

REFERENCES

- Chang, J.S., Lee K.S., and Lin, P.J., (2002). Biohydrogen production with fixed-bed bioreactors. International Journal of Hydrogen Energy, 27(11-12), 1167-1174.
- Chen, X., Sun, Y., Xiu, Z., Li, X., and Zhang, D., (2006). Stoichiometric analysis of biological hydrogen production by fermentative bacteria. International Journal of Hydrogen Energy, 31(4), 539-549.
- Das, D., and Veziroglu, T.N., (2001). Hydrogen production by biological processes: a survey of literature. International Journal of Hydrogen Energy, 26(1), 13-28.
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F., (1956). Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry, 28(3), 350-356.
- Fan, Y.T., Zhang, G.S., Guo, X.Y., Xing, Y., and Fan, M.H., (2006). Biohydrogen production from beer lees biomass by cow dung compost. International Journal of Hydrogen Energy, 30(5), 493-496.
- Fang, H.H.P., and Liu, H., (2002). Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technology, 82(1), 87-93.
- Gavala, H.N., Skiadas, I.V., and Ahring, B.K., (2006). Biological hydrogen production in suspended and attached growth anaerobic reactor systems. International Journal of Hydrogen Energy, 31(9), 1164-1175.
- Ginkel, S.T., Oh, and Logan, B.E., (2005). Biohydrogen gas production from food processing and domestic wastewaters. International Journal of Hydrogen Energy, 30(15), 1535-1542.
- Greenberg, A.E., Clesceri, L.S., and Eaton, A.D., (1992). Standard Methods for The Examination of Water and Wastewater. Washington, DC: American Public Health Association.
- Hallenbeck, P.C., and Benemann, T.N., (2002). Biological hydrogen production; fundamentals and limiting processes. International Journal of Hydrogen Energy, 27(11-12), 1185-1193.

- Han, S.K., and Shin, H.S., (2004). Biohydrogen production by anaerobic fermentation of food waste. International Journal of Hydrogen Energy, 29(6), 569-577.
- Hawkes, F.R., Dinsdale, R., Hawkes, D.L., and Hussy, I., (2002). Sustainable fermentative hydrogen production: challenges for process optimisation. International Journal of Hydrogen Energy, 27(11-12), 1339-1347.
- Horiuchi, J.I., Shimizu, T., Tada, K., Kanno, T., and Kobayashi, M., (2002). Selective production of organic acids in anaerobic acid reactor by pH control. Bioresource Technology, 82(3), 209-213.
- Jacobson, M.Z., (2002). Atmospheric Pollution History, Science, and Regulation. Cambridge: Cambridge University Press.
- Kim, S.H., Han, S.K., and Shin, H.S., (2004). Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. International Journal of Hydrogen Energy, 29(15), 1607-1616.
- Lay, J.J., Lee, Y.J., and Noike, T., (1999). Feasibility of biological hydrogen production from organic fraction of municipal solid waste. Water Research, 33(11), 2579-2586.
- Lin, C.Y., and Chang, R.C., (2004). Fermentative hydrogen production at ambient temperature. International Journal of Hydrogen Energy, 29(7), 715-720.
- Matcarf & Eddy. (2003). Wastewater Engineering: Treatment and Reuse. New York: McGraw-Hill.
- Mizuno, O., Dinsdale, R., Hawkes, D.L., and Noike, T., (2000). Enhancement of hydrogen production from glucose by nitrogen gas sparging. Bioresource Technology, 73(1), 59-65.
- Morimoto, M., Atsuko, M., Atif, A.A.Y., Ngan, M.A., Fakhru'l-Razi, A., Iyuke, S.E., and Bakir, A.M., (2004). Biological production of hydrogen from glucose by natural anaerobic microflora. International Journal of Hydrogen Energy, 29(7), 709-713.
- Qasim, S.R., (2003). Wastewater Treatment Plants Planning, Design and Operation. New York: McGraw-Hill.

- Oh, Y.K., Seol, E.H., Kim, J.R., and Park, S.H., (2003). Fermentative biohydrogen production by a new chemoheterotrophic bacterium *Citrobacter* sp. Y19. International Journal of Hydrogen Energy, 28(12), 1353-1359.
- Shin, H.S., Youn, J.H., and Kim, S.H., (2004). Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. International Journal of Hydrogen Energy, 29(13), 1355-1363.
- Stafford, D.A., Wheatley, B.I., and Hughes, D.E., (1980). Anaerobic Digestion. London: Applied Science Publishers LTD.
- Ueno, Y., Otsuka, S., and Morimoto, M., (1996). Hydrogen production from industrial wastewater by anaerobic microflora in chemostat culture. Journal of Fermentation and Bioengineering, 82(2), 194-197.
- Vijayaraghavan, K., Ahmad, D., and Ibrahim, M.K.B., (2006). Biohydrogen generation from jackfruit peel using anaerobic contact filter. International Journal of Hydrogen Energy, 31(5), 569-579.
- Yu, H., Zhu, Z., Hu, W., and Zhang, H., (2002). Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures. International Journal of Hydrogen Energy, 27(11-12), 1359-1365.
- Yu, H.Q., and Fang, H.H.P., (2001). Acidification of mid- and high-strength dairy wastewaters. Water Research, 35(15), 3697-3705.
- Yu, H.Q., and Fang, H.H.P., (2003). Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature. Water Research, 37(1), 55-66.
- Zhang, H., Bruns, M.A., and Logan, B.E., (2006). Biological hydrogen production by *Clostridium acetobutylicum* in an unsaturated flow reactor. Water Research, 40(4), 728-734.
- Zhang, T., Liu, H., and Fang, H.H.P., (2003). Biohydrogen production from starch in wastewater under thermophilic condition. Journal of Environmental Management, 69(2), 149-156.

