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APPENDICES

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

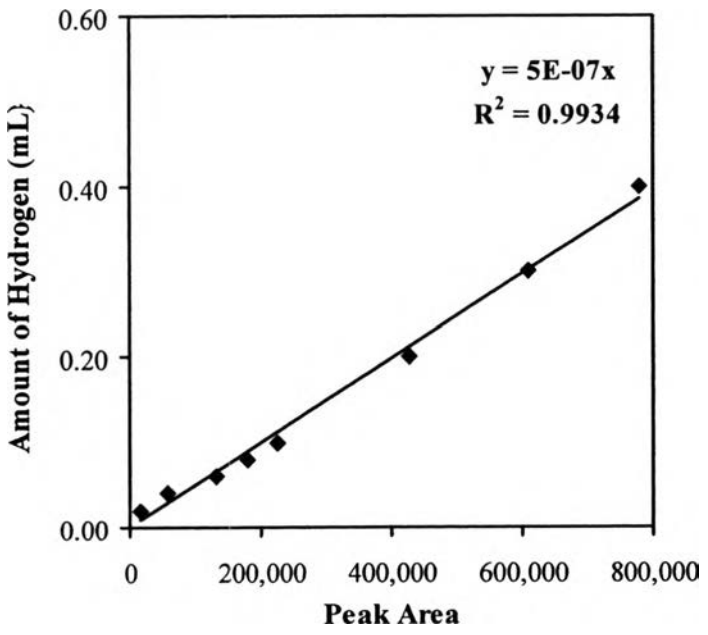

Figure A1 The relationship between amount of hydrogen (H_2) and peak area.

Equation

Amount of hydrogen = $5x10^{-7}$ 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

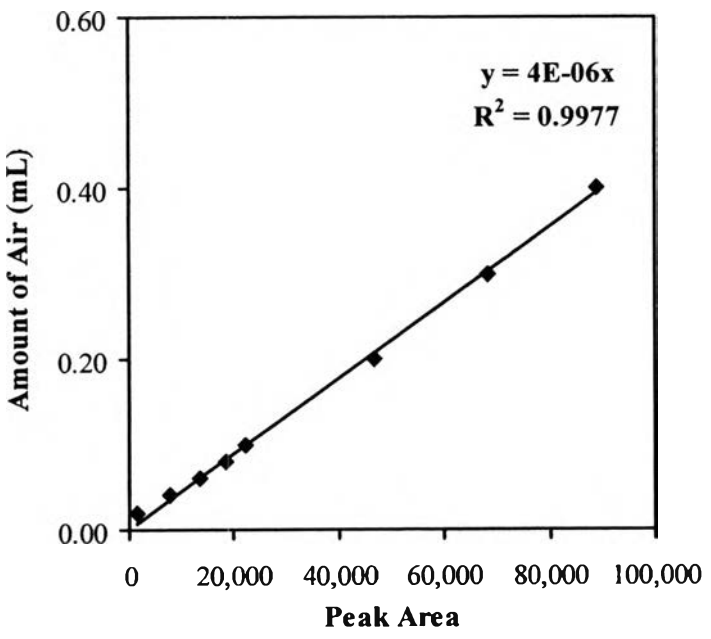

Figure A2 The relationship between amount of air and peak area.

Equation

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

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

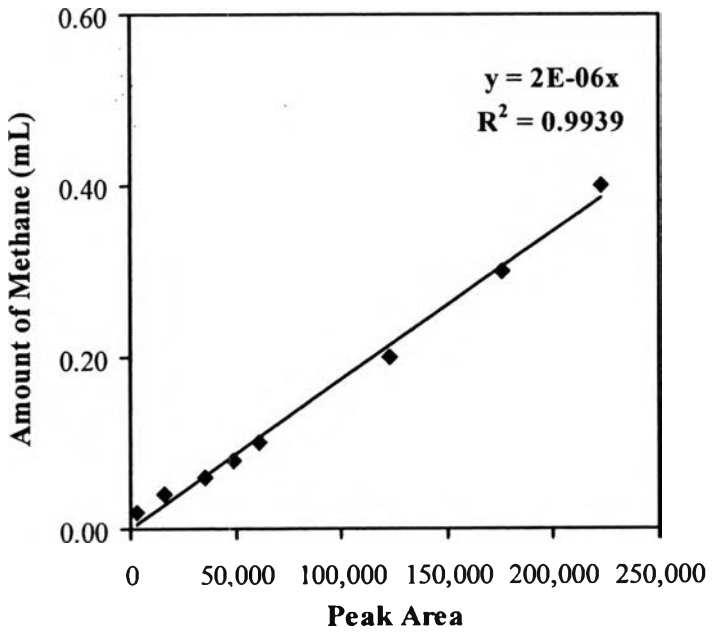

Figure A3 The relationship between amount of methane (CH_4) and peak area.

