CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of COD Loading Rate without and with pH Control

The effect of pH on biohydrogen production was first investigated in order to compare with the results from the previous work (Neramitsuk *et al.*, 2007). In the previous work, anaerobic sequencing batch reactor (ASBR) was operated without pH control. The experiment was initially examined as a function of COD loading rate in the range of 10 to 50 kg m^{-3} d^{-1} with 10 kg m^{-3} d^{-1} increment under mesophilic temperature (37°C). From literature review, the pH of the fermentation system significantly affected biohydrogen production (Fang and Liu, 2002, Shin *et al.*, 2004), and the optimum pH for biohydrogen production was reported at 5.5 (Fang and Liu, 2002). Therefore, the same range of COD loading rate from the previous work was used to operate the system with controlled pH at 5.5 under mesophilic temperature (37°C) and 24 h of HRT or 6 h for operation steps.

4.1.1 Gas Production Rate

For the system with pH control, gas production rate dramatically increased with increasing of COD loading rate from 0.41 L h^{-1} at 10 kg COD m^{-3} d^{-1} to 2.88 L h^{-1} at 40 kg COD m^{-3} d^{-1} and decreased rapidly to 1.34 L h^{-1} at COD loading rate of 50 kg COD m^{-3} d^{-1}, as shown in Figure 4.1. The decrease in gas production rate at high loading rate was observed in both the cases of systems with and without pH control. This might be due to the negative effect of toxicity from high amount of glucose in wastewater that was not completely consumed by microflora.

The comparative results between the system without pH control and the system with pH conrol at 5.5 show that at the same COD loading rate of 10, 20, 30 and 40 kg COD m^{-3} d^{-1}, the gas production rate increased when the system pH was controlled. At COD loading rate of 40 kg COD m^{-3} d^{-1}, the highest production rate of 2.88 L h^{-1} was obtained. At COD loading rate of 50 kg COD m^{-3} d^{-1}, the gas production rate from the system with pH control was lower than that from the system without pH control. This can be explained by the effect of toxicity both from high amount of glucose in wastewater and from too high amount of H_2SO_4 and NaOH used for pH adjustment. Furthermore, since the pH of the system with pH control needed to be maintained at 5.5 by adding H_2SO_4 and NaOH into the reactor, the addition of these compounds could negatively affect the hydrogen-producing bacteria due to salt formation.

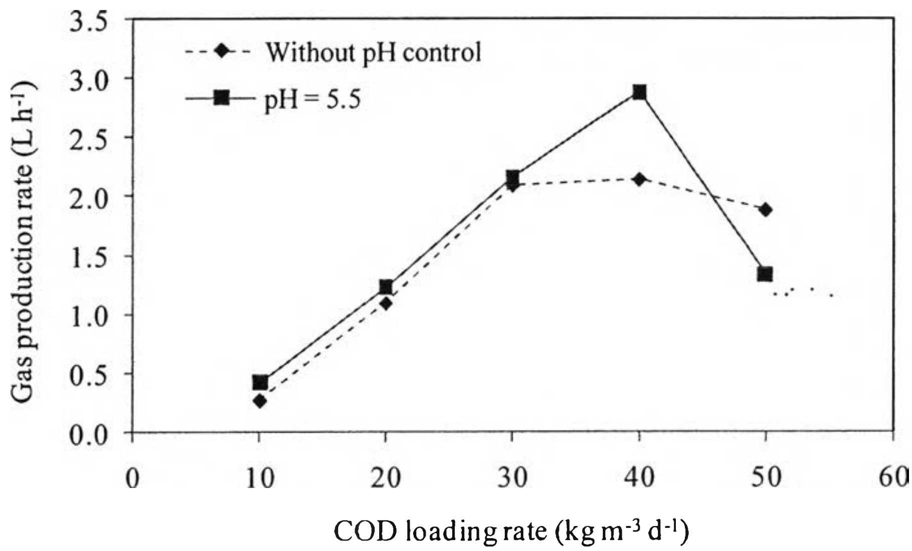


Figure 4.1 Effect of pH and COD loading rate on gas production rate at 37°C and 24 h HRT.

4.1.2 Gas Composition

The composition of produced gas was analyzed by gas chromatography and illustrated in Figure 4.2. The main components of the produced gas were hydrogen and carbon dioxide. Oxygen was detected with only few amounts that could be neglected, and no methane in the produced gas was detected at all operating conditions. This is due to the deactivation of methanogens at high COD loading rate and short hydraulic retention time operation. Under the system with pH control, the hydrogen percentage in the produced gas increased with COD loading rate from 20% at 10 kg COD m^{-3} d^{-1} to reach the maximum of 44% at 40 kg COD m^{-3} d^{-1}, after that it decreased to 30% at COD loading rate of 50 kg COD m^{-3} d^{-1}. At the COD loading rate of 50 kg COD m^{-3} d^{-1}, the hydrogen percentage under the system without pH control was higher than that under the system with pH control. The possible reason might be the same as previously explained for the gas production rate. Moreover, the carbon dioxide percentage was in the opposite trend to the hydrogen percentage and reached the minimum of 57% at COD loading rate of 40 kg COD m^{-3} d^{-1}.