APPENDICES

Appendix A Gas Chromatograph's Calibration Curves

Table A 1 Gas chromatograph's calibration curves for air

Amount of air (mL)	Peak area
0.1	4137115
0.2	9044634
0.3	13600000
0.4	18239458

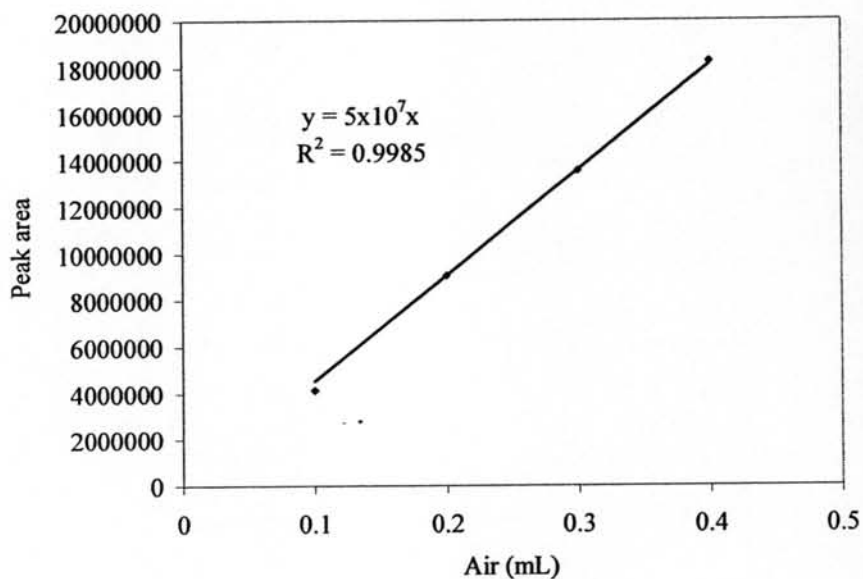


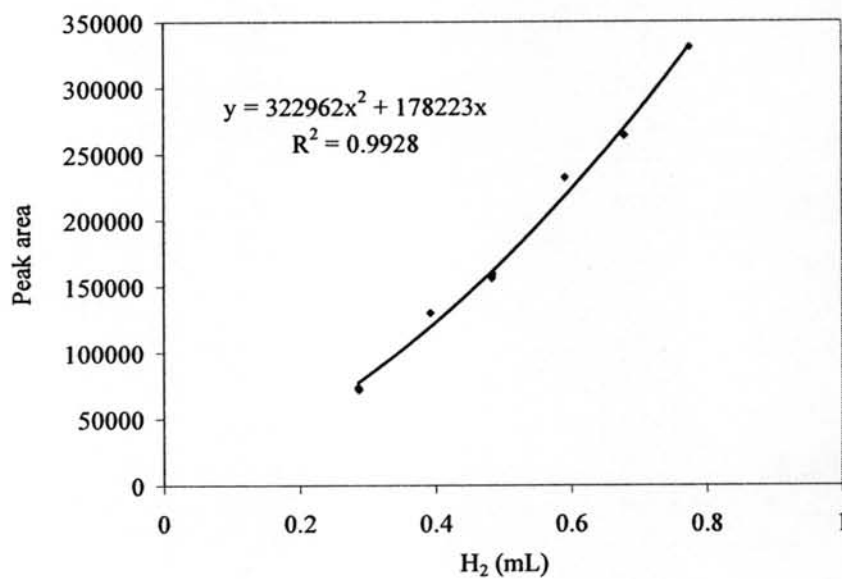
Figure A 1 The relationship between peak area and amount of air.

Relationship

$$\text{Peak area} = 5 \times 10^7 \times \text{amount of air (mL)}$$

Table A 2 Gas chromatograph's calibration curves for hydrogen (H₂)

Amount of H ₂ (mL)	Peak area
19.132	4109
18.805	5410
19.05	5104
58.66	23990
59.446	14260
59.588	18356
79.559	37565
79.784	35046

**Figure A 2** The relationship between peak area and amount of hydrogen (H₂).**Relationship**

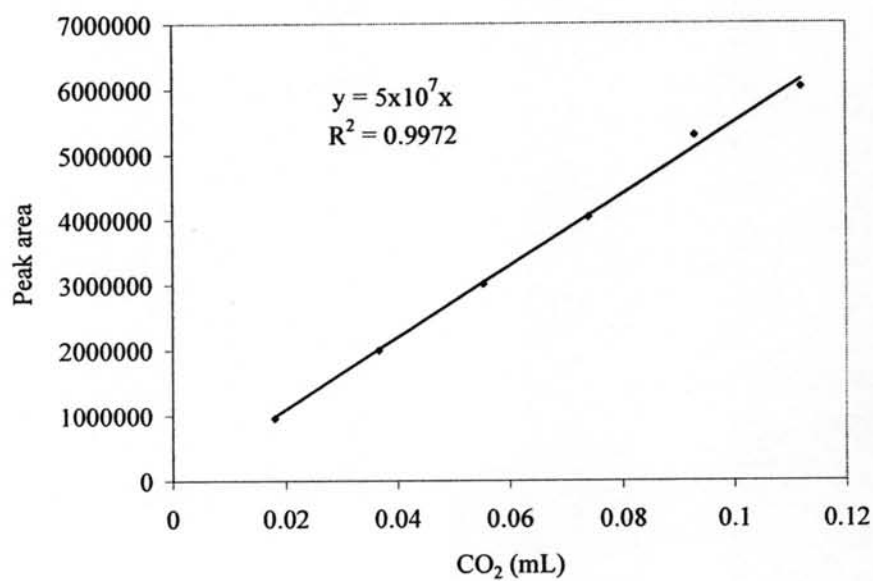
$$\text{Peak area} = 322,962 \times (X)^2 + 178,223 \times X$$

where

$$X = \text{amount of hydrogen (mL)}$$

Table A 3 Gas chromatograph's calibration curves for carbon dioxide (CO₂)

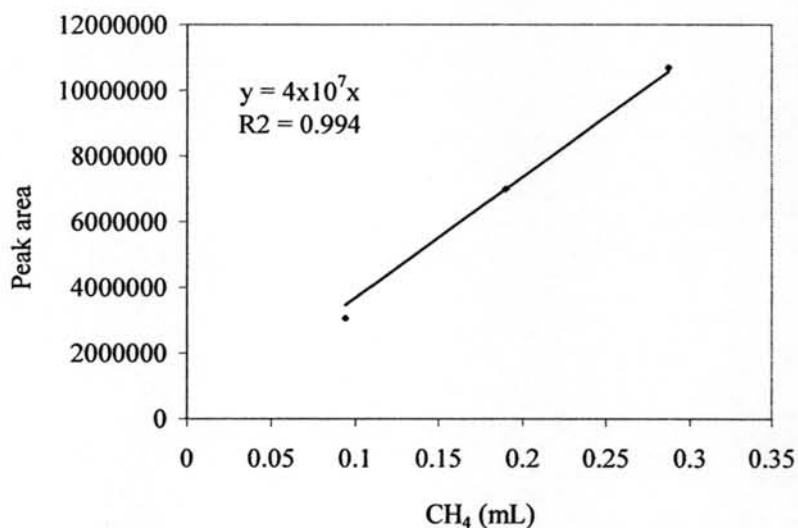
Amount of CO ₂ (mL)	Peak area
0.018	960193
0.0367	1993588
0.0554	3006185
0.074	4036503
0.093	5288153
0.112	6022949

**Figure A 3** The relationship between peak area and amount of carbon dioxide (CO₂).**Relationship**

$$\text{Peak area} = 5 \times 10^7 \times \text{amount of carbon dioxide (mL)}$$

Table A 4 Gas chromatograph's calibration curves for methane (CH₄)

Amount of CH ₄ (mL)	Peak area
0.094	3063075
0.19	7001020
0.287	10691500

**Figure A4** The relationship between peak area and amount of methane (CH₄).**Relationship**

$$\text{Peak area} = 5 \times 10^7 \times \text{amount of methane (mL)}$$

Appendix B Volatile Fatty Acids (VFA) Quantification by Distillation Method**B 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

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

$$= 17.78 \text{ M}$$

- Preparation of acetic acid at concentration of 2,000 mg L⁻¹

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

$$= 0.0333 \text{ M}$$

- Dilution of acetic acid

$$M_1 V_1 = M_2 V_2$$

$$V_1 = \frac{M_2 V_2}{M_1}$$

$$= \frac{0.0333 \times 1}{17.78}$$

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

B 2. Standard Sodium Hydroxide (0.1) Preparation

$$\text{Concentration of fresh NaOH (solid)} = 99\%$$

$$\text{Molecular weight of acetic acid} = 40$$

- Preparation of acetic acid at concentration of 0.1 M

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

$$= 4.04 \text{ g}$$

B 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)

$$\begin{aligned}
 1) \quad & \text{Distillate} \quad 50 \quad \text{mL} \quad \text{NaOH} \quad 11.7 \quad \text{mL} \\
 & \text{Used NaOH} \quad = \quad 11.7 \times 10^{-3} \times 0.1 \\
 & \quad \quad \quad = \quad 1.17 \times 10^{-3} \quad \text{mol} \\
 \therefore \text{Acetic acid in distillate} & = \quad 1.17 \times 10^{-3} \quad \text{mol} \\
 & = \quad 1.17 \times 10^{-3} \times 60.5 \\
 & = \quad 0.07 \quad \text{g}
 \end{aligned}$$

\therefore Concentration of acetic acid in distillate

$$\begin{aligned}
 & = \quad 0.07/50 \\
 & = \quad 1.405 \times 10^{-3} \quad \text{g mL}^{-1} \\
 & = \quad 1,405 \quad \text{mg L}^{-1}
 \end{aligned}$$

$$\begin{aligned}
 2) \quad & \text{Distillate} \quad 25 \quad \text{mL} \quad \text{NaOH} \quad 5.7 \quad \text{mL} \\
 & \text{Used NaOH} \quad = \quad 5.7 \times 10^{-3} \times 0.1 \\
 & \quad \quad \quad = \quad 5.7 \times 10^{-4} \quad \text{mol} \\
 \therefore \text{Acetic acid in distillate} & = \quad 5.7 \times 10^{-4} \quad \text{mol} \\
 & = \quad 5.7 \times 10^{-4} \times 60.5 \\
 & = \quad 0.034 \quad \text{g}
 \end{aligned}$$