Equation

Amount of methane = $2x10^{-6}$ x 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

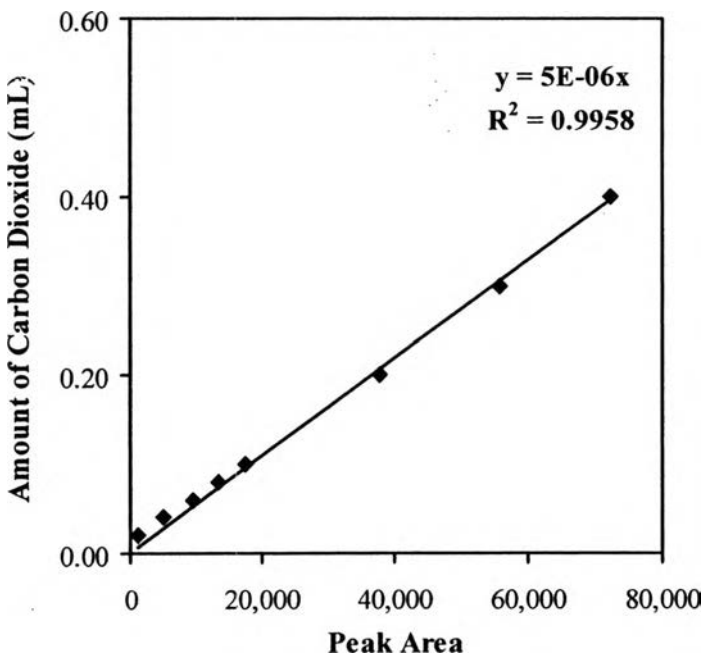

Figure A4 The relationship between amount of carbon dioxide (CO_2) and peak area.

Equation

Amount of carbon dioxide = $5x10^{-6}$ x Peak area

Table A5 Gas chromatograph's calibration curves for acetic acid

Acetic acid concentration (ppm)	$\frac{\text{Peak area of acetic acid}}{\text{Peak area of n-propanol}}$
0	0
1,000	0.4916
2,000	0.8158
3,000	1.2596
4,000	1.5860

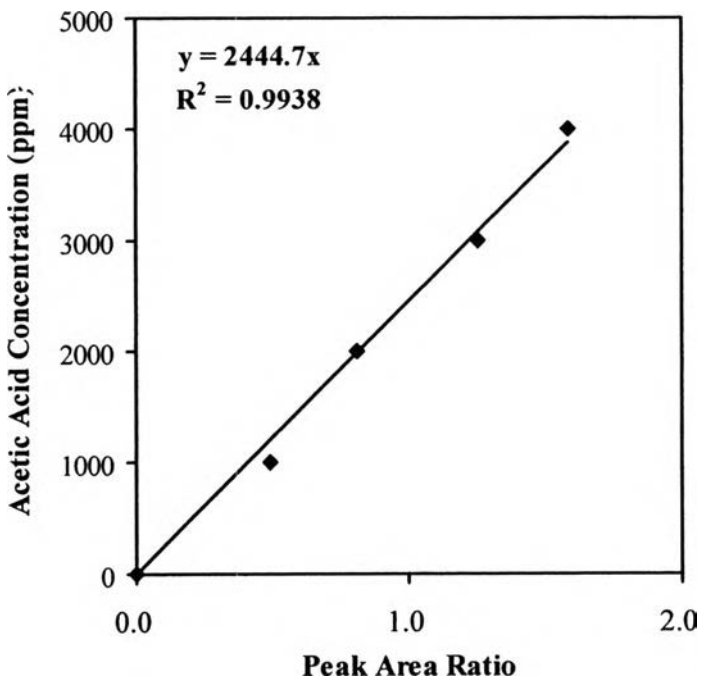

Figure A5 The relationship between acetic acid concentration and peak area ratio.

Equation

Acetic acid concentration (ppm) = 2444.7 x Peak area ratio

Table A6 Gas chromatograph's calibration curves for valeric acid

Valeric acid concentration (ppm)	Peak area of valeric acid / Peak area of n-propanol
0	0
1,000	0.9740
2,000	2.3376
3,000	3.3325
4,000	4.4053