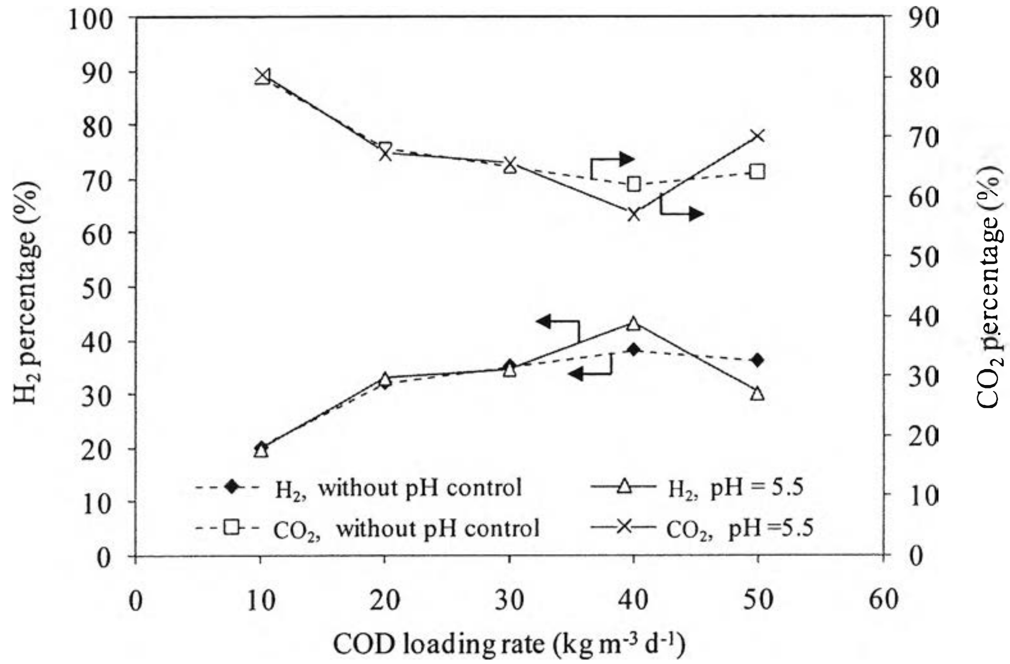


Figure 4.2 Effect of pH and COD loading rate on hydrogen and carbon dioxide percentages at 37°C and 24 h HRT.

4.1.3 Specific Hydrogen Production Rate

The specific hydrogen production rate was calculated from the gas production rate and the gas composition, as shown in Figure 4.3. According to the results, the specific H_2 production rate significantly depended on the concentration of glucose. The specific hydrogen production rate reached the maximum of 7.44 L H_2 L^{-1} d^{-1} at COD loading rate of 40 kg COD m^{-3} d^{-1} due to the highest gas production rate and the highest hydrogen percentage in the produced gas.

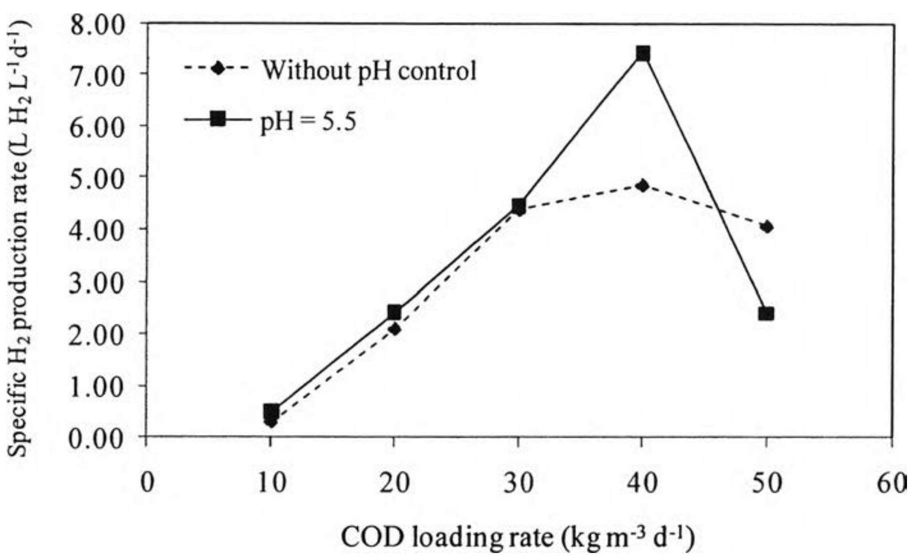


Figure 4.3 Effect of pH and COD loading rate on specific hydrogen production rate at 37°C and 24 h HRT.

4.1.4 Glucose Conversion

The experimental results of glucose conversion are shown in Figure 4.4. At COD loading rates of 10, 20, 30 and 40 kg m^{-3} d^{-1} under the systems both without and with pH control, glucose was converted more than 98% to hydrogen, carbon dioxide, VFA, alcohol, and biomass. The high percentage of glucose conversion is due to its small molecule, the smallest of carbohydrates, so it could be easily degraded by hydrogen-producing bacteria. The highest glucose conversion (99.65%) was found at COD loading rate of 40 kg m^{-3} d^{-1}. However, glucose conversion at COD loading rate of 50 kg m^{-3} d^{-1} decreased to 84.99 and 88.46% under systems without and with pH control, respectively. The decrease in conversion percentage possibly results from the effect of toxicity from high amount of glucose (high COD loading rate) in wastewater as reported in many literatures.