\therefore Concentration of acetic acid in distillate

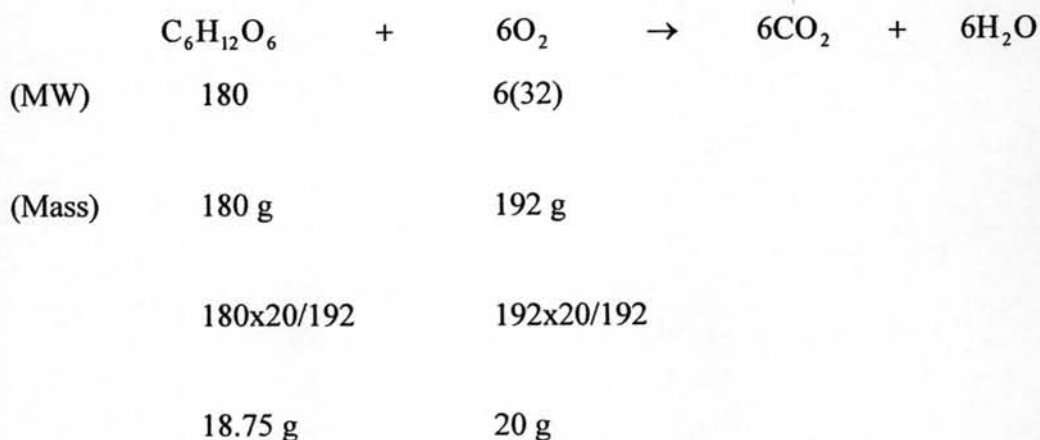
$$\begin{aligned}
 & = \quad 0.034/25 \\
 & = \quad 1.368 \times 10^{-3} \quad \text{g mL}^{-1} \\
 & = \quad 1,368 \quad \text{mg L}^{-1}
 \end{aligned}$$

$$\text{Average} \quad = \quad 1,387 \quad \text{mg L}^{-1}$$

$$\begin{aligned}
 \text{Recovery factor (f)} & = \quad 1,387/2,000 \\
 & = \quad 0.6935
 \end{aligned}$$

Appendix C Feed and Seed Sludge Preparation

C 1. Glucose Preparation (Example: COD = 20 g L⁻¹)



Desired COD = 20 g L⁻¹

Desired Glucose = 18.75 g L⁻¹

For 4 L of reactor,

Amount of glucose used = 18.75 × 4 = 75 g

C 2. Suspended Solid (SS) of Seed Sludge Determination

(1)	Weight of filter (B)	=	114.5 mg
	Weight of filter + dried residue at 100°C (A)	=	761.8 mg
	Sample volume	=	20 mL

$$\frac{\text{mg (SS)}}{\text{L}} = \frac{(A - B) \times 1,000}{\text{sample volume (mL)}}$$

$$SS = \frac{(761.8 - 114.5) \times 1,000}{20}$$

$$SS = 32,365 \text{ mg L}^{-1}$$

(2)	Weight of filter (B)	=	111.7 mg
	Weight of filter + dried residue at 100°C (A)	=	273.3 mg
	Sample volume	=	5 mL

$$\frac{\text{mg (SS)}}{\text{L}} = \frac{(A - B) \times 1,000}{\text{sample volume (mL)}}$$

$$\text{SS} = \frac{(273.3 - 111.7) \times 1,000}{5}$$

$$\text{SS} = 32,320 \text{ mg L}^{-1}$$

Average

$$\text{SS} = 32,342.5 \text{ mg L}^{-1}$$

C 3. Determination of 0.5% (W/V) of Seed Sludge

$$0.5\% \text{ (W/V)} = 0.5 \text{ g/100 mL}$$

$$32,342 \text{ mg L}^{-1} = 0.0323425 \text{ g L}^{-1}$$

- Find volume of seed sludge for adding to 4 L of reactor

$$M_1 V_1 = M_2 V_2$$

$$0.0323425 V_1 = (0.005)(4)$$

$$V_1 = 0.618 \text{ L}$$

Appendix D Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl)

Preparation for pH Control System

D 1. 1 M of NaOH Preparation

$$\text{Concentration of fresh NaOH (solid)} = 99\%$$

$$\text{Molecular weight of acetic acid} = 40$$

- Preparation of acetic acid at concentration of 1 M

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

$$= 40.404 \text{ g}$$

D 2. 1 M of HCl Preparation

$$\text{Concentration of fresh HCl (liquid)} = 37\%$$

$$\text{Density of HCl} = 1.19 \text{ g mL}^{-1}$$

$$\text{Molecular weight of HCl} = 36.46$$

- Determination of fresh HCl concentration in term of molar

$$= \frac{0.37 \text{ L of HCl}}{\text{L of solution}} \times \frac{1.17 \text{ g of HCl}}{\text{mL of HCl}} \times \frac{1 \text{ mole of HCl}}{36.46 \text{ g of HCl}}$$

$$= 11.873 \text{ M}$$

- Dilution of HCL

$$M_1 V_1 = M_2 V_2$$

$$V_1 = M_2 V_2 / M_1$$

$$= 1 \times 1 / 11.873$$

$$= 0.08422 \quad \text{L}$$

$$= 84.22 \quad \text{mL}$$

Appendix E Experimental Data of The Effect of COD Loading

E 1. Volatile Suspended Solids (VSS) Determination

Set A COD Loading Rate = 10 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

$$\text{Volume of solution} = 40 \quad \text{mL}$$

$$\text{Weight of filter paper} = 0.1211 \quad \text{g}$$

$$\text{Weight of residue + filter paper (100°C)} = 0.1515 \quad \text{g}$$

$$\text{Weight of residue + filter paper (500°C)} = 0.1225 \quad \text{g}$$

(VSS)

$$\text{VSS} = (0.1515 - 0.1225) / 40 = 7.25 \times 10^{-4} \quad \text{g mL}^{-1}$$

$$= 725 \quad \text{mg L}^{-1}$$

Set B COD Loading Rate = 20 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

$$\text{Volume of solution} = 30 \quad \text{mL}$$

$$\text{Weight of filter paper} = 0.1209 \quad \text{g}$$

$$\text{Weight of residue + filter paper (100°C)} = 0.1483 \quad \text{g}$$

$$\text{Weight of residue + filter paper (500°C)} = 0.1251 \quad \text{g}$$

$$\begin{aligned} \text{(VSS)} \\ \text{VSS} &= (0.1483-0.1251)/30 = 7.73 \times 10^{-4} \text{ g mL}^{-1} \\ &= 773 \text{ mg L}^{-1} \end{aligned}$$

Set C COD Loading Rate = 10 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

$$\begin{aligned} \text{Volume of solution} &= 30 \text{ mL} \\ \text{Weight of filter paper} &= 0.1211 \text{ g} \\ \text{Weight of residue + filter paper (100°C)} &= 0.1465 \text{ g} \\ \text{Weight of residue + filter paper (500°C)} &= 0.1226 \text{ g} \end{aligned}$$

$$\begin{aligned} \text{(VSS)} \\ \text{VSS} &= (0.1465-0.1226)/30 = 7.97 \times 10^{-4} \text{ g mL}^{-1} \\ &= 797 \text{ mg L}^{-1} \end{aligned}$$

Set D COD Loading Rate = 10 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

$$\begin{aligned} \text{Volume of solution} &= 30 \text{ mL} \\ \text{Weight of filter paper} &= 0.1211 \text{ g} \\ \text{Weight of residue + filter paper (100°C)} &= 0.1453 \text{ g} \\ \text{Weight of residue + filter paper (500°C)} &= 0.1219 \text{ g} \end{aligned}$$

$$\begin{aligned} \text{(VSS)} \\ \text{VSS} &= (0.1453-0.1219)/30 = 7.80 \times 10^{-4} \text{ g mL}^{-1} \\ &= 780 \text{ mg L}^{-1} \end{aligned}$$