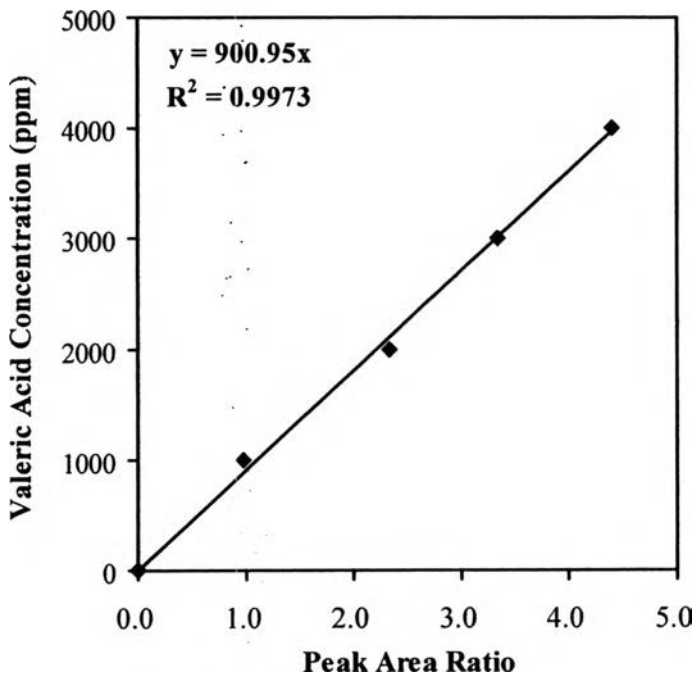

Figure A6 The relationship between valeric acid concentration and peak area ratio.

Equation

Valeric acid concentration (ppm) = 900.95 x Peak area ratio

Table A7 Gas chromatograph's calibration curves for propionic acid

Propionic acid concentration (ppm)	Peak area of propionic acid / Peak area of n-propanol
0	0
1,000	0.6819
2,000	1.4916
3,000	2.3378
4,000	3.2784

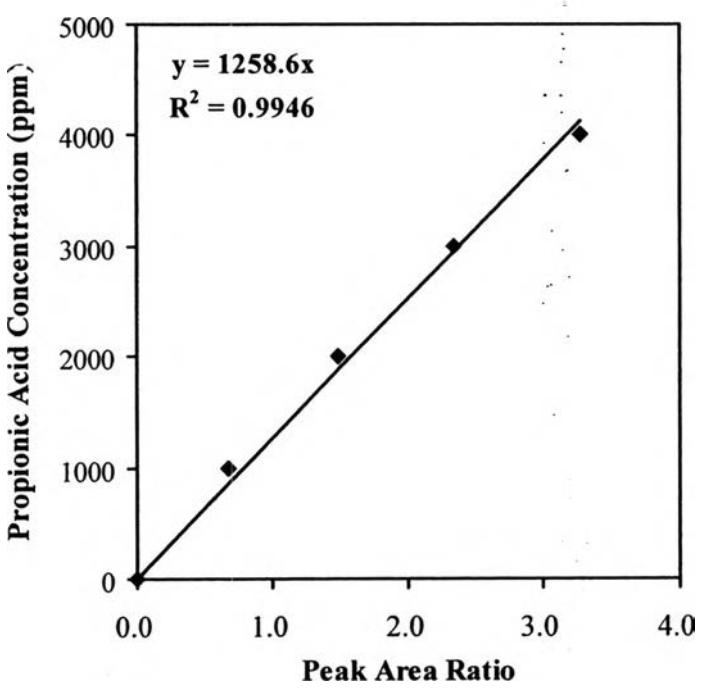

Figure A7 The relationship between propionic acid concentration and peak area ratio.

Equation

Propionic acid concentration (ppm) = 1258.6 x Peak area ratio

Table A8 Gas chromatograph's calibration curves for butyric acid

Butyric acid concentration (ppm)	Peak area of butyric acid / Peak area of n-propanol
0	0
1,000	0.5328
2,000	1.3043
3,000	1.8347
4,000	2.4220

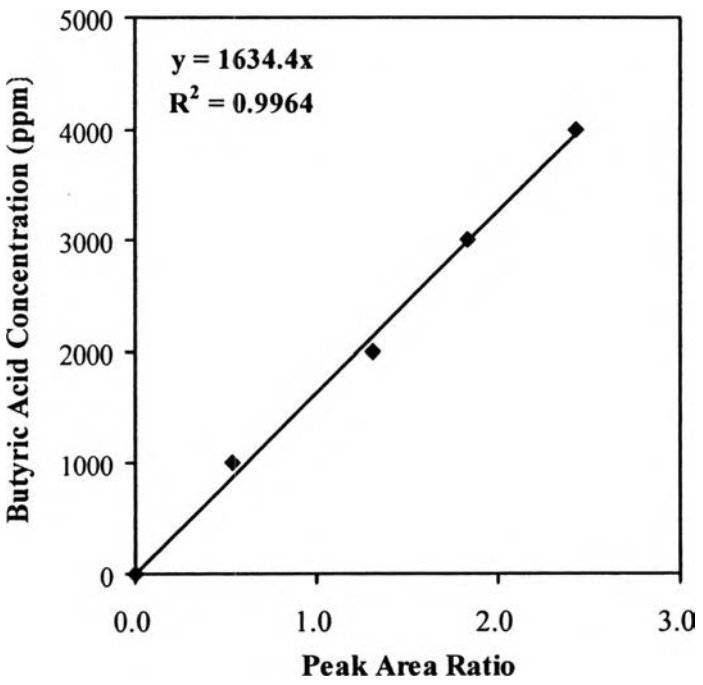

Figure A8 The relationship between butyric acid concentration and peak area ratio.

