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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

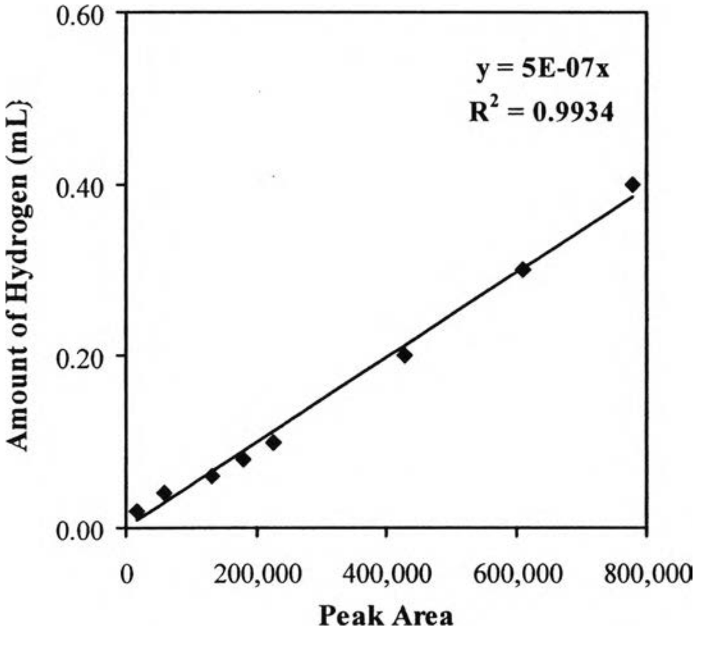

Figure A1 The relationship between amount of hydrogen (H_2) and peak area.

Equation

$$\text{Amount of hydrogen} = 5\times10^{-7} \times \text{Peak area}$$

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

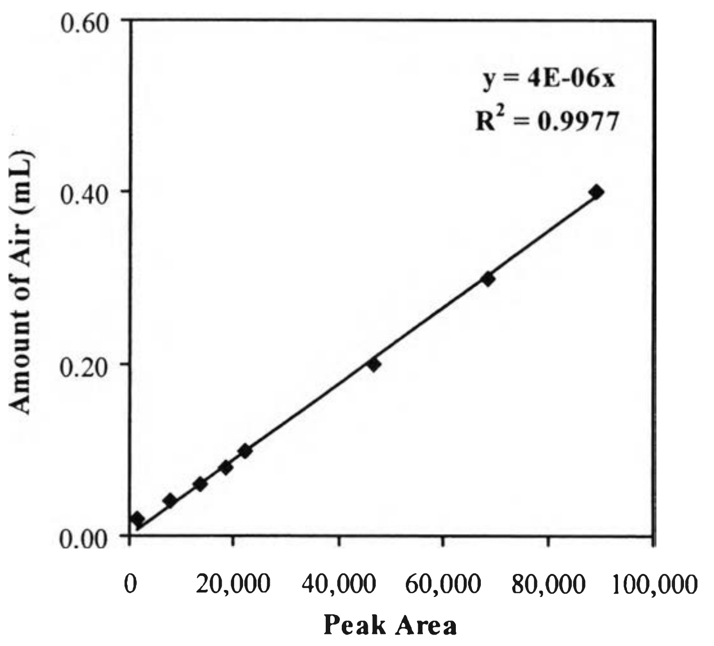

Figure A2 The relationship between amount of air and peak area.

Equation

Amount of air = $4x10^{-6}$ 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

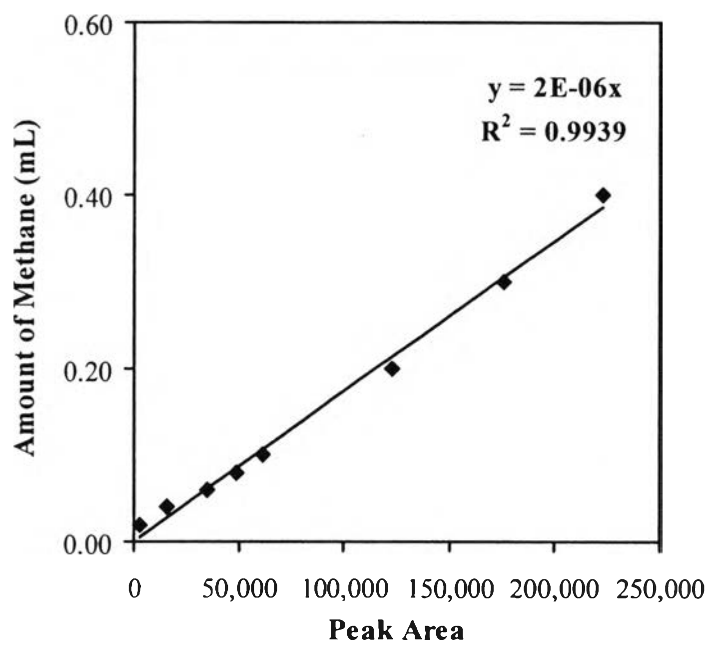

Figure A3 The relationship between amount of methane (CH_4) and peak area.