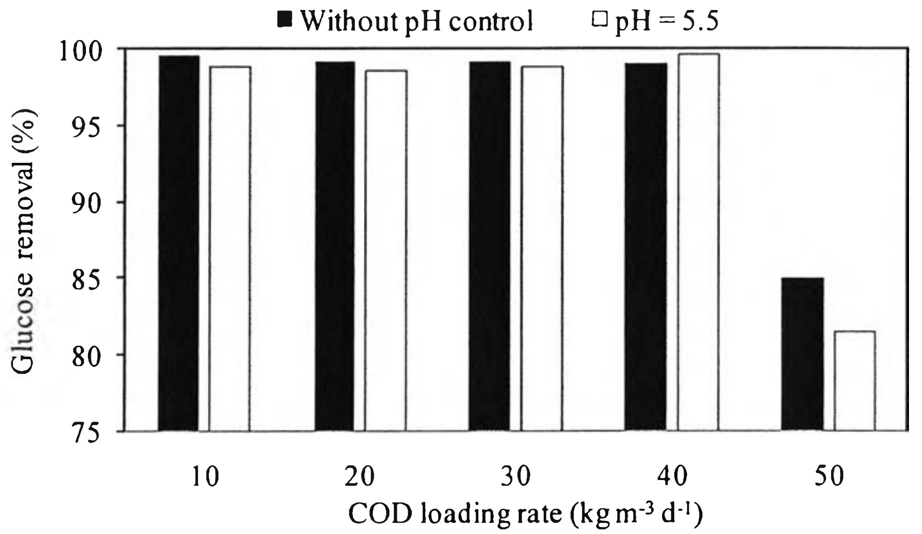


Figure 4.4 Effect of pH and COD loading rate on glucose removal rate at 37°C and 24 h HRT.

4.1.5 VFA Composition

Because the VFA composition analysis was not performed in the previous work (Neramitsuk *et al.*, 2007), the composition of VFA was analyzed in both two systems (with and without pH control). The composition of VFA in effluent liquid after the system reached steady state for the systems without and with pH control is shown in Figures 4.5 and 4.6, respectively. The distribution of VFA and alcohol in the effluent is strongly dependent on the wastewater concentration and pH. From the results, butyric and acetic acids are the main products of the anaerobic fermentation. For the system without pH control, the percentages of butyric and acetic acids increased with COD loading rate and reached the maximum at COD loading rate of 40 kg m^{-3} d^{-1}. At this point, the percentages of acetic and butyric acids were 37.42 and 41.93 %, respectively. The percentages of propionic acid, valeric acid, and ethanol in both systems without and with pH control decreased when higher percentages of butyric and acetic acids were obtained. Corresponding to the hydrogen production, these results indicate that hydrogen production is favored when butyric and acetic acid fermentation predominates. This might be because glucose in feedstock was mainly converted to hydrogen and carbon dioxide via the following equations (Horiuchi *et al.*, 2002):

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (4.1)

(Glucose) (Acetic acid)

$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ (4.2)

(Glucose) (Butyric acid)

From these equations, hydrogen is produced during the production of acetic and butyric acids from glucose while hydrogen is consumed during the production of propionic acid via the following equation:

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (4.3)

(Glucose) (Propionic acid)

Thus for biohydrogen production, the production of propionic acid should be avoided (Hawkes *et al.*, 2002).

From the results, since hydrogen and carbon dioxide are formed along the production of butyric and acetic acids, the microflora in the anaerobic sludge may have contained a large number of acidogenic bacteria that perform hydrogen producion during butyric and acetic acid fermentation.

For the system with pH control, the trend of VFA composition is similar to the system without pH control. The concentration of liquid fermentation products is shown in Figure 4.6. The main soluble products are butyric and acetic acids, while the propionic acid concentration is significantly lower. The ethanol concentration is also relatively low for all operating conditions. The maximum percentage of butyric and acetic acid was found at COD loading rate of 40 kg m^{-3} d^{-1}, and the percentages of acetic and butyric acids increased from 37.42 % to 37.81 % and from 41.93 % to 43.18 %, respectively, as compared with the system without pH control. The explanation for the increase in butyric and acetic acid percentages is that at pH of 5.5, acidogenic bacteria are dominant microbial populations so most of glucose is converted to butyric acid, acetic acid, hydrogen, and carbon dioxide. The trend of butyric acid/acetic acid ratios (B/A) shown in Figure 4.7 is similar to the hydrogen production, confirming that the hydrogen production is favored when the butyric acid and acetic acid fermentation predominates. The highest specific hydrogen production rate and B/A ratio were simultaneously achieved at COD loading rate of 40 kg m^{-3} d^{-1}. The B/A ratio was also reported to increase with respect to the hydrogen production (Han *et al.*, 2004).