Equation

Butyric acid concentration (ppm) = 1634.4 x Peak area ratio

Table A9 Gas chromatograph's calibration curves for ethanol

Ethanol concentration (ppm)	$\frac{\text{Peak area of ethanol}}{\text{Peak area of n-propanol}}$
0	0
1,000	0.5682
2,000	1.1800
3,000	1.8570
4,000	2.4495

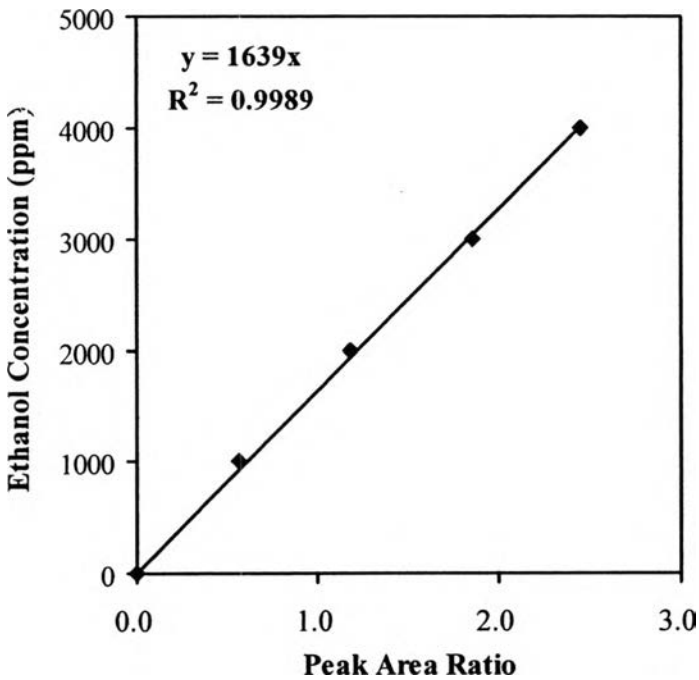

Figure A9 The relationship between ethanol concentration and peak area ratio.

Equation

Ethanol concentration (ppm) = 1639 x Peak area ratio

Appendix B Seed Sludge Preparation

Determine the amount of total suspended solid in seed sludge

From Equation 3.2

$$\frac{\text{mg total suspended solids}}{\text{L}} = \frac{(A - B) \times 10^6}{\text{Sample volume, mL}}$$

Sample volume = 5 mL

Weight of filter disk (B) = 0.1245 g

Weight of filter disk + dried residual at 110°C (A) = 0.4283 g

$$TSS = \frac{(0.4283 - 0.1245) \times 10^6}{5}$$

TSS = 60,760 mg/L

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 = 5 g/L

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

$N_1V_1 = N_2V_2$

60.760 x V1 = 5 x 4

V1 = 0.329 L

Appendix C Preparation of 1 M NaOH solution for pH Control System

Concentration of NaOH (solid) = 99%

Molecular weight of NaOH = 40

Preparation of NaOH at concentration of 1 M

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

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

Molecular weight of acetic acid = 60

Determination of fresh acetic acids concentration in term of molar

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

$$= 17.78 \text{ M}$$

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

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

$$= 0.0333 \text{ M}$$

Dilution of acetic acid

$N_1V_1 = N_2V_2$

$V_1 = N_2V_2/N_1$

$= (0.0333 \text{x} 1)/17.78$

$= 1.873 \text{x} 10^{-3}$ L

D 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

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

$$= 4.04 \text{ g}$$

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

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 = $11.7 \times 10^{-3} \times 0.1$

= 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

= 1,405 mg/L

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

= 1,368 mg/L

Average = 1,387 mg/L

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

= 0.6935

Appendix E Raw Data of Effect of Number of Cycles per Day

E 1. At initial feed COD value of 20,000 kg/m^3d

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	0.0128	0.0524	0.0082	0.0734	17.43	71.40	11.17
2	0.0096	0.0522	0.0116	0.0734	13.12	71.05	15.82
3	0.0090	0.0505	0.0121	0.0716	12.53	70.55	16.92
4	0.0058	0.0497	0.0142	0.0697	8.25	71.30	20.45
5	0.0031	0.0463	0.0155	0.0648	4.74	71.40	23.86
6	0.0006	0.0483	0.0165	0.0654	0.98	73.82	25.20
7	0.0001	0.0435	0.0167	0.0603	0.17	72.11	27.72
8	0.0001	0.0471	0.0203	0.0674	0.13	69.82	30.05
9	0.0002	0.0471	0.0211	0.0684	0.22	68.95	30.83
10	0.0002	0.0455	0.0210	0.0668	0.36	68.14	31.51
11	0.0002	0.0448	0.0211	0.0661	0.31	67.80	31.89
12	0.0001	0.0431	0.0211	0.0643	0.22	67.05	32.73
Avg.	0.0002	0.0445	0.0211	0.0658	0.29	67.66	32.04