Equation

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

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

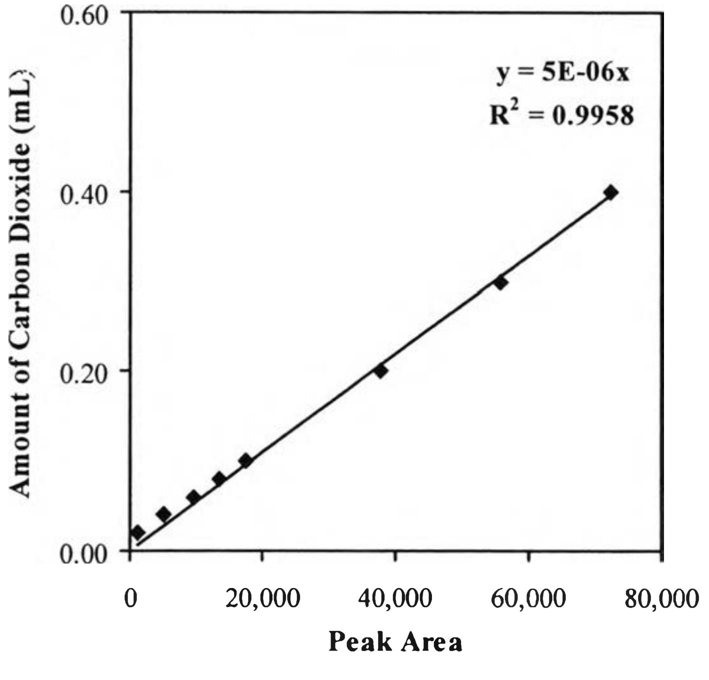

Figure A4 The relationship between amount of carbon dioxide (CO_2) and peak area.

Equation

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

Table A5 Gas chromatograph's calibration curves for acetic acid

Acetic acid concentration (ppm)	Peak area of acetic acid / Peak area of n-propanol
0	0
1,000	0.4916
2,000	0.8158
3,000	1.2596
4,000	1.5860

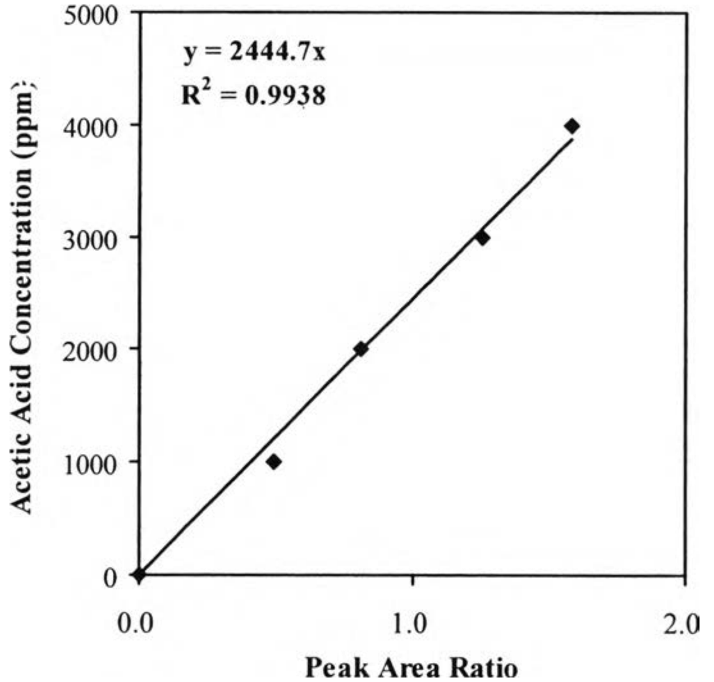

Figure A5 The relationship between acetic acid concentration and peak area ratio.

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

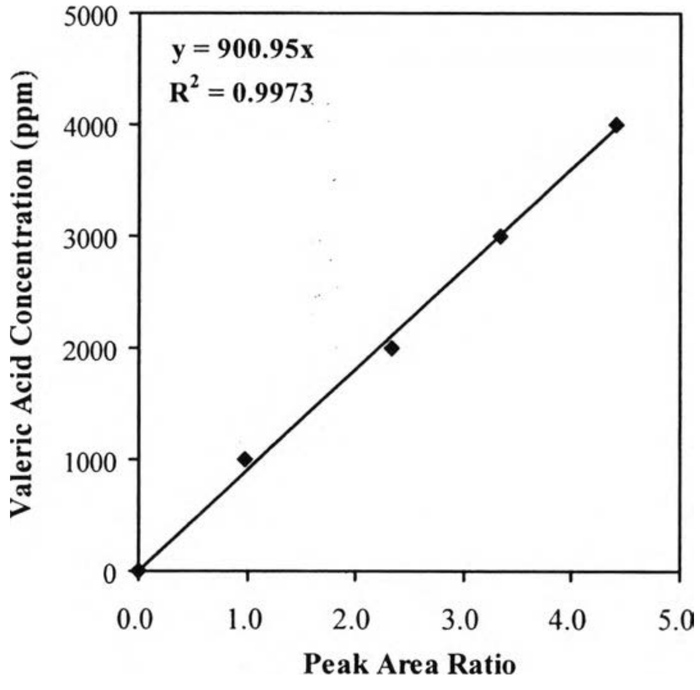

Figure A6 The relationship between valeric acid concentration and peak area ratio.

