,962

CURRICULUM VITAE

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Proceedings:

1. Limwattanalert, N., Chavadej, S., Sreethawong, T., and Rangsunvigit, P. (2011, April 26) Hydrogen Production from Ethanol Wastewater by Using Upflow Anaerobic Sludge Blanket Reactor. Proceedings of The 2nd Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 17th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.