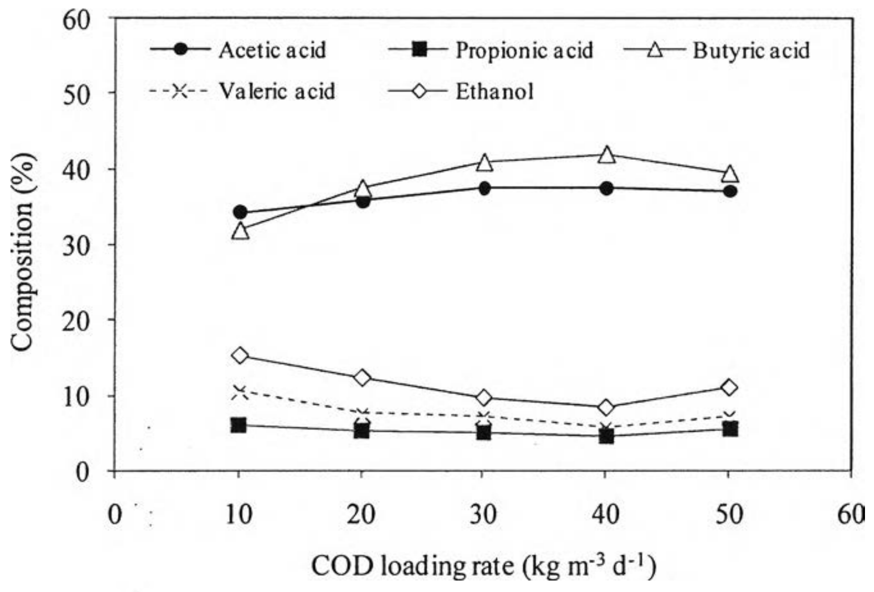


Figure 4.5 Effect of COD loading rate on VFA composition at 37°C and 24 h HRT under the system without pH control.

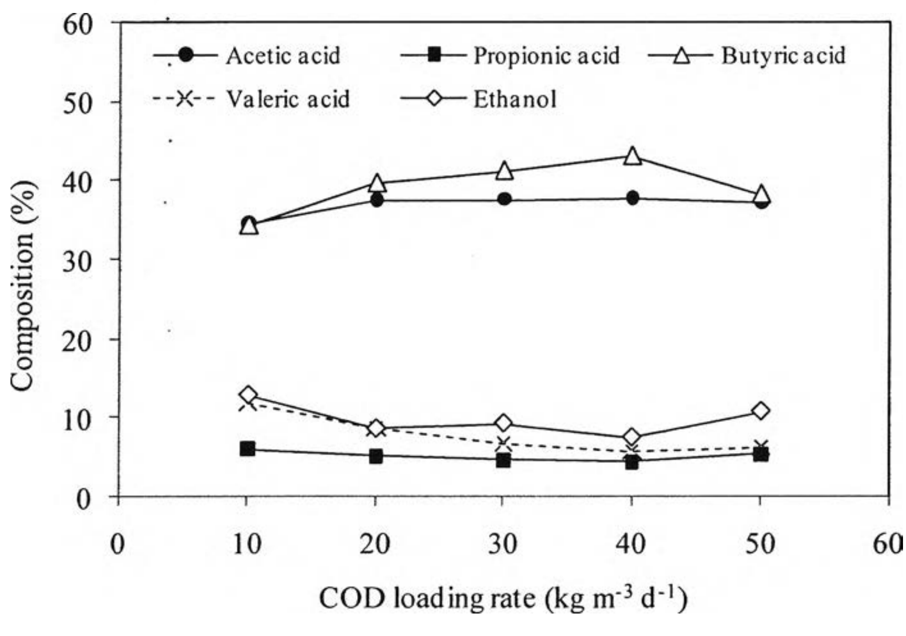


Figure 4.6 Effect of COD loading rate on VFA composition at 37°C and 24 h HRT under the system with pH control.

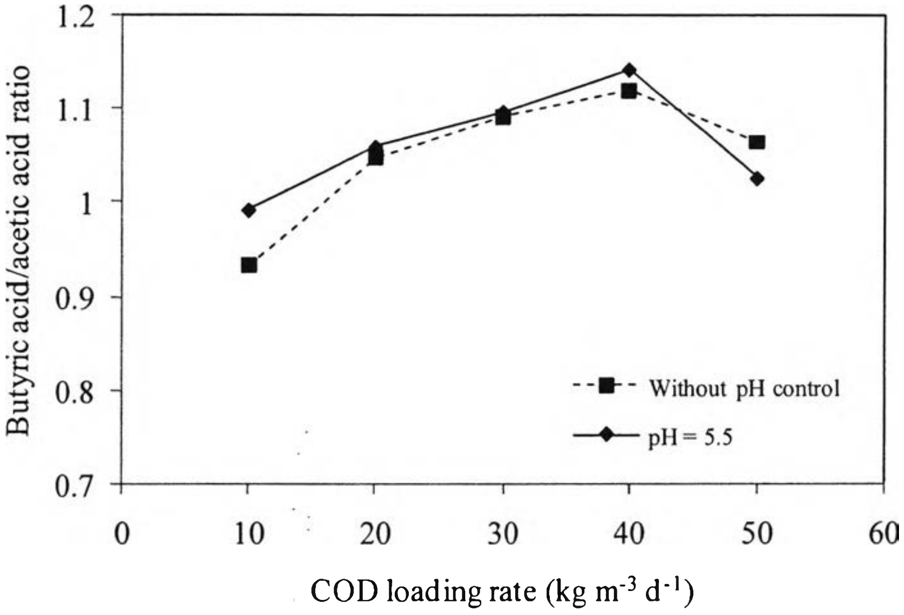


Figure 4.7 Effect of pH and COD loading rate on butyric acid/acetic acid ratio at 37°C and 24 h HRT.

4.1.6 Production of VFA

Production of VFA is quantified approximately as acetic acid by using distillation-titration method as a standard method (Greenberg *et al.,* 1992). From Equations 1 and 2, the biohydrogen production from glucose always accompanies with VFA production. The data of VFA production under the systems without and with pH control when the systems reached steady state are shown in Figure 4.8. For the system with pH control, the VFA production increased with an increase in COD loading rate from 5,278 mg L^{-1} at 10 kg m^{-3} d^{-1} to 17,304 mg L^{-1} at 40 kg m^{-3} d^{-1} and decreased rapidly to 5,768 mg L^{-1} at 50 kg m^{-3} d^{-1}.