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)	$\frac{\text{Peak area of propionic acid}}{\text{Peak area of n-propanol}}$
0	0
1,000	0.6819
2,000	1.4916
3,000	2.3378
4,000	3.2784

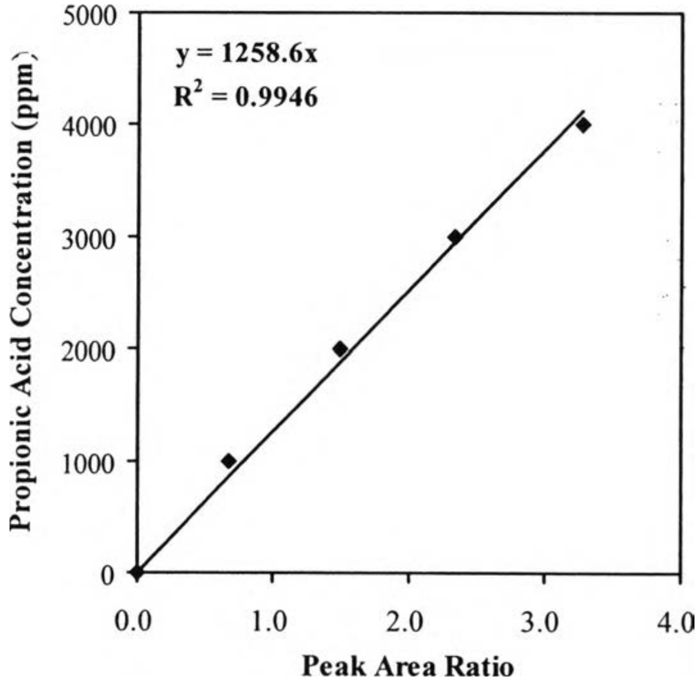

Figure A7 The relationship between propionic acid concentration and peak area ratio.

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

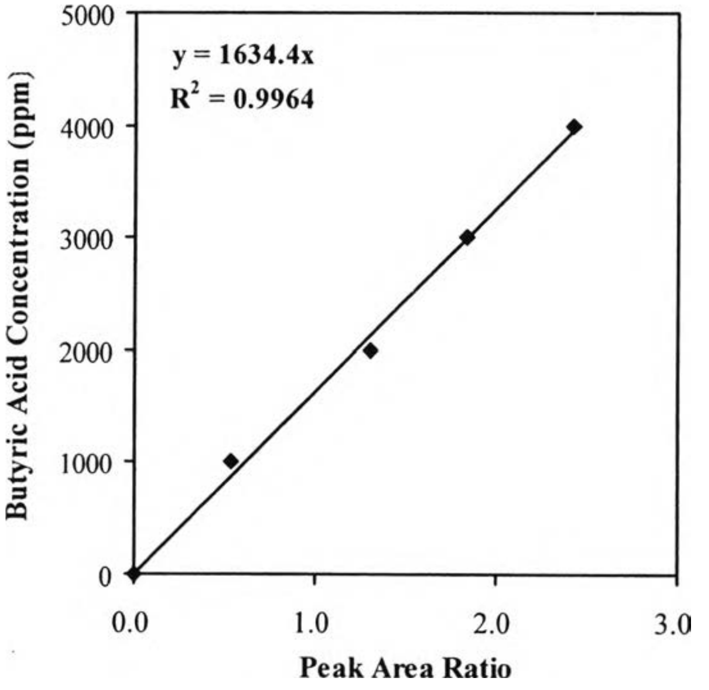

Figure A8 The relationship between butyric acid concentration and peak area ratio.

Equation

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

Table A9 Gas chromatograph's calibration curves for ethanol

Ethanol concentration (ppm)	Peak area of ethanol / Peak area of n-propanol
0	0
1,000	0.5682
2,000	1.1800
3,000	1.8570
4,000	2.4495

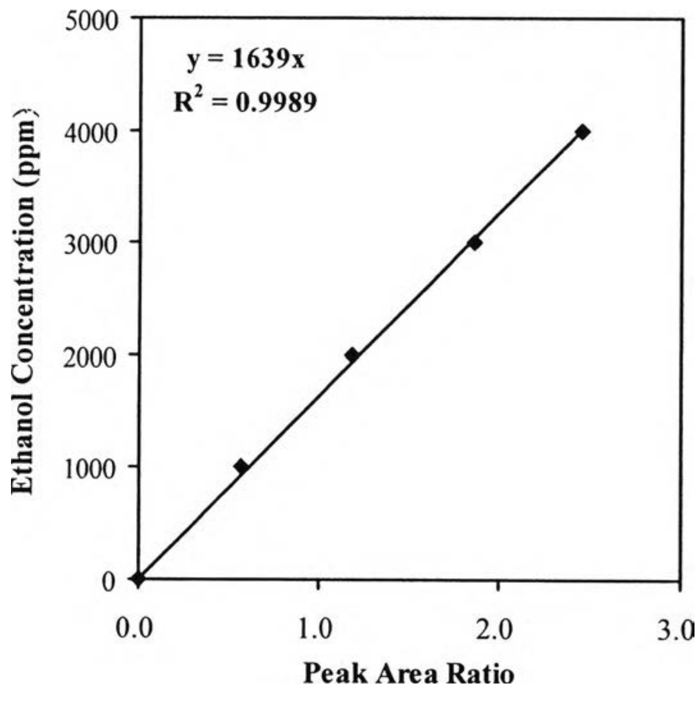

Figure A9 The relationship between ethanol concentration and peak area ratio.