E 2. Valatile Fatty Acids as Acetic Acid Determination by Distillation

Formula

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

where

$$\begin{aligned} \text{N} &= \text{Normality of NaOH solution} \\ \text{f} &= \text{recovery factor} \end{aligned}$$

Set A COD Loading Rate = $10 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,

Temperature = 37°C

Distillate = 5 mL

NaOH 0.1 M = 4.4 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{4.4 \times 0.1 \times 60,000}{5 \times 0.6935}$$

$$= 7,613.554 \quad \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

Set B COD Loading Rate = $20 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,

Temperature = 37°C

Distillate = 5 mL

NaOH 0.1 M = 7.1 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{7.1 \times 0.1 \times 60,000}{5 \times 0.6935}$$

$$= 1,2285.5083 \quad \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

Set C COD Loading Rate = $30 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,

Temperature = 37°C

Distillate = 10 mL

NaOH 0.1 M = 38 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{38 \times 0.1 \times 60,000}{10 \times 0.6935}$$

$$= 32,876.7123 \quad \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

Set D COD Loading Rate = $40 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,

Temperature = 37°C

Distillate = 10 mL

NaOH 0.1 M = 40 mL

$$\frac{\text{mg valitile acids as acetic acid}}{\text{L}} = \frac{40 \times 0.1 \times 60,000}{10 \times 0.6935}$$

$$= 34,607.0656 \quad \frac{\text{mg VFA as acetic acid}}{\text{L}}$$

E 3. Glucose Determination in Effluent using UV Spectrophotometer

Formula

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

Set A COD Loading Rate = 10 kg m⁻³ d¹, pH = Not Controlled,

Temperature = 37°C

$$A(\text{sample blank}) = 0.375$$

$$A(\text{reagent blank}) = 0.026$$

$$A(\text{total blank}) = 0.401$$

$$A(\text{test}) = 0.536$$

$$\Delta A = A(\text{test}) - A(\text{total blank}) = 0.536 - 0.401 = 0.135$$

$$\text{mg glucose/mL} = (0.1351 \times 2 \times 10 \times 0.029) / 2$$

$$= 0.03919 \quad \text{g/L}$$

$$\% \text{glucose removal} = (9.375 - 0.03919) \times 100 / 9.375$$

$$= 99.582\%$$

Set B COD Loading Rate = 20 kg m⁻³ d¹, pH = Not Controlled,

Temperature = 37°C

$$A(\text{sample blank}) = 0.695$$

$$A(\text{reagent blank}) = 0.027$$

$$A(\text{total blank}) = 0.722$$

$$A(\text{test}) = 1.246$$

$$\Delta A = A(\text{test}) - A(\text{total blank}) = 1.246 - 0.722 = 0.5024$$

$$\text{mg glucose/mL} = (0.5024 \times 2 \times 10 \times 0.029) / 2$$

$$= 0.146 \quad \text{g/L}$$

$$\% \text{glucose removal} = (18.75 - 0.1457) \times 100 / 18.75$$

$$= 99.233\%$$

Set C COD Loading Rate = 30 kg m⁻³ d¹, pH = Not Controlled,

Temperature = 37°C

$$A(\text{sample blank}) = 1.068$$

$$A(\text{reagent blank}) = 0.026$$

$$A(\text{total blank}) = 1.094$$

$$\begin{aligned}
 A(\text{test}) &= 1.896 \\
 \Delta A &= A(\text{test}) - A(\text{total blank}) = 1.896 - 1.094 = 0.8020 \\
 \text{mg glucose/mL} &= (0.8020 \times 2 \times 10 \times 0.029) / 2 \\
 &= 0.2326 \quad \text{g/L} \\
 \% \text{glucose removal} &= (28.125 - 0.2326) \times 100 / 9.375 \\
 &= 99.173\%
 \end{aligned}$$

*Set D COD Loading Rate = 40 kg m⁻³ d¹, pH = Not Controlled,
Temperature = 37°C*

$$\begin{aligned}
 A(\text{sample blank}) &= 0.642 \\
 A(\text{reagent blank}) &= 0.026 \\
 A(\text{total blank}) &= 0.668 \\
 A(\text{test}) &= 1.8486 \\
 \Delta A &= A(\text{test}) - A(\text{total blank}) = 1.8486 - 0.668 = 1.1806 \\
 \text{mg glucose/mL} &= (1.1806 \times 2 \times 10 \times 0.029) / 2 \\
 &= 0.3424 \quad \text{g/L} \\
 \% \text{glucose removal} &= (37.5 - 0.3424) \times 100 / 37.5 \\
 &= 99.087\%
 \end{aligned}$$

E 4. Hydrogen Productivity (Yield of Hydrogen) Determination

*Set A COD Loading Rate = 10 kg m⁻³ d¹, pH = Not Controlled,
Temperature = 37°C*

$$\begin{aligned}
 \text{Amount of used glucose} &= 9.375 - 0.03919 \\
 &= 9.3358 \text{ g L}^{-1} \\
 (1 \text{ day}) \text{ Amount of used glucose} &= 9.3358 \times 4 \\
 &= 37.3432 \text{ g} \\
 \text{Mole of used glucose} &= 37.3432 / 180 \\
 &= 0.2075 \text{ mole} \\
 \text{Volume of H}_2 \text{ in 1 day} &= 0.2 \times 0.274 \times 24 \\
 &= 1.3152 \text{ L}
 \end{aligned}$$

Mole of H₂ produce in 1 day

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

$$n = \frac{1 \times 1.3152}{0.082 \times (273 + 25)}$$

$$= 0.0538 \text{ mole of H}_2$$

$$\text{Yield of hydrogen production} = \frac{0.0538 \text{ mole of H}_2}{0.2075 \text{ mole of glucose}}$$

$$= 0.2594 \frac{\text{mole of H}_2}{\text{mole of glucose}}$$

Set B COD Loading Rate = 20 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

$$\text{Amount of used glucose} = 18.75 - 0.1457$$

$$= 18.6043 \text{ g L}^{-1}$$

$$(1 \text{ day}) \text{ Amount of used glucose} = 18.6043 \times 4$$

$$= 74.4172 \text{ g}$$

$$\text{Mole of used glucose} = 74.4172 / 180$$

$$= 0.4134 \text{ mole}$$

$$\text{Volume of H}_2 \text{ in 1 day} = 1.0908 \times 0.32 \times 24$$

$$= 8.3773 \text{ L}$$

Mole of H₂ produce in 1 day

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

$$n = \frac{1 \times 8.3773}{0.082 \times (273 + 25)}$$

$$= 0.3428 \text{ mole of H}_2$$

$$\text{Yield of hydrogen production} = \frac{0.3428 \text{ mole of H}_2}{0.4134 \text{ mole of glucose}}$$

$$= 0.8292 \frac{\text{mole of H}_2}{\text{mole of glucose}}$$

Set C COD Loading Rate = 30 kg m⁻³ d¹, pH = Not Controlled,

Temperature = 37°C

$$\begin{aligned}
 \text{Amount of used glucose} &= 28.125-0.2326 \\
 &= 27.8924 \quad \text{g L}^{-1} \\
 \text{(1 day) Amount of used glucose} &= 27.8924 \times 4 \\
 &= 111.5696 \text{ g} \\
 \text{Mole of used glucose} &= 111.5696/180 \\
 &= 0.6198 \text{ mole} \\
 \text{Volume of H}_2 \text{ in 1 day} &= 2.085 \times 0.35 \times 24 \\
 &= 17.514 \text{ L}
 \end{aligned}$$

Mole of H₂ produce in 1 day

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

$$\begin{aligned}
 n &= \frac{1 \times 17.514}{0.082 \times (273 + 25)} \\
 &= 0.7167 \text{ mole of H}_2 \\
 \text{Yield of hydrogen production} &= \frac{0.7167 \text{ mole of H}_2}{0.6198 \text{ mole of glucose}} \\
 &= 1.156 \frac{\text{mole of H}_2}{\text{mole of glucose}}
 \end{aligned}$$