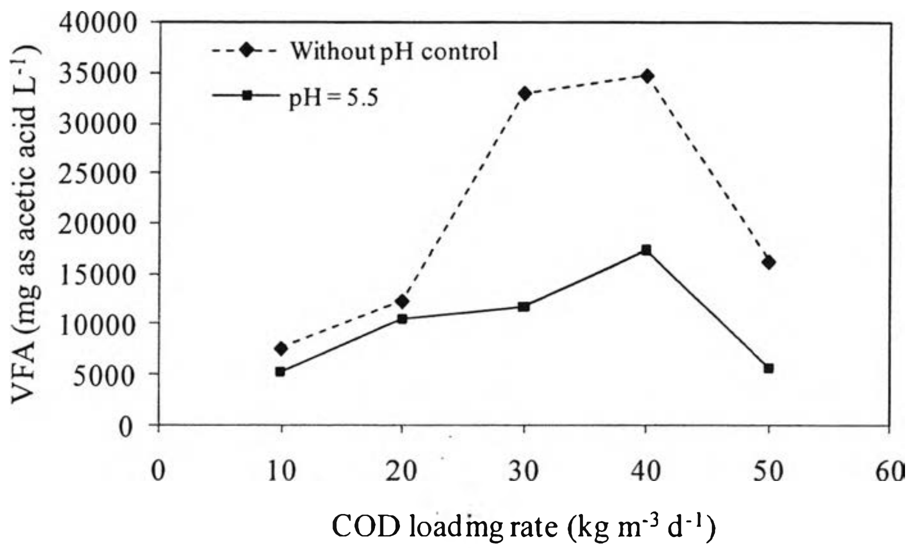


Figure 4.8 Effect of pH and COD loading rate on total VFA at 37°C and 24 h HRT.

The comparative results between this work and the previous work (un-controlled pH) show that the VFA production of system with pH control was lower than that of the system without pH control at the same COD loading rate. This is supposed that the metabolism of hydrogen-producing bacteria shifts to the formation hydrogen rather than acids, as shown in Figure 4.3.

4.1.7 Yield of Hydrogen Production

Yield of hydrogen production is defined as a molar ratio of produced hydrogen to glucose consumed. The comparative results between the systems without and with pH control at 5.5 are shown in Figure 4.9. For the system with pH control, the yield of hydrogen increased with COD loading rate from 0.145 mol H_2/mol glucose consumed at 10 kg m^{-3} d^{-1} to reach the maximum of 1.46 mol H_2/mol glucose consumed at 40 kg m^{-3} d^{-1}. When compared with the system without pH control, the maximum yield of hydrogen production decreased from 1.46 mol H_2/mol glucose consumed at COD loading rate of 40 kg m^{-3} d^{-1} to 1.16 mol H_2/mol glucose consumed at COD loading rate of 30 kg m^{-3} d^{-1}. This might be because the pH control enhances the activity of hydrogen-producing bacteria to produce hydrogen more effectively than that of the system without pH control. The increase in the yield of hydrogen production is in the same trend as the increase in butyric and acetic acid production (Figure 4.5 and 4.6) because the hydrogen forms mainly through Equations 4.1 and 4.2. The optimum H_2 yield should be achieved with acetate as the fermentation end product. In practice, high H_2 yields are usually associated with butyrate production, and low yields with the production of propionate, valeric acid, and alcohol.

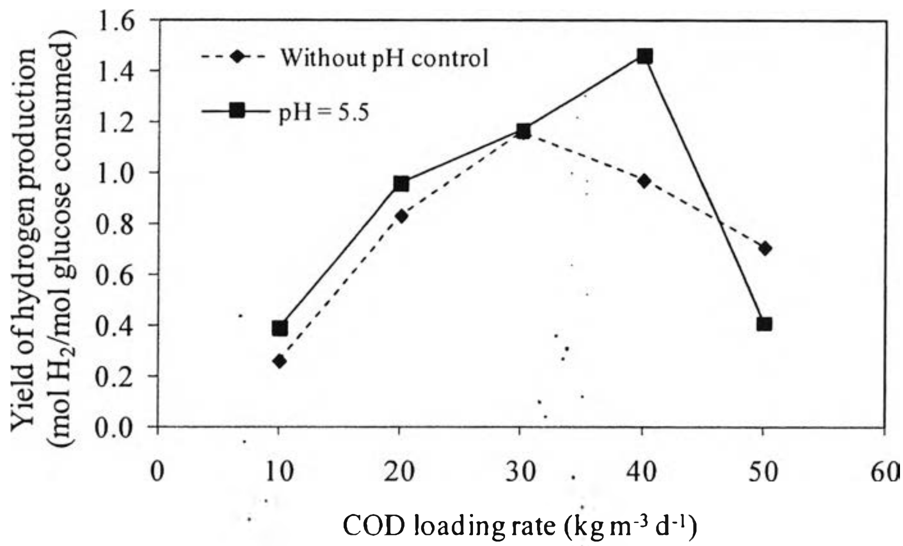


Figure 4.9 Effect of pH and COD loading rate on yield of hydrogen production at 37°C and 24 h HRT.