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 3.3

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

Sample volume	= 5	mL
Weight of filter disk (B)	= 0.1246 g	
Weight of filter disk + dried residual at 105°C (A)	= 0.3453 g	

$$TSS = \frac{(0.3453 - 0.1246) \times 10^6}{5}$$

TSS = 44,140 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

$$N_1V_1 = N_2V_2$$

$$44.140 \times V1 = 5 \times 4$$

$$V1 = 0.453 \text{ L}$$

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

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

= 17.78 M

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

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

= 0.0333 M

Dilution of acetic acid

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

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

= 4.04 g

D 3. <u>Recovery Factor (f) Determination</u>

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. <u>4 Cycles Per day</u>

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0062	0.0184	0.0224	0.0470	13.3	39.0	47.7
2	0.0043	0.0089	0.0142	0.0274	15.6	32.5	51.9
3	0.0056	0.0198	0.0198	0.0452	9.0	31.6	59.4
4	0.0001	0.0204	0.0373	0.0578	0.2	44.7	55.1
5	0.0008	0.0195	0.0252	0.0455	2.0	47.7	50.3
6	0.0064	0.0181	0.0206	0.0451	9.5	26.6	63.9
7	0.0043	0.0201	0.0435	0.0679	5.8	27.0	67.2
8	0.0017	0.0229	0.0500	0.0746	2.4	31.7	66.0
9	0.0089	0.0146	0.0476	0.0711	11.5	18.8	69.7
10	0.0052	0.0157	0.0539	0.0748	6.9	21.1	71.9
11	0.0016	0.0224	0.0535	0.0775	2.1	29.0	69.0
12	0.0004	0.0263	0.0533	0.0800	0.6	34.9	64.5
13	0.0001	0.0290	0.0486	0.0777	0.2	37.0	62.8
Avg.	0.0003	0.0277	0.0510	0.0790	0.4	36.0	63.6

Gas production rate	=	0.78	L/h
Hydrogen production rate	=	0.0031	L/h
Specific hydrogen production rate	=	19	mL H_2/L d
VFA concentration	=	1,428	mg/L as acetic acid
Hydrogen yield	=	5	mL H_2/g COD removed
COD removal efficiency	=	48	%
MLVSS	=	9,460	mg/L
VSS	=	1,933	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	9.24	0.97.
Acetic acid	154.65	16.18
Propionic acid	73.16	7.66
Butyric acid	533.42	55.82
Valeric acid	185.09	19.37

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0014	0.0204	0.0529	0.0747	1.9	27.2	70.9
2	0.0103	0.0121	0.0558	0.0782	13.1	15.5	71.4
3	0.0275	0.0021	0.0498	0.0794	34.6	2.6	62.7
4	0.0276	0.0014	0.0500	0.0790	34.9	1.8	63.3
5	0.0226	0.0028	0.0516	0.0770	29.4	3.7	66.9
6	0.0177	0.0085	0.0472	0.0734	24.1	11.6	64.3
7	0.0202	0.0068	0.0550	0.0820	24.6	8.4	67.0
8	0.0186	0.0066	0.0530	0.0782	23.8	8.4	67.8
9	0.0177	0.0062	0.0529	0.0768	23.1	8.0	68.9
Avg.	0.0182	0.0064	0.0530	0.0775	23.5	8.1	68.4

Gas production rate	=	0.78	L/h
Hydrogen production rate	=	0.1833	L/h
Specific hydrogen production rate	=	1,100	mL H_2/L d
VFA Concentration	=	7,614	mg/L as acetic acid
Hydrogen yield	=	170	mL H_2/g COD removed
COD removal efficiency	=	56	%
MLVSS	=	8,900	mg/L
VSS	=	3,090	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	765.44	18.30
Propionic acid	399.61	9.55
Butyric acid	2,136.32	51.06
Valeric acid	882.39	21.09

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0306	0	0.0484	0.0790	38.7	0	61.3
2	0.0304	0	0.0483	0.0787	38.6	0	61.4
3	0.0306	0	0.0471	0.0777	39.4	0	60.6
4	0.0323	0	0.0515	0.0838	38.5	0	61.5
5	0.0338	0	0.0497	0.0835	40.5	0	59.5
6	0.0314	0	0.0475	0.0789	39.8	0	60.2
7	0.0297	0	0.0470	0.0767	38.7	0	61.3
8	0.0298	0	0.0490	0.0788	37.8	0	62.2
9	0.0287	0	0.0493	0.0780	36.8	0	63.2
Avg.	0.0293	0	0.0492	0.0784	37.3	0	62.7

Gas production rate	=	0.75	L/h
Hydrogen production rate	=	0.2798	L/h
Specific hydrogen production rate	=	1,679	mL H_2/L d
VFA concentration	=	8,133	mg/L as acetic acid
Hydrogen yield	=	141	ml H_2/g COD removed
COD removal efficiency	=	67	%

MLVSS	=	7,400	mg/L
VSS	=	494	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	610.44	14.29
Propionic acid	316.79	7.42
Butyric acid	2,323.46	54.39
Valeric acid	1,021.50	23.91

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0248	0	0.0548	0.0796	31.1	0	44.06
2	0.0260	0	0.0533	0.0793	32.7	0	53.56
3	0.0236	0.0002	0.0502	0.0739	31.9	0.2	55.26
4	0.0252	0.0005	0.0538	0.0796	31.7	0.6	57.47
5	0.0242	0.0013	0.0521	0.0776	31.1	1.7	66.64
6	0.0193	0.0001	0.0439	0.0633	30.5	0.2	62.20
Avg.	0.0218	0.0007	0.0480	0.0705	30.8	0.9	68.3

Gas production rate	=	0.54	L/h
Hydrogen production rate	=	0.1663	L/h
Specific hydrogen production rate	=	998	mL H_2/L d
VFA concentration	=	9,560	mg/L as acetic acid
Hydrogen yield	=	62	ml H_2/g COD removed
COD removal efficiency	=	66	%

MLVSS	=	7,240	mg/L
VSS	=	590	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	473.54	8.14
Propionic acid	332.14	5.71
Butyric acid	3,731.50	64.14
Valeric acid	1,280.52	22.01