Set D COD Loading Rate = 40 kg m⁻³ d¹, pH = Not Controlled,

Temperature = 37°C

$$\begin{aligned}
 \text{Amount of used glucose} &= 37.5-0.3424 \\
 &= 37.1576 \quad \text{g L}^{-1} \\
 \text{(1 day) Amount of used glucose} &= 37.1576 \times 4 \\
 &= 148.6304 \text{ g} \\
 \text{Mole of used glucose} &= 148.6304/180 \\
 &= 0.826 \text{ mole} \\
 \text{Volume of H}_2 \text{ in 1 day} &= 2.1435 \times 0.38 \times 24 \\
 &= 19.5487 \text{ L}
 \end{aligned}$$

Mole of H₂ produce in 1 day

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

$$n = \frac{1 \times 19.5487}{0.082 \times (273 + 25)}$$

$$= 0.7999 \text{ mole of H}_2$$

$$\text{Yield of hydrogen production} = \frac{0.7999 \text{ mole of H}_2}{0.826 \text{ mole of glucose}}$$

$$= 0.9684 \frac{\text{mole of H}_2}{\text{mole of glucose}}$$

E 5. COD Removal Determination

Set A COD Loading Rate = 10 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

Feed COD	=	16,000	mg L ⁻¹
Product COD	=	7,300	mg L ⁻¹
%COD removal	=	(16,000-7,300)/16,000	
	=	54.375%	

Set B COD Loading Rate = 20 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

Feed COD	=	19,500	mg L ⁻¹
Product COD	=	6,000	mg L ⁻¹
%COD removal	=	(19,500-6,000)/19,500	
	=	69.231%	

Set C COD Loading Rate = 30 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

Feed COD	=	32,000	mg L ⁻¹
Product COD	=	10,100	mg L ⁻¹
%COD removal	=	(32,000-10,100)/32,000	
	=	68.438%	

Set D COD Loading Rate = 40 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

Feed COD = 37,700 mg L⁻¹

Product COD = 19,900 mg L⁻¹

%COD removal = (37,700-19,900)/37,700

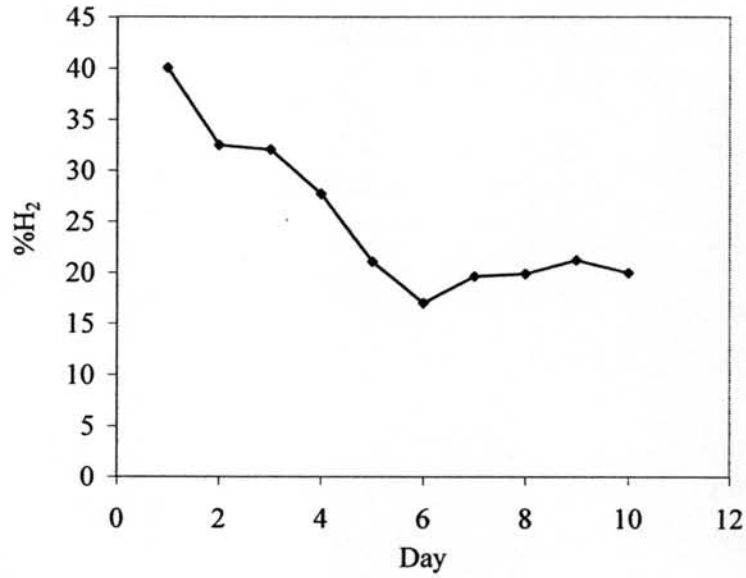
= 48.579%

E 6. Raw Data of Hydrogen Content in Produced Gas for Judging the Steady State of the Experiments

Set A COD Loading Rate = 10 kg m⁻³ d⁻¹, pH = Not Controlled,

Temperature = 37°C

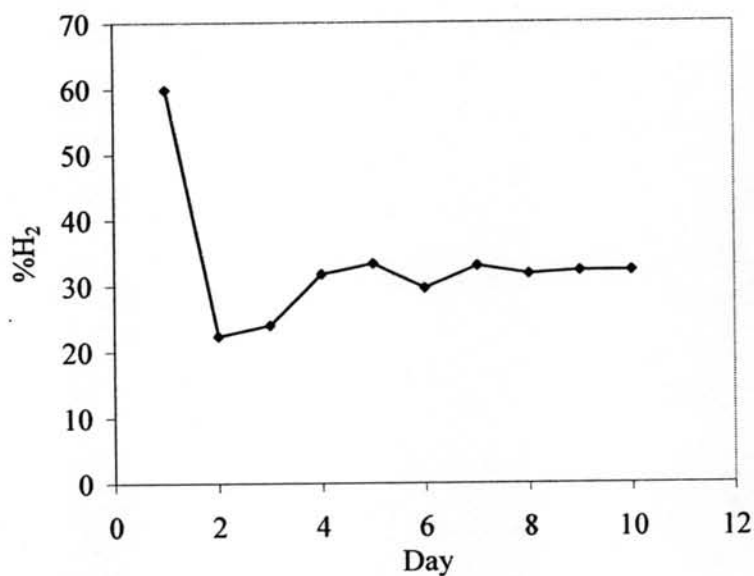
Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	40.05	0.0556	0.083224	0	10916	4161185	0
2	32.478	0.0419	0.087109	0	8046	4355450	0
3	32.065	0.0478	0.101273	0	9274	5063641	0
4	27.693	0.0469	0.122457	0	9078	6122838	0
5	21.096	0.0236	0.088271	0	4388	4413545	0
6	17.048	0.0265	0.128939	0	4964	6446952	0
7	19.682	0.0291	0.104294	0	4459	4876582	0
8	19.925	0.0227	0.091225	0	4213	4561327	0
9	21.252	0.0257	0.095231	0	4812	4761526	0
10	20.02	0.0271	0.108265	0	5078	5413251	0



Set B COD Loading Rate = 20 kg m⁻³ d⁻¹, pH = Not Controlled,

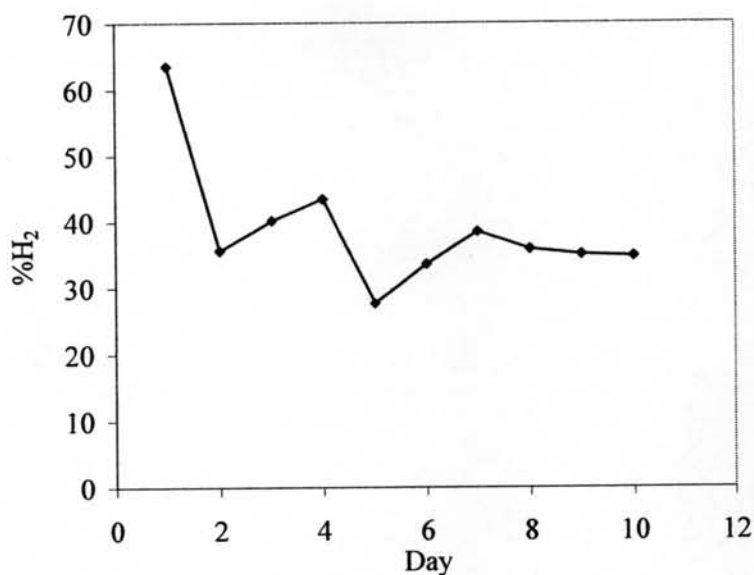
Temperature = 37°C

Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	59.83607016	0.1368	0.09182464	0	30451	4591232	0
2	22.34316661	0.03	0.10426924	0	5641	5213462	0
3	24.00103851	0.0345	0.10924378	0	6549	5462189	0
4	31.76089383	0.06	0.12891156	0	11986	6445578	0
5	33.34352769	0.0459	0.0917579	0	8871	4587895	0
6	29.63013688	0.054	0.12824688	0	10571	6412344	0
7	32.99037716	0.0612	0.12430864	0	12123	6215432	0
8	31.79134367	0.0571	0.12250864	0	11231	6125432	0
9	32.20801257	0.0458	0.09640064	0	8846	4820032	0
10	32.265437	0.0528	0.1108426	0	10324	5542130	0