4.1.8 Biomass Production (VSS)

Hydrogen-producing bacteria can be quantified by VSS method as a standard method (Greenberg *et al.*, 1992). Figure 4.10 shows the experimental data of biomass production for the two systems. For the system with pH control, the biomass concentration in terms of VSS gradually increased with increasing COD loading rate in the range of 10-40 kg m^{-3} d^{-1}. This is consistent with the results from fermentative biohydrogen production by a pure culture (Oh *et al.*, 2003). The correlative results of the systems with and without pH control show that the biomass concentration increased when the pH was controlled. This is plausibly because these conditions are suitable for glucose conversion by bacteria and subsequent bacterial growth. The increase in VSS corresponds to the increases in gas production rate, yield of hydrogen production, and VFA composition, as shown in Figure 4.1, Figure

4.9, and Figure 4.6, respectively. However, when the COD loading rate was further increased up to 50 kg COD m^{-3} d^{-1}, the biomass concentration was decreased to 810 mg L^{-1} due to the excess glucose in the system, which considerably affected the hydrogen-producing bacteria. Furthermore, the salt formation from the compounds added for pH control could affect the growth and activity of microflora.

Notice: In this part of experiment the amount of VSS was measured during the decant step, so it was low if compared with other studies.

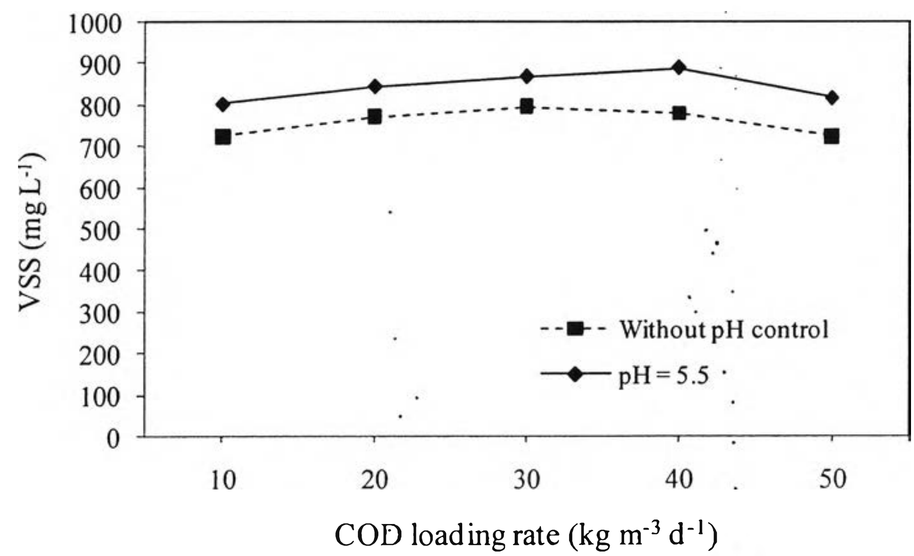


Figure 4.10 Effect of pH and COD loading rate on VSS at 37°C and 24 h HRT.

4.1.9 COD Removal

COD removal is normally related to the H_2 and VFA production. The experimental results of percentage of COD removal are shown in Figure 4.11. The percentage of COD removal for the system with pH control at 5.5 increased with COD loading rate and reached the maximum value of 80.24% at COD loading rate of 40 kg m^{-3} d^{-1}, consistent with the decrease in VFA production. On the other hand, the COD removal rapidly decreased to 42.06% at COD loading rate of 50 kg m^{-3} d^{-1}. This is resulted from the residual glucose and high concentration of volatile fatty acids produced during the fermentation. The comparative results of COD removal at 40 kg COD m^{-3} d^{-1} show that the system with pH control gave the higher COD removal than that without pH control. The higher COD removal means that the liquid product contains lower content of organic compounds, VFA in this case. Therefore, the system with pH control produced VFA less than the system without pH control. The explanation is that the microflora convert the glucose to gas more selectively than to VFA due to the better activity at the optimum conditions (pH = 5.5), resulting in higher gas production rate (Figure 4.1).

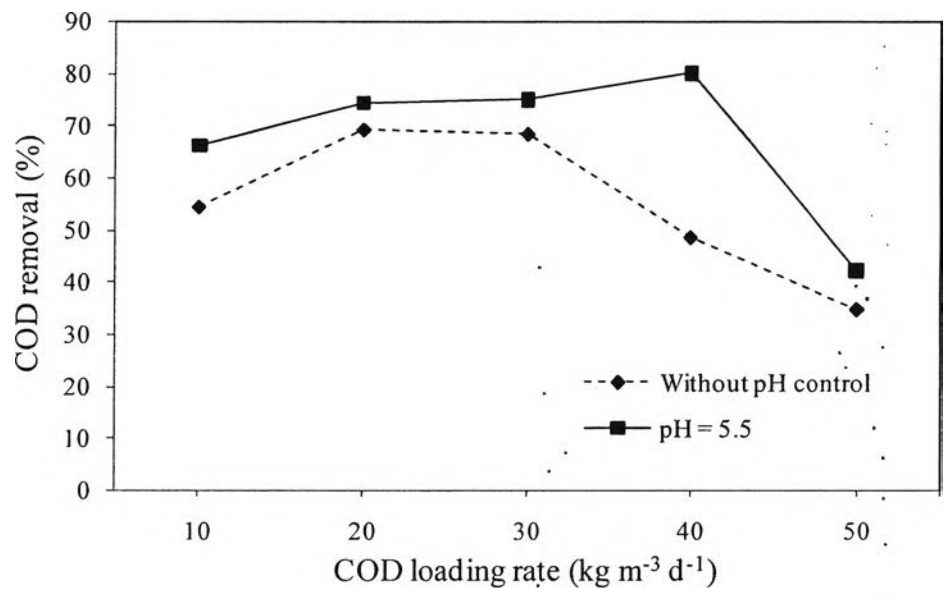


Figure 4.11 Effect of pH and COD loading rate on COD removal at 37°C and 24 h HRT.