E 2. <u>6 Cycles per Day</u>

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0080	0.0134	0.0258	0.0472	16.9	28.5	54.7
2	0.0076	0.0149	0.0299	0.0524	14.4	28.4	57.1
3	0.0057	0.0190	0.0353	0.0600	9.5	31.6	58.9
4	0.0017	0.0272	0.0375	0.0664	2.5	41.0	56.5
5	0.0009	0.0350	0.0376	0.0735	1.2	47.7	51.1
6	0.0039	0.0100	0.0195	0.0334	10.8	31.2	57.9
7	0.0097	0.0147	0.0482	0.0726	13.3	20.3	66.4
8	0.0069	0.0138	0.0432	0.0639	10.8	21.7	67.5
9	0.0067	0.0152	0.0481	0.0700	9.6	21.7	68.8
10	0.0066	0.0158	0.0507	0.0731	9.0	21.6	69.4
11	0.0064	0.0164	0.0532	0.0760	8.4	21.6	70.0
Avg.	0.0065	0.0161	0.0520	0.0746	8.7	21.6	69.7

Gas production rate	=	0.57	L/h
Hydrogen production rate	=	0.0496	L/h
Specific hydrogen production rate	=	298	mL H_2/L d
VFA concentration	=	1,687	mg/L as acetic acid
Hydrogen yield	=	35	ml H_2/g COD removed
COD removal efficiency	=	56	%
MLVSS	=	26,020	mg/L
VSS	=	1,387	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	380.64	44.65
Propionic acid	55.00	6.45
Butyric acid	303.83	35.64
Valeric acid	112.98	13.25

COD loading rate = 22.5 kg/ m^3d   pH = 5.5   Temperature = 37°C

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0208	0	0.0441	0.0649	32.0	0	68.0
2	0.0109	0	0.0257	0.0366	29.7	0	70.3
3	0.0216	0	0.0416	0.0632	34.2	0	65.8
4	0.0283	0	0.0518	0.0801	35.3	0	64.7
5	0.0292	0	0.0494	0.0786	37.2	0	62.8
6	0.0254	0	0.0523	0.0777	32.7	0	67.3
7	0.0301	0	0.0518	0.0819	36.8	0	63.2
8	0.0307	0	0.0514	0.0821	37.4	0	62.6
9	0.0280	0	0.0482	0.0762	36.7	0	63.3
10	0.0230	0	0.0393	0.0623	36.9	0	63.1
Avg.	0.0255	0	0.0438	0.0693	36.8	0	63.2

Gas production rate	=	1.06	L/h
Hydrogen production rate	=	0.3901	L/h
Specific hydrogen production rate	=	2,340	mL H_2/L d
VFA concentration	=	5,061	mg/L as acetic acid
Hydrogen yield	=	186	ml H_2/g COD removed
COD removal efficiency	=	57	%
MLVSS	=	11,740	mg/L
VSS	=	2,450	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	395.80	19.75
Propionic acid	121.71	6.07
Butyric acid	921.15	45.96
Valeric acid	565.44	28.21

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

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0272	0	0.0460	0.0732	37.1	0	62.9
2	0.0250	0	0.0466	0.0716	35.0	0	65.0
3	0.0248	0	0.0498	0.0746	33.2	0	66.8
4	0.0257	0	0.0513	0.0770	33.4	0	66.6
5	0.0266	0	0.0493	0.0759	35.1	0	64.9
6	0.0269	0	0.0459	0.0728	37.0	0	63.0
7	0.0318	0	0.0468	0.0786	40.5	0	59.5
8	0.0325	0	0.0458	0.0783	41.5	0	58.5
9	0.0338	0	0.0479	0.0817	41.3	0	58.7
Avg.	0.0332	0	0.0469	0.0801	41.4	0	58.6

Gas production rate	=	1.53	L/h
Hydrogen production rate	=	0.6334	L/h
Specific hydrogen production rate	=	3801	mL H_2/L d
VFA concentration	=	6,965	mg/L as acetic acid
Hydrogen yield	=	186	ml H_2/g COD removed
COD removal efficiency	=	67	%

MLVSS	=	9,840	mg/L
VSS	=	2,350	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	481.85	12.52
Propionic acid	272.99	7.10
Butyric acid	2,150.22	55.89
Valeric acid	942.39	24.49

COD loading rate = 37.5 kg/ m^3d   pH = 5.5   Temperature = 37°C

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0338	0	0.0435	0.0773	43.7	0	56.3
2	0.0295	0	0.0504	0.0799	37.0	0	63.0
3	0.0258	0	0.0504	0.0762	33.8	0	66.2
4	0.0274	0	0.0512	0.0786	34.9	0	65.1
5	0.0259	0.0001	0.0468	0.0728	35.6	0.1	64.4
6	0.0288	0.0003	0.0538	0.0829	34.7	0.4	64.9
7	0.0280	0.0004	0.0516	0.0800	35.0	0.5	64.5
8	0.0275	0.0007	0.0501	0.0783	35.2	0.8	64.0
Avg.	0.0278	0.0006	0.0509	0.0793	35.1	0.6	64.3

Gas production rate	=	1.41	L/h
Hydrogen production rate	=	0.4949	L/h
Specific hydrogen production rate	=	2969	mL H_2/L d
VFA concentration	=	8,565	mg/L as acetic acid

Hydrogen yield	=	160	ml H_2/g COD removed
COD removal efficiency	=	62	%
MLVSS	=	11,140	mg/L
VSS	=	3,260	mg/L

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

VFA	concentration (ppm)	%
Ethanol	0	0
Acetic acid	865.91	10.17
Propionic acid	880.89	10.34
Butyric acid	4,479.07	52.60
Valeric acid	2,289.94	26.89