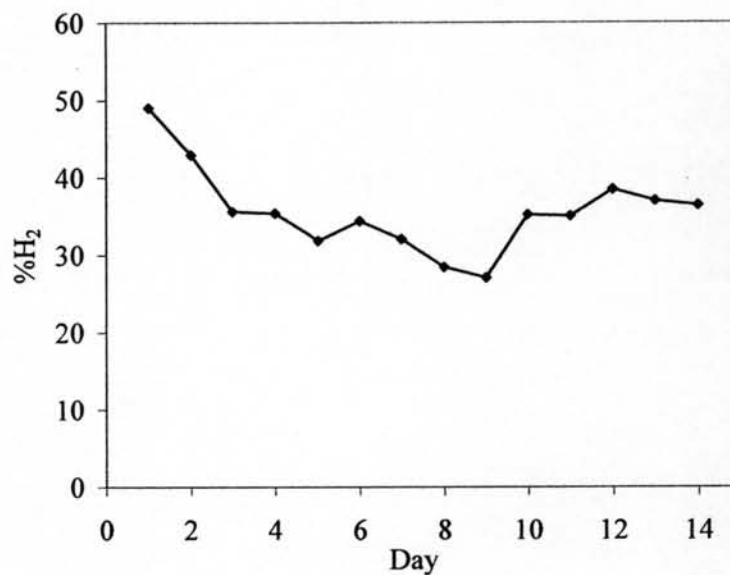
*Set C COD Loading Rate = 30 kg m⁻³ d⁻¹, pH = Not Controlled,
Temperature = 37°C*

Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	63.56143781	0.1539	0.08822794	0	35083	4411397	0
2	35.64508548	0.0614	0.11085376	0	12167	5542688	0
3	40.18381412	0.0614	0.09139784	0	12182	4569892	0
4	43.44774287	0.0811	0.10556102	0	16585	5278051	0
5	27.65161111	0.0465	0.1216638	0	8993	6083190	0
6	33.62977813	0.033	0.06512732	0	6235	3256366	0
7	38.49664857	0.0571	0.0912246	0	11235	4561230	0
8	35.88434503	0.0524	0.09362468	0	10234	4681234	0
9	35.0437791	0.0506	0.09379082	0	9845	4689541	0
10	34.74316877	0.0443	0.08320708	0	8542	4160354	0



*Set D COD Loading Rate = 40 kg m⁻³ d⁻¹, pH = Not Controlled,
Temperature = 37°C*

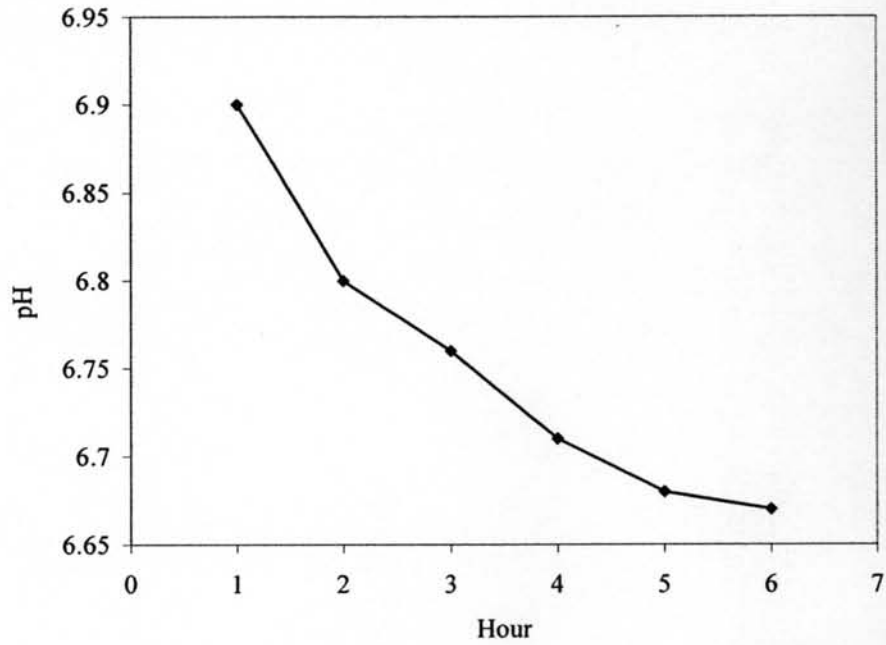
Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	49.012	0.1562	0.16249464	0	35726	8124732	0
2	42.956	0.1232	0.163604	0	26878	8180201	0
3	35.639	0.0626	0.11304914	0	12434	5652457	0
4	35.446	0.0678	0.123475	0	13582	6173726	0
5	31.835	0.0588	0.1259045	0	11599	6295225	0
6	34.402	0.06474	0.123447	0	12892	6172358	0
7	27.072	0.0492	0.13253918	0	9563	6626959	0
8	35.056	0.0661	0.1224527	0	13207	6122635	0
9	38.497	0.0763	0.121897	0	15482	6094842	0
10	31.380	0.0614	0.1342557	0	12162	6712785	0
11	36.457	0.0702	0.122352	0	14124	6117590	0
12	38.304	0.0702	0.11306884	0	15631	5653442	0
13	38.213	0.0731	0.118197	0	14765	5909842	0



E 7. Raw Data of pH of the System

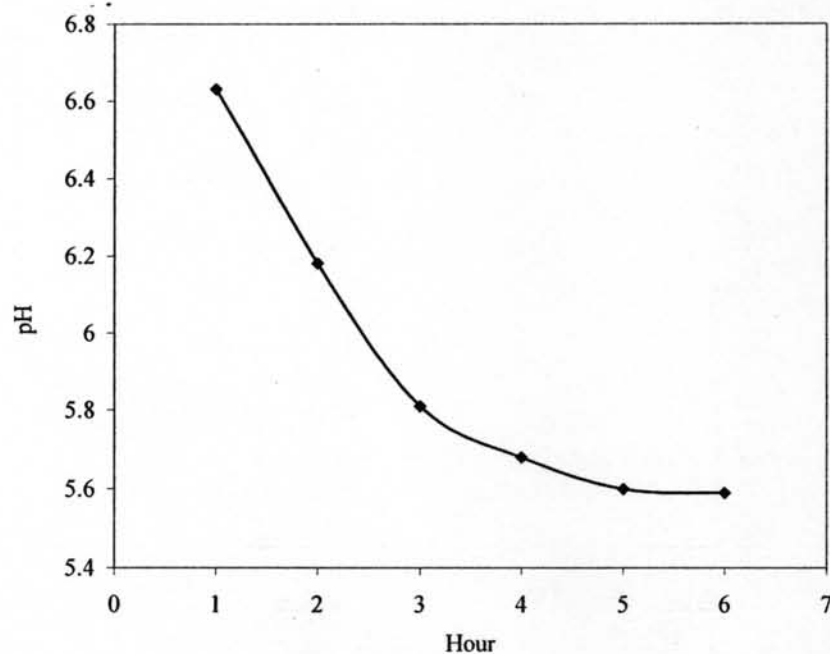
*Set A COD Loading Rate = $10 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,
Temperature = 37°C*