4.2 Effect of COD:N Ratio without and with pH Control

The effect of COD:N ratio on biohydrogen production was investigated in order to achieve the optimum amount of supplementary nutrient, especially nitrogen content, that is the most suitable for hydrogen production. Nutrient supplementation has been used for improving the treatment of wastewater containing relatively resistant organic wastes (Nakano et al., 2001). To study the influence of supplementary nutrient on hydrogen production, the amount of nitrogen in feed solution was chosen as a variable. The amount of NH_4HCO_3, the main nitrogen source in feedstock, was adjusted to 2.62 g L^{-1} for COD:N ratio of 100:1.4 and 10.48 g L^{-1} for COD:N ratio of 100:3.3. Note that the experiments in the first part at the COD loading rate of 40 kg m^{-3} d^{-1} were conducted under mesophilic temperature (37°C), 24 h HRT, and COD:N ratio of 100:2.4. In this part, the COD:N ratio was therefore varied by increasing from 100:2.4 to 100:3.3 (N content increased) and decreasing from 100:2.4 to 100:1.4 (N content decreased). Both experiments were also operated under systems without and with pH control.

4.2.1 Gas Production Rate

As shown in Figure 4.12, for the system without pH control, gas production rate increased from 1.28 to 2.14 L h^{-1} when COD:N ratio was changed from 100:1.4 to 100:2.4 and rapidly decreased to 1.10 L h^{-1} at COD:N of 100:3.3. This trend was also observed in the system with pH control, in which gas production rate increased from 1.86 to 2.88 L h^{-1} when COD:N ratio was changed from 100:1.4 to 100:2.4 and greatly decreased to 1.33 L h^{-1} at COD:N of 100:3.3. From the Figure 4.12, the increase in gas production rate with COD:N ratio in the range of 100:1.4 to 100:2.4 was observed in both systems. This might be because the nutrient supplementation was necessary for anaerobic fermentation. The addition of nitrogen in feed can enhance the hydrogen production by stimulating the microbial flora in the wastes and improving their degradation activity (Lee et al., 1993). The decrease in gas production rate at high N content might be results from the accumulation of excess ammonium used as a nitrogen source in feed solution.

The comparative results between the systems without and with pH control show that the gas production rate increased when the system pH was controlled at all operating conditions, especially at COD:N ratios of 100:1.4 and 100:2.4, of which the gas production rate increased to 1.86 and 2.88 L h^{-1}, respectively. The possible reason might be the same as previously explained for the effect of COD loading rate.

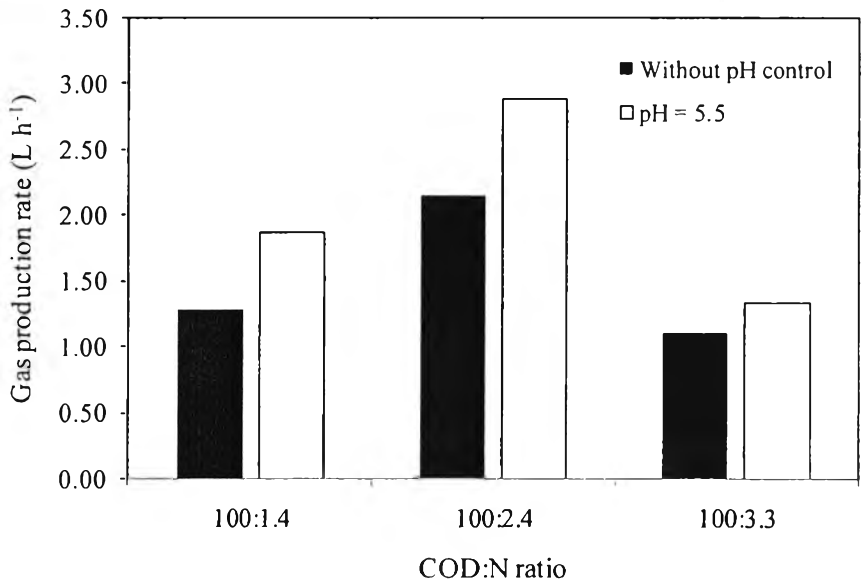


Figure 4.12 Effect of pH and COD:N ratio on gas production rate at 37°C and 24 h HRT.

4.2.2 Production of VFA

The data of VFA production under systems without and with pH control at COD:N ratios of 100:1.4, 100:2.4, and 100:3.3 are shown in Figure 4.13. For the system without pH control, the VFA production increased from 22,872 to 34,607 mg as acetic acid L^{-1} with increasing N content in term of COD:N ratio from 100:1.4 to 100:2.4 and decreased to 25,494 mg as acetic acid L^{-1} when the ratio was 100:3.3. On the other hand, for the pH-controlled system, the VFA production slightly increased with COD:N ratio to reach the maximum of 19,322 mg as acetic acid L^{-1} at COD:N ratio of 100:3.3. The slight increase in VFA production with COD:N ratio at all conditions might be because under pH-controlled condition, the system pH did not significant affect the activity of microflora since it was maintained at 5.5. Therefore, the N content in feed solution is a decisive factor affecting microflora activity. When the amount of nitrogen in feed solution increased, the acid production therefore increased. The possible explanation for this trend is that the increase in nitrogen content in the system can drive the reaction pathway to acid production.