Appendix F Raw Data of Effect of Nutrient Supplementation

COD loading rate = 37.5 kg/ m^3d (6 cycles/d) pH = 5.5

Temperature = 37°C COD:N ratio = 100:2.2

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0266	0.0010	0.0582	0.0857	31.1	1.1	67.9
2	0.0258	0.0009	0.0533	0.0800	32.2	1.2	66.6
3	0.0206	0.0009	0.0389	0.0604	34.1	1.5	64.4
4	0.0237	0.0024	0.0564	0.0826	28.7	3.0	68.3
5	0.0280	0.0030	0.0507	0.0817	34.3	3.7	62.0
6	0.0289	0.0034	0.0506	0.0829	34.9	4.1	61.0
7	0.0334	0.0027	0.0451	0.0811	41.2	3.2	55.6
8	0.0340	0.0015	0.0483	0.0838	40.6	1.8	57.6
9	0.0335	0.0009	0.0490	0.0834	40.1	1.1	58.8
10	0.0326	0.0006	0.0460	0.0793	41.2	0.7	58.1
Avg.	0.0331	0.0008	0.0475	0.0814	40.6	1.2	58.2

Gas production rate	=	2.33	L/h
Hydrogen production rate	=	0.95	L/h
Specific hydrogen production rate	=	5,676	mL H_2/L d
VFA concentration	=	5,451	mg/L as acetic acid
Hydrogen yield	=	438	ml H_2/g COD removed
COD removal efficiency	=	54	%
MLVSS	=	10,840	mg/L
VSS	=	3,180	mg/L

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

VFA	**concentration (ppm)**	**%**
Ethanol	0	0
Acetic acid	544.43	29.27
Propionic acid	46.44	2.50
Butyric acid	1,101.75	59.23
Valeric acid	167.40	9.00

COD loading rate = 37.5 kg/ m^3d (6 cycles/d) pH = 5.5

Temperature = 37°C COD:N ratio = 100:3.3

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0229	0.0011	0.0492	0.0732	31.3	1.5	67.2
2	0.0275	0.0004	0.0548	0.0827	33.3	0.4	66.3
3	0.0259	0.0005	0.0524	0.0788	32.9	0.6	66.5
4	0.0302	0.0007	0.0517	0.0826	36.6	0.8	62.6
5	0.0327	0.0007	0.0500	0.0834	39.2	0.8	59.9
6	0.0219	0.0007	0.0458	0.0684	32.0	1.0	67.0
7	0.0249	0.0007	0.0524	0.0780	31.9	0.9	67.2
8	0.0254	0.0005	0.0557	0.0816	31.1	0.6	68.3
9	0.0230	0.0007	0.0519	0.0756	30.4	1.0	68.6
Avg.	0.0244	0.0006	0.0533	0.0783	31.1	0.9	68.0

Gas production rate	=	2.19	L/h
Hydrogen production rate	=	0.68	L/h
Specific hydrogen production rate	=	4,087	mL H_2/L d
VFA concentration	=	12,112	mg/L as acetic acid
Hydrogen yield	=	296	ml H_2/g COD removed

COD removal efficiency	=	52	%
MLVSS	=	7,860	mg/L
VSS	=	2,790	mg/L

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

VFA	concentration (ppm)	%
Ethanol	852.28	11.43
Acetic acid	329.30	4.42
Propionic acid	329.88	4.42
Butyric acid	3,849.01	51.61
Valeric acid	2,096.96	28.12

COD loading rate = 37.5 kg/ m^3d (6 cycles/d) pH = 5.5

Temperature = 37°C COD:N ratio = 100:4.4

Day	Amount of each component (mL)			Total amount (mL)	Produced gas composition (%)		
	H_2	CH_4	CO_2		H_2	CH_4	CO_2
1	0.0227	0.0013	0.0496	0.0736	30.8	1.8	67.4
2	0.0241	0.0012	0.0519	0.0772	31.2	1.6	67.2
3	0.0244	0.0002	0.0495	0.0742	32.9	0.3	66.8
4	0.0290	0.0002	0.0495	0.0788	36.8	0.3	62.9
5	0.0281	0	0.0521	0.0802	35.0	0	65.0
6	0.0185	0.0001	0.0437	0.0623	29.6	0.2	70.2
7	0.0226	0	0.0569	0.0795	28.5	0	71.5
8	0.0215	0.0002	0.0579	0.0796	27.0	0.3	72.7
9	0.0218	0.0004	0.0578	0.0801	27.3	0.5	72.7
Avg.	0.0220	0.0002	0.0575	0.0797	27.6	0.1	72.3

Gas production rate	=	2.07	L/h
Hydrogen production rate	=	0.57	L/h
Specific hydrogen production rate	=	3,428	mL H_2/L d
VFA concentration	=	15,573	mg/L as acetic acid
Hydrogen yield	=	317	ml H_2/g COD removed
COD removal efficiency	=	46	%
MLVSS	=	11,600	mg/L
VSS	=	7,660	mg/L

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

VFA	concentration (ppm)	%
Ethanol	1,266.78	13.80
Acetic acid	380.40	4.15
Propionic acid	418.11	4.56
Butyric acid	4,535.62	49.43
Valeric acid	2,575.63	28.07

Appendix G Example of Calculation

I 1. Mixed Liquor Volatile Suspended Solids (MLVSS) Determination

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

Number of cycles per day = 4 cycles/d

Volume of sample	=	5	mL
Weight of residue + filter paper (105°C)	=	0.1768	g
Weight of residue + filter paper (500°C)	=	0.1295	g

$$\text{MLVSS} = \frac{(0.1768 - 0.1295\ \text{g}) \times 10^6}{5\ \text{mL}} = 9{,}460\ \text{mg/L}$$