Hour	1	2	3	4	5	6
pH	6.9	6.8	6.76	6.71	6.67	6.67



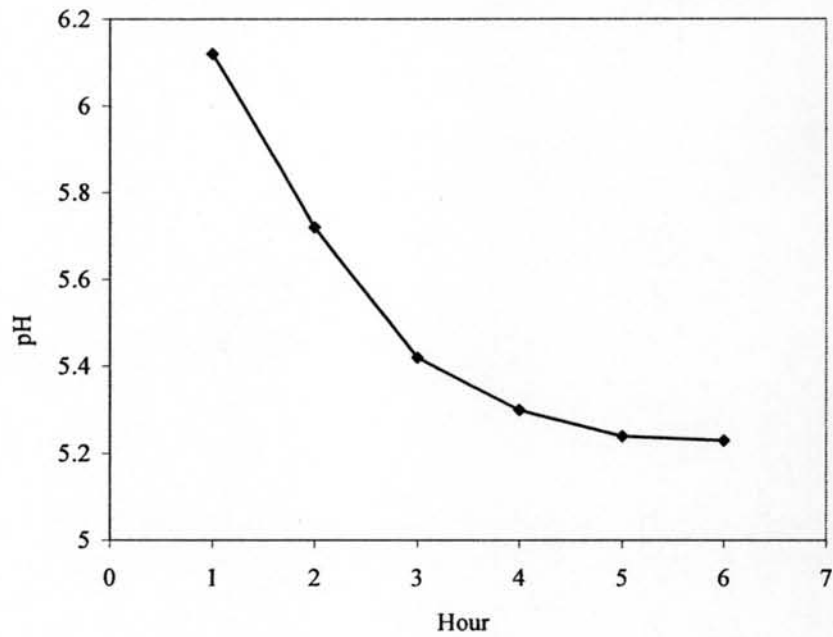
*Set B COD Loading Rate = $20 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,
Temperature = 37°C*

Hour	1	2	3	4	5	6
pH	6.63	6.18	5.81	5.63	5.60	5.59



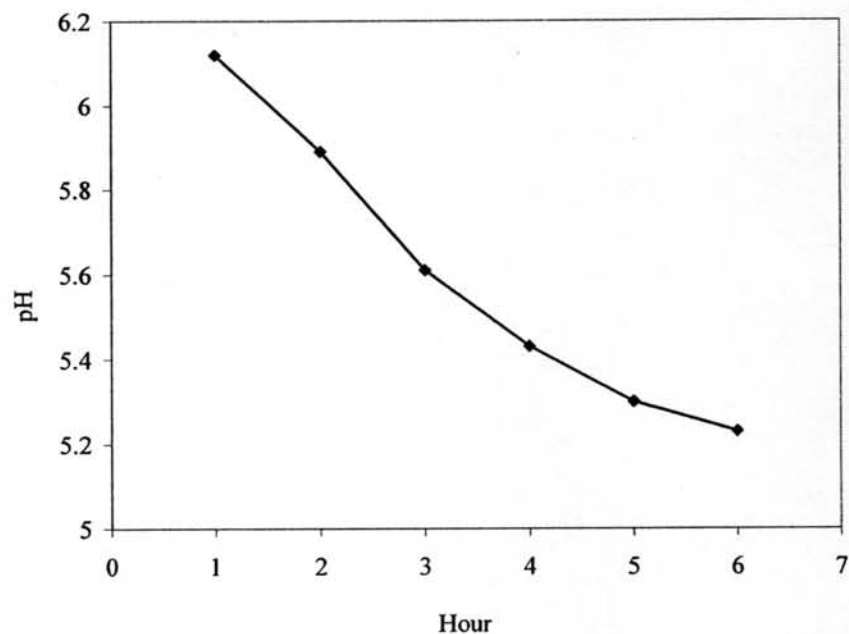
*Set C COD Loading Rate = $30 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,
Temperature = 37°C*

Hour	1	2	3	4	5	6
pH	6.12	5.72	5.42	5.30	5.24	5.23



*Set D COD Loading Rate = $40 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = Not Controlled,
Temperature = 37°C*

Hour	1	2	3	4	5	6
pH	6.12	5.86	5.67	5.47	5.36	5.23



**Appendix F Experimental Data of The Effect of COD Loading at Optimum pH
(pH = 5.5)**

F 1. Volatil Suspended Solids (VSS) Determination

Set A COD Loading Rate = $40 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = 5.5, Temperature = 37°C

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)

VSS = $(0.1676 - 0.1246) / 50 = 8.60 \times 10^{-4} \text{ g mL}^{-1}$

= 860 mg L^{-1}

Set B COD Loading Rate = $50 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = 5.5, Temperature = 37°C

Volume of solution = 50 mL

Weight of filter paper = 0.1231 g

Weight of residue + filter paper (100°C) = 0.1639 g

Weight of residue + filter paper (500°C) = 0.1230 g

$$\begin{aligned}
 & \text{(VSS)} \\
 \text{VSS} &= (0.1639-0.1230)/50 = 8.18 \times 10^{-4} \text{ g mL}^{-1} \\
 &= 818 \text{ mg L}^{-1}
 \end{aligned}$$

F 2. Valatile Fatty Acids as Acetic Acid Determination by Distillation

Formula

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

where

N = Normality of NaOH solution

f = recovery factor

Set A COD Loading Rate = 40 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

Distillate = 10 mL

NaOH 1 M = 2 mL

$$\begin{aligned}
 \frac{\text{mg valitile acids as acetic acid}}{\text{L}} &= \frac{2 \times 1 \times 60,000}{10 \times 0.6935} \\
 &= 17,303.53 \frac{\text{mg VFA as acetic acid}}{\text{L}}
 \end{aligned}$$

Set B COD Loading Rate = 50 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

Distillate = 15 mL

NaOH 1 M = 1 mL

$$\begin{aligned}
 \frac{\text{mg valitile acids as acetic acid}}{\text{L}} &= \frac{1 \times 1 \times 60,000}{15 \times 0.6935} \\
 &= 5,767.844 \frac{\text{mg VFA as acetic acid}}{\text{L}}
 \end{aligned}$$

F 3. Glucose Determination in Effluent using UV Spectrophotometer

Formula

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

Set A COD Loading Rate = $40 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = 5.5, Temperature = 37°C

$$A(\text{sample blank}) = 0.348$$

$$A(\text{reagent blank}) = 0.026$$

$$A(\text{total blank}) = 0.374$$

$$A(\text{test}) = 0.4888$$

$$\Delta A = A(\text{test}) - A(\text{total blank}) = 0.4888 - 0.374 = 0.1148$$

$$\text{mg glucose/mL} = (0.1148 \times 2 \times 10 \times 0.029) / 2$$

$$= 0.0333 \text{ g/L}$$

$$\% \text{glucose removal} = (9.375 - 0.0333) \times 100 / 9.375$$

$$= 99.645\%$$

Set B COD Loading Rate = $50 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = 5.5, Temperature = 37°C

(Note: Dilute sample 100 times)

$$A(\text{sample blank}) = 0.815$$

$$A(\text{reagent blank}) = 0.027$$

$$A(\text{total blank}) = 0.842$$

$$A(\text{test}) = 1.638$$

$$\Delta A = A(\text{test}) - A(\text{total blank}) = 1.638 - 0.842 = 0.799$$

$$\text{mg glucose/mL} = (0.799 \times 2 \times 150 \times 0.029) / 2$$

$$= 3.476 \text{ g/L}$$

$$\% \text{glucose removal} = (18.75 - 3.476) \times 100 / 18.75$$

$$= 81.462\%$$

F 4. Hydrogen Productivity (Yield of Hydrogen) Determination

Set A COD Loading Rate = $40 \text{ kg m}^{-3} \text{ d}^{-1}$, pH = 5.5, Temperature = 37°C

$$\text{Amount of used glucose} = 37.5 - 0.0333$$

$$= 37.4667 \text{ g L}^{-1}$$

$$(1 \text{ day}) \text{ Amount of used glucose} = 37.467 \times 4$$

$$= 149.868 \text{ g}$$

$$\text{Mole of used glucose} = 149.868 / 180$$

$$= 0.8326 \text{ mole}$$

$$\text{Volume of H}_2 \text{ in 1 day} = 0.43 \times 2.876 \times 24$$

$$= 29.68 \text{ L}$$

Mole of H₂ produce in 1 day

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

$$n = \frac{1 \times 29.68}{0.082 \times (273 + 25)}$$

$$= 1.215 \text{ mole of H}_2$$

$$\text{Yield of hydrogen production} = \frac{1.215 \text{ mole of H}_2}{0.8326 \text{ mole of glucose}}$$