Gas production rate	=	0.11	L/h
Hydrogen production rate	=	0	L/h
Specific hydrogen production rate	=	1.94	mL H_2/L d
VFA Concentration	=	11,420	mg/L as acetic acid
Hydrogen yield	=	0.85	mL H_2/g COD removed
COD removal efficiency	=	14.3	%
MLVSS	=	11,180	mg/L
VSS	=	587	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	715	8.93
Propionic acid	925.50	11.56
Butyric acid	4,239.48	52.97
Valeric acid	2,123.81	26.54

COD loading rate = 22.5 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	0.0001	0.0406	0.0207	0.0614	0.18	66.10	33.72
2	0.0006	0.0431	0.0163	0.0600	1.02	71.86	27.12
3	0.0013	0.0489	0.0194	0.0695	1.81	70.32	27.87
4	0.0021	0.0608	0.0175	0.0804	2.58	75.68	21.74
5	0.0022	0.0486	0.0181	0.0689	3.18	70.59	26.23
6	0.0057	0.0520	0.0178	0.0755	7.59	68.90	23.51
7	0.0067	0.0547	0.0164	0.0778	8.58	70.29	21.14
8	0.0046	0.0553	0.0165	0.0764	6.01	72.42	21.57
9	0.0058	0.0523	0.0162	0.0743	7.84	70.33	21.83
Avg.	0.0057	0.0541	0.0164	0.0762	7.48	71.01	21.51

Gas production rate	=	0.28	L/h
Hydrogen production rate	=	0.02	L/h
Specific hydrogen production rate	=	125.6	mL H_2/L d
VFA concentration	=	14,102	mg/L as acetic acid
Hydrogen yield	=	31.52	ml H_2/g COD removed
COD removal efficiency	=	18	%
MLVSS	=	11,480	mg/L
VSS	=	1,150	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	616.51	7.36
Propionic acid	912.21	10.89
Butyric acid	4,496.20	53.65
Valeric acid	2,355.44	28.11

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	0.0016	0.0323	0.0140	0.0479	3.44	67.40	29.16
2	0.0033	0.0380	0.0122	0.0535	6.13	71.07	22.80
3	0.0067	0.0430	0.0136	0.0633	10.53	67.99	21.48
4	0.0054	0.0427	0.0136	0.0617	8.69	69.29	22.02
5	0.0062	0.0458	0.0127	0.0647	9.64	70.79	19.57
6	0.0153	0.0395	0.0054	0.0602	25.42	65.62	8.96
7	0.0160	0.0393	0.0054	0.0607	26.31	64.78	8.91
8	0.0135	0.0407	0.0070	0.0612	22.07	66.50	11.43
9	0.0108	0.0363	0.0081	0.0552	19.54	65.76	14.71
10	0.0114	0.0434	0.0101	0.0649	17.60	66.86	15.54
11	0.0114	0.0440	0.0102	0.0656	17.44	67.04	15.52
12	0.0131	0.0486	0.0077	0.0694	18.87	70.00	11.12
13	0.0121	0.0451	0.0072	0.0644	18.83	69.99	11.18
Avg.	0.0122	0.0459	0.0084	0.0665	18.38	69.01	12.61

Gas production rate	=	0.76	L/h
Hydrogen production rate	=	0.14	L/h
Specific hydrogen production rate	=	838	mL H_2/L d
VFA concentration	=	15,314	mg/L as acetic acid
Hydrogen yield	=	119.88	ml H_2/g COD removed
COD removal efficiency	=	23.3	%
MLVSS	=	10,780	mg/L
VSS	=	1,198	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	1,605.95	16.38
Propionic acid	888.02	9.06
Butyric acid	4,892.24	49.91
Valeric acid	2,415.83	24.65

COD loading rate = 37.5 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	0.0054	0.0427	0.0136	0.0617	8.69	69.29	22.02
2	0.0062	0.0458	0.0127	0.0647	9.64	70.79	19.57
3	0.0067	0.0430	0.0136	0.0633	10.53	67.99	21.48
4	0.0163	0.0518	0.0061	0.0742	22.01	69.83	8.17
5	0.0212	0.0427	0.0037	0.0676	31.34	63.21	5.45
6	0.0171	0.0350	0.0037	0.0558	30.64	62.74	6.61
7	0.0210	0.0448	0.0045	0.0703	29.89	63.67	6.44
8	0.0159	0.0347	0.0036	0.0542	29.35	64.05	6.61
9	0.0204	0.0440	0.0045	0.0689	29.62	63.89	6.49
Avg.	0.0191	0.0412	0.0042	0.0645	29.62	63.87	6.51