Number of cycles/day	COD loading rate (kg/m^3d)	Volume of solution (mL)	Weight (105°C) (g)	Weight (500°C) (g)	MLVSS (mg/L)
4	10	5	0.1768	0.1295	9,460
	15	5	0.1781	0.1336	8,900
	20	5	0.1747	0.1377	7,400
	25	5	0.1727	0.1365	7,240
6	15	5	0.2707	0.1406	26,020
	22.5	5	0.2215	0.1628	11,740
	30	5	0.1958	0.1466	9,840
	37.5	5	0.1961	0.1404	11,140

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

Number of cycles per day = 4 cycles/d COD:N ratio = 100:2.2

Volume of sample = 5 mL

Weight of residue + filter paper (105°C) = 0.2162 g

Weight of residue + filter paper (500°C) = 0.1620 g

$$\text{MLVSS} = \frac{(0.2162 - 0.1620\text{ g}) \times 10^6}{5\text{ mL}} = 10{,}840 \text{ mg/L}$$

COD ratio	Volume of solution (mL)	Weight (105°C) (g)	Weight (500°C) (g)	MLVSS (mg/L)
100:2.2	5	0.2162	0.1620	10,840
100:3.3	5	0.1765	0.1372	7,860
100:4.4	5	0.2219	0.1639	11,600

I 2. Effluent Volatile Suspended Solids (VSS) Determination

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

Number of cycles per day = 4 cycles/d

Volume of sample = 15 mL

Weight of residue + filter paper (105°C) = 0.1511 g

Weight of residue + filter paper (500°C) = 0.1221 g

$$VSS = \frac{(0.1511 - 0.1221\ g) \times 10^6}{15\ mL} = 1{,}933\ mg/L$$

Number of cycles/day	COD loading rate (kg/m^3d)	Volume of solution (mL)	Weight (105°C) (g)	Weight (500°C) (g)	MLVSS (mg/L)
4	10	15	0.1511	0.1221	1,933
	15	10	0.1565	0.1256	3,090
	20	35	0.1373	0.1200	494
	25	30	0.1345	0.1168	590
6	15	15	0.1477	0.1269	1,387
	22.5	10	0.1529	0.1284	2,450
	30	10	0.1496	0.1261	2,350
	37.5	10	0.1571	0.1245	3,260

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

Number of cycles per day = 4 cycles/d COD:N ratio = 100:2.2

Volume of sample = 10 mL

Weight of residue + filter paper (105°C) = 0.1565 g

Weight of residue + filter paper (500°C) = 0.1247 g

$$VSS = \frac{(0.1565 - 0.1247\ g) \times 10^6}{10\ mL} = 3{,}180\ mg/L$$

COD ratio	Volume of solution (mL)	Weight (105°C) (g)	Weight (500°C) (g)	MLVSS (mg/L)
100:2.2	10	0.1565	0.1247	3,180
100:3.3	10	0.1520	0.1241	2,790
100:4.4	5	0.1478	0.1095	7,660

I 3. 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 = 10 kg/ m^3d pH = 5.5 Temperature = 37°C

Number of cycles per day = 4 cycles/d

Distillate = 10 mL

NaOH 0.1 M = 1.65 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{1.65 \text{ x } 0.1 \text{ x } 60{,}000}{10 \text{ x } 0.6935}$$

$$= 1{,}428 \ \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

Number of cycles/day	COD loading rate (kg/m^3d)	Volume of distillate (mL)	Volume of 0.1 M NaOH (mL)	VFA (mg/L as acetic acid)
4	10	10	1.65	1,933
	15	10	8.80	3,090
	20	10	9.40	494
	25	10	11.05	590
6	15	10	1.95	1,687
	22.5	10	5.85	5,061
	30	10	8.05	6,965
	37.5	10	9.90	8,565

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

Number of cycles per day = 4 cycles/d COD:N ratio = 100:2.2

Distillate = 10 mL

NaOH 0.1 M = 6.30 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{6.30 \times 0.1 \times 60{,}000}{10 \times 0.6935}$$

$$= 5{,}451 \ \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

COD ratio	Volume of distillate (mL)	Volume of 0.1 M NaOH (mL)	VFA (mg/L as acetic acid)
100:2.2	10	6.30	5,451
100:3.3	10	14	12,112
100:4.4	10	18	15,573

I 4. Hydrogen Yield Determination

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

Number of cycles per day = 4 cycles/d

Gas production rate = 0.78 L/h

Hydrogen fraction in produced gas = 0.004

Hydrogen production rate $= \frac{0.78\ \text{L}}{\text{h}} \times 0.004 \times \frac{1{,}000\ \text{mL}}{\text{L}} \times \frac{24\ \text{h}}{\text{d}}$

= 75 mL/d

COD removed per day $= \left(16{,}791 - 8{,}738 \frac{\text{mg}}{\text{L}}\right) \times \frac{1\ \text{g}}{1{,}000\ \text{mg}} \times \frac{2\ \text{L}}{\text{d}}$

= 16 g/d

Hydrogen yield $= \frac{\text{Hydrogen production rate}}{\text{COD removed per day}}$