$$= 1.46 \frac{\text{mole of H}_2}{\text{mole of glucose}}$$

Set B COD Loading Rate = 50 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

$$\text{Amount of used glucose} = 46.875 - 3.476$$

$$= 43.399 \text{ g L}^{-1}$$

$$(1 \text{ day}) \text{ Amount of used glucose} = 43.399 \times 4$$

$$= 173.596 \text{ g}$$

$$\text{Mole of used glucose} = 173.596 / 180$$

$$= 0.964 \text{ mole}$$

$$\text{Volume of H}_2 \text{ in 1 day} = 0.3 \times 1.328 \times 24$$

$$= 9.562 \text{ L}$$

Mole of H₂ produce in 1 day

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

$$n = \frac{1 \times 9.562}{0.082 \times (273 + 25)}$$

$$= 0.391 \text{ mole of H}_2$$

$$\text{Yield of hydrogen production} = \frac{0.391 \text{ mole of H}_2}{0.964 \text{ mole of glucose}}$$

$$= 0.406 \frac{\text{mole of H}_2}{\text{mole of glucose}}$$

F 5. COD Removal Determination

Set A COD Loading Rate = 40 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

$$\begin{aligned} \text{Feed COD} &= 41,500 \text{ mg L}^{-1} \\ \text{Product COD} &= 8,200 \text{ mg L}^{-1} \\ \text{\%COD removal} &= (41,500-8,200)/41,500 \\ &= 80.241\% \end{aligned}$$

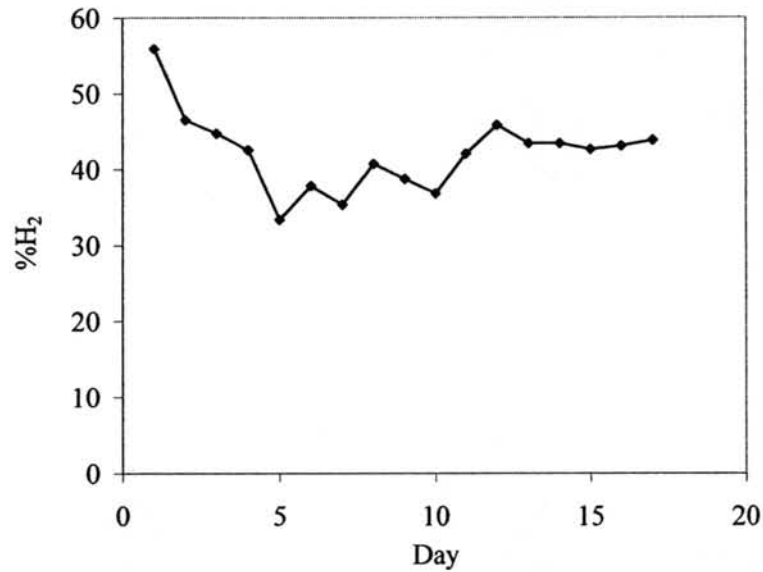
Set B COD Loading Rate = 50 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

$$\begin{aligned} \text{Feed COD} &= 53,500 \text{ mg L}^{-1} \\ \text{Product COD} &= 31,000 \text{ mg L}^{-1} \\ \text{\%COD removal} &= (53,500-31,000)/53,500 \\ &= 42.056\% \end{aligned}$$

F 6. Raw Data of Hydrogen Content in Produced Gas for Judging the Steady State

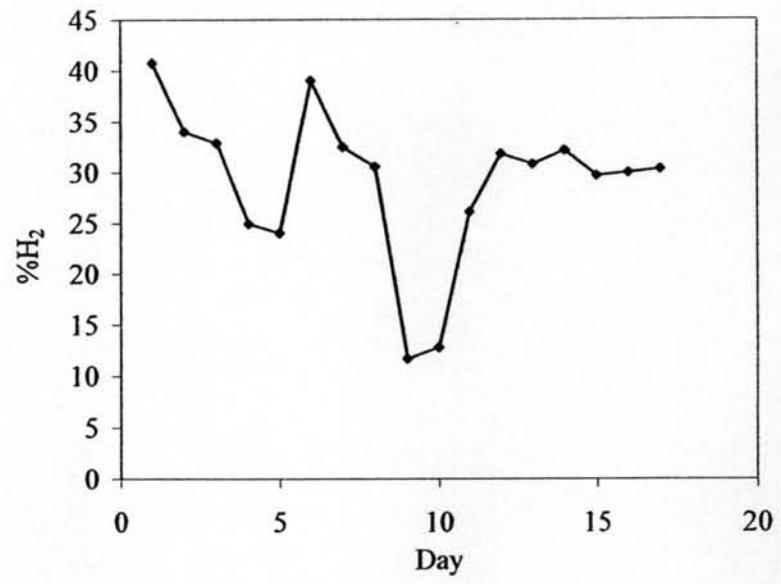
Set A COD Loading Rate = 40 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	55.92518325	0.0622	0.04902002	0	12354	2451001	0
2	46.45882489	0.0679	0.07825092	0	13604	3912546	0
3	44.73693549	0.0711	0.08782908	0	14324	4391454	0
4	42.5235306	0.0623	0.08420712	0	12372	4210356	0
5	33.36837998	0.0452	0.09025758	0	8717	4512879	0
6	37.80323442	0.0443	0.07288574	0	8542	3644287	0
7	35.35384982	0.0469	0.08575882	0	9071	4287941	0
8	40.70471146	0.0703	0.10240728	0	14127	5120364	0
9	38.73670561	0.0694	0.10975824	0	13933	5487912	0
10	36.80960134	0.0463	0.0794824	0	8945	3974120	0
11	42.03269394	0.0707	0.0975024	0	14232	4875120	0
12	45.82339602	0.06	0.07093748	0	11874	3546874	0
13	43.43384211	0.0464	0.06042914	0	8974	3021457	0
14	43.43095241	0.0524	0.06825128	0	10325	3412564	0
15	42.64228554	0.0493	0.06631294	0	9754	3315647	0
16	43.08033642	0.0569	0.07517882	0	11203	3758941	0
17	43.83650902	0.0507	0.06495702	0	9875	3247851	0



Set B COD Loading Rate = 50 kg m⁻³ d⁻¹, pH = 5.5, Temperature = 37°C

Day	%H ₂	Amount of each component (mL)			Peak area		
		H ₂	CO ₂	CH ₄	H ₂	CO ₂	CH ₄
1	40.75108578	0.0631	0.0917425	0	12543	4587125	0
2	34.01897701	0.0573	0.1111354	0	11294	5556770	0
3	32.93008613	0.054	0.10998378	0	10586	5499189	0
4	24.97538411	0.0462	0.13878214	0	8941	6939107	0
5	24.05166934	0.0469	0.14809686	0	9087	7404843	0
6	39.04549075	0.0809	0.12629422	0	16545	6314711	0
7	32.52147805	0.0633	0.1313406	0	12595	6567030	0
8	30.58035015	0.0556	0.1262161	0	10906	6310805	0
9	11.71190281	0.0126	0.09498286	0	2315	4749143	0
10	12.78057133	0.0186	0.1269334	0	3429	6346670	0
11	26.112346	0.0425	0.12025826	0	8167	6012913	0
12	31.81851624	0.0506	0.1084269	0	9845	5421345	0
13	30.83937031	0.0622	0.13949024	0	12354	6974512	0
14	32.17366315	0.0676	0.14250974	0	13542	7125487	0
15	29.69693313	0.0507	0.1200247	0	9874	6001235	0
16	29.9983578	0.0525	0.12250958	0	10254	6125479	0
17	30.35112937	0.0512	0.11749224	0	9987	5874612	0



CURRICULUM VITAE

Name : Hannarong Neramitsuk

Birth date : March 17, 1983

Nationality : Thai

University Education :

2001-2004 Bachelor Degree of Chemical Engineering, Faculty of Engineering, King Mongkut's Institute of Technology North Bangkok, Bangkok, Thailand

Working Experience:

2004

Position: Internship Student

Company name: Packgo Access Company