Gas production rate	=	1.09	L/h
Hydrogen production rate	=	0.32	L/h
Specific hydrogen production rate	=	1,947	mL H_2/L d
VFA concentration	=	16,352	mg/L as acetic acid
Hydrogen yield	=	177.72	ml H_2/g COD removed
COD removal efficiency	=	27.4	%
MLVSS	=	10,567	mg/L
VSS	=	1,208	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	2,087.47	21.30
Propionic acid	875.00	8.93
Butyric acid	5,600.15	57.13
Valeric acid	2,737.10	27.92

E 2. At initial feed COD value of 40,000 kg/m^3d

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

Days	Amount of each compo-nent (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CO_2	CH_4		H_2	CO_2	CH_4
1	0.0003	0.0519	0.0216	0.0738	0.42	70.31	29.27
2	0.0001	0.0583	0.0196	0.0781	0.19	74.68	25.13
3	0.0013	0.0633	0.0129	0.0774	1.62	81.73	16.65
4	0.0012	0.0605	0.0116	0.0733	1.64	82.59	15.77
5	0.0027	0.0578	0.0119	0.0723	3.68	79.91	16.40
6	0.0036	0.0604	0.0129	0.0769	4.71	78.49	16.80
7	0.0038	0.0600	0.0104	0.0742	5.13	80.82	14.05
8	0.0029	0.0599	0.0103	0.0731	4.03	81.89	14.08
9	0.0030	0.0645	0.0106	0.0780	3.85	82.63	13.52
10	0.0014	0.0639	0.0121	0.0774	1.84	82.56	15.60
11	0.0014	0.0619	0.0127	0.0760	1.86	81.41	16.73
12	0.0050	0.0589	0.0082	0.0721	6.97	81.62	11.41
13	0.0053	0.0607	0.0087	0.0748	7.13	81.17	11.69
14	0.0055	0.0648	0.0094	0.0797	6.85	81.34	11.81
Avg.	0.0053	0.0614	0.0088	0.0755	6.98	81.38	11.63

Gas production rate	=	0.3	L/h
Hydrogen production rate	=	0.02	L/h
Specific hydrogen production rate	=	125.72	mL H_2/L d
VFA concentration	=	13,670	mg/L as acetic acid
Hydrogen yield	=	20.78	ml H_2/g COD removed
COD removal efficiency	=	22.4	%
MLVSS	=	15,500	mg/L
VSS	=	1,027	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	1,536.84	11.58
Propionic acid	1,670.99	12.60
Butyric acid	7,467.17	56.28
Valeric acid	2,591.91	19.54

COD loading rate = 45 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	0.0021	0.0373	0.0238	0.0632	3.27	59.02	37.71
2	0.0082	0.0624	0.0070	0.0775	10.57	80.41	9.02
3	0.0065	0.0627	0.0066	0.0758	8.61	82.70	8.69
4	0.0093	0.0628	0.0041	0.0762	12.23	82.42	5.35
5	0.0062	0.0645	0.0043	0.0750	8.29	86.03	5.68
6	0.0090	0.0683	0.0039	0.0813	11.10	84.10	4.80
7	0.0144	0.0584	0.0018	0.0746	19.33	78.31	2.37
8	0.0141	0.0642	0.0016	0.0799	17.67	80.31	2.02
9	0.0137	0.0618	0.0020	0.0774	17.73	79.74	2.53
10	0.0122	0.0584	0.0011	0.0716	17.00	81.49	1.51
Avg.	0.0133	0.0614	0.0016	0.0763	17.47	80.51	2.02

Gas production rate	=	1.29	L/h
Hydrogen production rate	=	0.22	L/h
Specific hydrogen production rate	=	1,347	mL H_2/L d
VFA concentration	=	16,957	mg/L as acetic acid
Hydrogen yield	=	117.18	ml H_2/g COD removed
COD removal efficiency	=	24.9	%
MLVSS	=	11,820	mg/L
VSS	=	1,326.67	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	1,917.43	13.64
Propionic acid	1,822.70	12.96
Butyric acid	7,648.64	54.39
Valeric acid	2,673.27	19.01

COD loading rate = 60 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	0.0168	0.0600	0	0.0769	21.90	78.10	0
2	0.0061	0.0385	0	0.0445	13.63	86.37	0
3	0.0170	0.0481	0	0.0651	26.09	73.91	0
4	0.0253	0.0535	0	0.0788	32.10	67.90	0
5	0.0265	0.0594	0	0.0859	30.81	69.19	0
6	0.0270	0.0581	0	0.0851	31.71	68.29	0
7	0.0279	0.0536	0	0.0815	34.29	65.71	0
8	0.0305	0.0577	0	0.0882	34.57	65.43	0
9	0.0299	0.0547	0	0.0846	35.31	64.69	0
Avg.	0.0294	0.0553	0	0.0848	34.72	65.28	0