$= \frac{75\ \text{mL/d}}{16\ \text{g/d}}$

= 5 mL H_2/g COD removed

Number of cycles/day	COD loading rate (kg/m^3d)	Hydrogen production rate (mL/d)	COD removed per day (g/d)	Hydrogen yield (mL H2/g COD removed)
4	10	75	16	5
	15	4,399	26	170
	20	6,714	47	141
	25	3,992	64	62
6	15	1,190	34	35
	22.5	9,362	50	186
	30	15,152	81	186
	37.5	11,878	74	160

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

Number of cycles per day = 4 cycles/d COD:N ratio = 100:2.2

Gas production rate = 2.33 L/h

Hydrogen fraction in produced gas = 0.406

Hydrogen production rate $= \frac{2.33\text{ L}}{\text{h}} \times 0.406 \times \frac{1{,}000\text{ mL}}{\text{L}} \times \frac{24\text{ h}}{\text{d}}$

$= 22{,}703$ mL/d

COD removed per day $= \left(16{,}091 - 7{,}460 \frac{\text{mg}}{\text{L}}\right) \times \frac{1\text{g}}{1{,}000\text{mg}} \times \frac{6\text{L}}{\text{d}}$

$= 52$ g/d

Hydrogen yield $= \frac{\text{Hydrogen production rate}}{\text{COD removed per day}}$

$= \frac{22{,}703\text{ mL/d}}{52\text{ g/d}}$

$= 438$ mL H_2/g COD removed

COD ratio	Hydrogen production rate (mL/d)	COD removed per day (g/d)	Hydrogen yield (mL H2/g COD removed)
100:2.2	· 22,703	52	438
100:3.3	16,346	55	296
100:4.4	13,712	43	317

I 5. <u>Specific Hydrogen Production Rate (SHPR)</u>

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

Number of cycles per day = 4 cycles/d

Hydrogen production rate = 75 mL/d

MLVSS $= 9{,}460 \frac{\text{mg}}{\text{L}} \times 4\text{ L} \times \frac{1\text{ g}}{1{,}000\text{ mg}}$

$= 38$ g VSS

SHPR (mL H_2/g VSS d) $= \frac{\text{Hydrogen production rate (mL / d)}}{\text{MLVSS (g VSS)}}$

$= \frac{75 \text{ mL / d}}{38 \text{ g VSS}}$

$= 2$ mL H_2/g VSS d

SHPR (mL H2/g VSS d) $= \frac{\text{Hydrogen production rate (mL / d)}}{\text{Working volume of reactor (L)}}$

$= \frac{75 \text{ mL / d}}{4 \text{ L}}$

$= 19$ mL H_2/L d

Number of cycles/day	COD loading rate (kg/m^3d)	Hydrogen production rate (mL/d)	MLVSS (g VSS)	SHPR (mL H_2/ g VSS d)	SHPR (mL H_2/L d)
4	10	75	38	2	19
	15	4,399	36	124	1,100
	20	6,714	30	227	1,679
	25	3,992	29	138	998
6	15	1,190	104	11	298
	22.5	9,362	47	199	2,340
	30	15,152	39	388	3,801
	37.5	11,878	44	267	2,969

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

Number of cycles per day = 4 cycles/d   COD:N ratio = 100:2.2

Hydrogen production rate $= 22{,}703$ mL/d

MLVSS $= 10{,}840 \frac{\text{mg}}{\text{L}} \times 4 \text{ L} \times \frac{1 \text{ g}}{1{,}000 \text{ mg}}$

$= 43$ g VSS

SHPR (mL H_2/g VSS d) $= \dfrac{\text{Hydrogen production rate (mL/d)}}{\text{MLVSS (g VSS)}}$

$= \dfrac{22{,}703 \text{ mL/d}}{43 \text{ g VSS}}$

$=$ 524 mL H_2/g VSS d

SHPR (mL H2/g VSS d) $= \dfrac{\text{Hydrogen production rate (mL/d)}}{\text{Working volume of reactor (L)}}$

$= \dfrac{22{,}703 \text{ mL/d}}{4 \text{ L}}$

$=$ 5,676 mL H_2/L d

COD ratio	Hydrogen production rate (mL/d)	MLVSS (g VSS)	SHPR (mL H_2/ g VSS d)	SHPR (mL H_2/L d)
100:2.2	22,703	43	524	5,676
100:3.3	16,346	31	520	4,087
100:4.4	13,712	46	296	3,428

I 6. <u>COD Removal Efficiency Determination</u>

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

Number of cycles per day = 4 cycles/d

Feed COD = 16,791 mg/L

Effluent COD = 8,738 mg/L

COD removal efficiency $= \dfrac{16{,}791 - 8{,}738 \text{ mg/L}}{16{,}791 \text{ mg/L}}$

$=$ 48%

Number of cycles/day	COD loading rate (kg/m^3d)	Feed COD (mg/L)	Effluent COD (mg/L)	COD removal efficiency (%)
4	10	16,791	8,738	48
	15	15,283	6,643	56
	20	17,736	5,873	67
	25	19,377	6,595	66
6	15	19,982	1.95	56
	22.5	19,474	5.85	57
	30	20,244	8.05	67
	37.5	15,984	6,097	62

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

Number of cycles per day = 4 cycles/d COD:N ratio = 100:2.2

Feed COD = 16,091 mg/L

Effluent COD = 7,460 mg/L

$$\text{COD removal efficiency} = \frac{16{,}091 - 7{,}460 \text{ mg/L}}{16{,}091 \text{ mg/L}}$$

$$= 54\%$$

COD ratio	Feed COD (mg/L)	Effluent COD (mg/L)	COD removal efficiency (%)
100:2.2	16,091	7,460	54
100:3.3	17,674	8,473	52
100:4.4	15,491	8,288	46

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