Gas production rate	=	1.59	L/h
Hydrogen production rate	=	0.55	L/h
Specific hydrogen production rate	=	3,308	mL H_2/L d
VFA concentration	=	18,385	mg/L as acetic acid
Hydrogen yield	=	172.47	ml H_2/g COD removed
COD removal efficiency	=	30.4	%
MLVSS	=	12,240	mg/L
VSS	=	513.33	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	2,290.89	13.22
Propionic acid	1,501.24	8.66
Butyric acid	9,200.71	53.08
Valeric acid	4,342.23	25.05

COD loading rate = 75 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	0.0289	0.0550	0.0005	0.0844	34.25	65.17	0.59
2	0.0278	0.0543	0.0007	0.0828	33.57	65.62	0.80
3	0.0227	0.0496	0.0013	0.0736	30.83	67.39	1.79
4	0.0247	0.0544	0.0017	0.0808	30.53	67.34	2.13
5	0.0231	0.0573	0	0.0804	28.70	71.30	0
6	0.0230	0.0568	0	0.0798	28.84	71.16	0
7	0.0226	0.0633	0	0.0859	26.33	73.67	0
Avg.	0.0229	0.0591	0	0.0820	27.96	72.04	0

Gas production rate	=	1.35	L/h
Hydrogen production rate	=	0.38	L/h
Specific hydrogen production rate	=	2,265	mL H_2/L d
VFA concentration	=	23,187	mg/L as acetic acid
Hydrogen yield	=	105.43	ml H_2/g COD removed
COD removal efficiency	=	27.2	%
MLVSS	=	12,240	mg/L
VSS	=	513.33	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	3,003.05	14.43
Propionic acid	1,282.19	6.16
Butyric acid	11,019.48	52.95
Valeric acid	5,507.67	26.46

E 3. At initial feed COD value of 60,000 kg/m^3d

COD loading rate = 45 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	0.0057	0.0416	0.0035	0.0507	11.15	81.94	6.91
2	0.0198	0.0590	0.0021	0.0809	24.42	72.94	2.63
3	0.0162	0.0343	0.0003	0.0508	31.97	67.53	0.50
4	0.0229	0.0576	0.0010	0.0816	28.09	70.64	1.27
5	0.0208	0.0575	0.0009	0.0793	26.27	72.58	1.15
6	0.0255	0.0567	0.0004	0.0826	30.83	68.69	0.47
7	0.0198	0.0590	0.0003	0.0790	24.99	74.65	0.36
8	0.0193	0.0590	0.0003	0.0785	24.51	75.12	0.37
9	0.0193	0.0640	0	0.0832	23.13	76.87	0
Avg.	0.0194	0.0607	0.0002	0.0803	24.21	75.55	0.24

Gas production rate	=	0.73	L/h
Hydrogen production rate	=	0.95	L/h
Specific hydrogen production rate	=	1,060	mL H_2/L d
VFA concentration	=	18,991	mg/L as acetic acid
Hydrogen yield	=	74.26	ml H_2/g COD removed
COD removal efficiency	=	31.5	%
MLVSS	=	12,080	mg/L
VSS	=	1,160	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	434.49	8.57
Propionic acid	543.19	10.72
Butyric acid	2,712.09	53.51
Valeric acid	13,78.40	27.20

COD loading rate = 67.5 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	0.0193	0.0585	0.0003	0.0781	24.75	74.88	0.37
2	0.0238	0.0591	0	0.0829	28.73	71.27	0
3	0.0233	0.0613	0	0.0846	27.53	72.47	0
4	0.0202	0.0615	0	0.0818	24.75	75.25	0
5	0.0203	0.0607	0	0.0810	25.11	74.89	0
6	0.0196	0.0601	0	0.0797	24.64	75.36	0
7	0.0192	0.0611	0	0.0803	23.88	76.12	0
8	0.0200	0.0610	0	0.0810	24.68	75.32	0
9	0.0198	0.0607	0	0.0806	24.61	75.39	0
Avg.	0.0197	0.0610	0	0.0806	24.39	75.61	0

Gas production rate	=	0.69	L/h
Hydrogen production rate	=	0.17	L/h
Specific hydrogen production rate	=	1,010	mL H_2/L d
VFA concentration	=	19,769	mg/L as acetic acid
Hydrogen yield	=	47.74	ml H_2/g COD removed
COD removal efficiency	=	31.1	%
MLVSS	=	11,040	mg/L
VSS	=	1,209.86	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	945.97	9.52
Propionic acid	1,087.90	10.95
Butyric acid	5,275.50	53.09
Valeric acid	2,628.11	26.45

COD loading rate = 90 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	0.0231	0.0591	0	0.0822	28.11	71.89	0
2	0.0254	0.0610	0	0.0864	29.36	70.64	0
3	0.0250	0.0594	0	0.0845	29.64	70.36	0
4	0.0250	0.0588	0	0.0838	29.83	70.17	0
5	0.0226	0.0603	0	0.0829	27.28	72.72	0
6	0.0159	0.0434	0	0.0593	26.77	73.23	0
7	0.0218	0.0619	0	0.0837	26.09	73.91	0
8	0.0204	0.0638	0	0.0841	24.21	75.79	0
9	0.0188	0.0636	0	0.0824	22.87	77.13	0
Avg.	0.0204	0.0631	0	0.0834	24.39	75.61	0

Gas production rate	=	0.84	L/h
Hydrogen production rate	=	0.20	L/h
Specific hydrogen production rate	=	1,229	mL H_2/L d
VFA concentration	=	20,764	mg/L as acetic acid
Hydrogen yield	=	44.96	ml H_2/g COD removed
COD removal efficiency	=	30.2	%
MLVSS	=	12,080	mg/L
VSS	=	1,253.33	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	1,317.23	11.29
Propionic acid	1,422.32	12.20
Butyric acid	6,080.74	52.14
Valeric acid	2,842.79	24.37

COD loading rate = 112.5 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	0.0114	0.0625	0	0.0746	15.32	83.81	0.87
2	0.0316	0.0568	0	0.0887	35.61	63.95	0.44
3	0.0315	0.0532	0	0.0847	37.22	62.78	0
4	0.0289	0.0588	0	0.0878	32.94	67.06	0
5	0.0298	0.0573	0	0.0871	34.19	65.81	0
6	0.0275	0.0581	0	0.0856	32.09	67.91	0
7	0.0288	0.0565	0	0.0853	33.73	66.27	0
8	0.0293	0.0573	0	0.0865	33.85	66.15	0
9	0.0278	0.0550	0	0.0829	33.61	66.39	0
Avg.	0.0287	0.0573	0	0.0860	33.73	66.27	0

Gas production rate	=	1.49	L/h
Hydrogen production rate	=	0.50	L/h
Specific hydrogen production rate	=	3,019	mL H_2/L d
VFA concentration	=	22,322	mg/L as acetic acid
Hydrogen yield	=	83.27	ml H_2/g COD removed
COD removal efficiency	=	32	%
MLVSS	=	12,740	mg/L
VSS	=	860	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	2,300.38	13.33
Propionic acid	1,209.70	7.01
Butyric acid	9,069.50	52.57
Valeric acid	4,673.41	27.09

E 4. At initial feed COD value of 120,000 kg/m^3d

COD loading rate = 90 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	0.0033	0.0489	0	0.0523	6.40	93.60	0
2	0.0024	0.0426	0.0147	0.0598	4.09	71.29	24.62
3	0.0008	0.0404	0.0118	0.0529	1.46	76.30	22.24
4	0.0004	0.0380	0.0102	0.0485	0.77	78.29	20.94
5	0.0003	0.0352	0.0094	0.0449	0.63	78.42	20.95
6	0.0002	0.0400	0.0075	0.0478	0.47	83.83	15.70
7	0.0209	0.0591	0	0.0800	26.10	73.90	0
8	0.0247	0.0645	0	0.0893	27.72	72.28	0
9	0.0216	0.0560	0	0.0776	27.86	72.14	0
Avg.	0.0224	0.0599	0	0.0823	27.23	72.77	0

Gas production rate	=	0.36	L/h
Hydrogen production rate	=	0.098	L/h
Specific hydrogen production rate	=	588	mL H_2/L d
VFA concentration	=	27,833	mg/L as acetic acid
Hydrogen yield	=	56	ml H_2/g COD removed
COD removal efficiency	=	11.66	%
MLVSS	=	11,960	mg/L
VSS	=	880	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	1141.28	8.44
Propionic acid	848.56	6.27
Butyric acid	7984.86	59.01
Valeric acid	3555.54	26.28

COD loading rate = 135 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	0.0001	0.0224	0.0124	0.0349	0.28	64.22	35.50
2	0.0000	0.0167	0.0094	0.0262	0.00	63.94	36.06
3	0.0000	0.0168	0.0086	0.0254	0.00	66.18	33.82
4	0.0001	0.0156	0.0077	0.0234	0.46	66.72	32.82
5	0.0199	0.0619	0.0004	0.0822	24.22	75.30	0.47
6	0.0191	0.0586	0.0002	0.0779	24.47	75.21	0.32
7	0.0170	0.0587	0	0.0757	22.44	77.56	0
8	0.0165	0.0555	0	0.0720	22.93	77.07	0
9	0.0178	0.0588	0	0.0766	23.23	76.77	0
Avg.	0.0171	0.0576	0	0.0747	22.87	77.13	0

Gas production rate	=	0.24	L/h
Hydrogen production rate	=	0.0549	L/h
Specific hydrogen production rate	=	329	mL H_2/L d
VFA concentration	=	32,790	mg/L as acetic acid
Hydrogen yield	=	36.60	ml H_2/g COD removed
COD removal efficiency	=	6.66	%
MLVSS	=	11,080	mg/L
VSS	=	2,510	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	1833.39	8.29
Propionic acid	3348.20	15.14
Butyric acid	11513.59	52.07
Valeric acid	5418.50	24.50

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