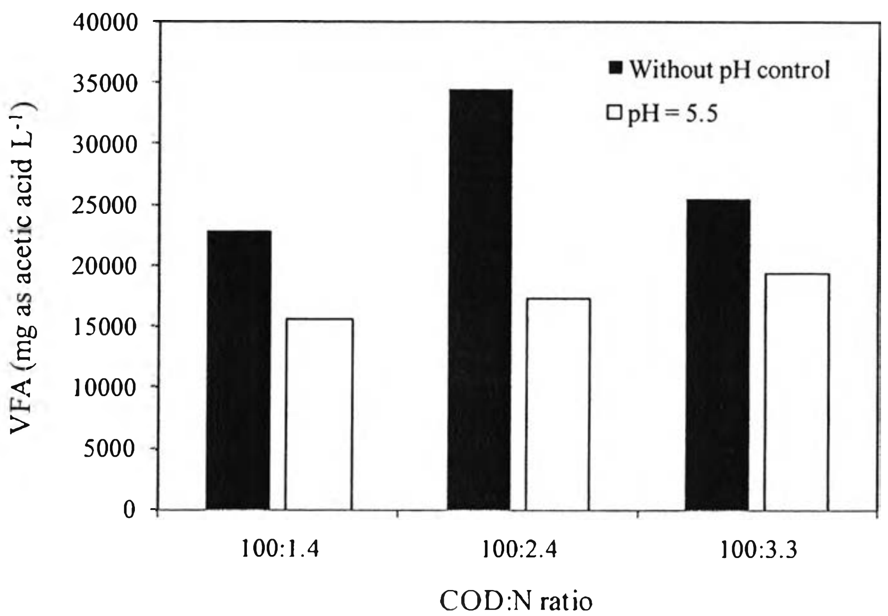


Figure 4.13 Effect of pH and COD:N on VFA production at 37°C and 24 h HRT.

The comparative results of systems without and with pH control show that the VFA production from the system with pH control was lower than that from the system without pH control at the same COD loading rate. This can be supposed that the pH control can decrease the VFA production since the microflora activity did not significantly change.

4.2.3 Gas Composition

From the experimental observation, hydrogen and carbon dioxide were also the main components of the produced gas. Oxygen was detected with only few amounts that could be neglected, and no methane in the produced gas was detected at all operating conditions. Under the system without pH control, the hydrogen percentage in the produced gas increased with COD:N ratio from 20% at COD:N of 100:1.4 to 38% at COD:N of 100:2.4, as shown in Figure 4.14. For system with pH control, the percentage of hydrogen reached the maximum of 44% at COD:N of 100:2.4. However, when the COD:N ratio was adjusted to 100:3.3, the percentage of hydrogen in produced gas decreased to 32% under system without pH control and 25% under system with pH control due to the excess amount of nitrogen in feed, which could drive the reaction pathway to acid production instead of hydrogen production, and the salt formation from compounds added for pH control. The optimum COD:N ratio for anaerobic fermentation was reported at COD:N ratio of 100:2.2, which is comparatively close to the condition exhibiting a maximum percentage of hydrogen production at COD:N of 100:2.4 in this work.

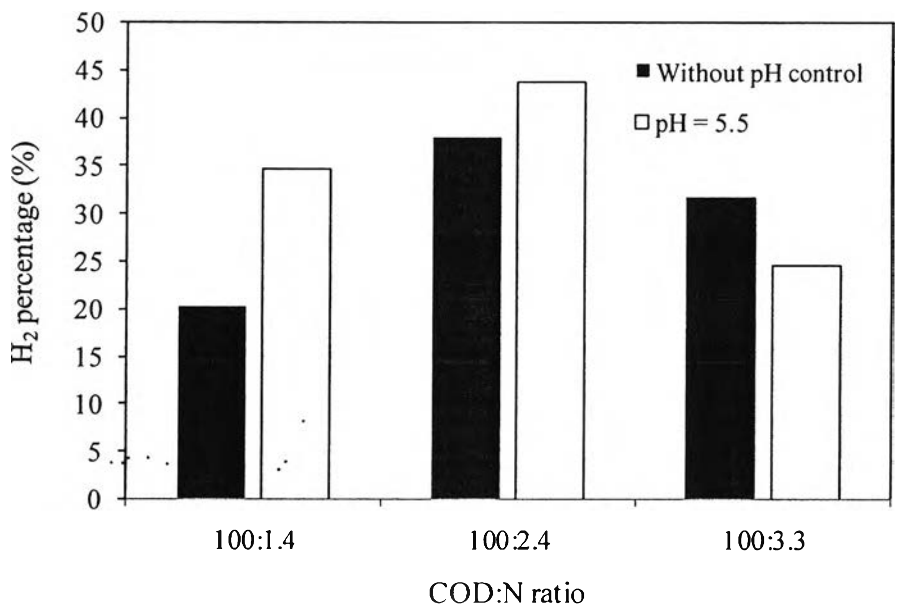


Figure 4.14 Effect of pH and COD:N on gas composition at 37°C and 24 h HRT.

4.2.4 Specific Hydrogen Production Rate

The specific hydrogen production rate shown in Figure 4.15 was calculated from the gas production rate and the gas composition. From the results, the COD:N ratio had a significant influence on the specific hydrogen production rate. The specific hydrogen production rate reached the maximum of 7.44 L H_2 L^{-1} d^{-1} at COD:N ratio of 100:2.4 under system with pH control due to the highest hydrogen percentage in the produced gas.

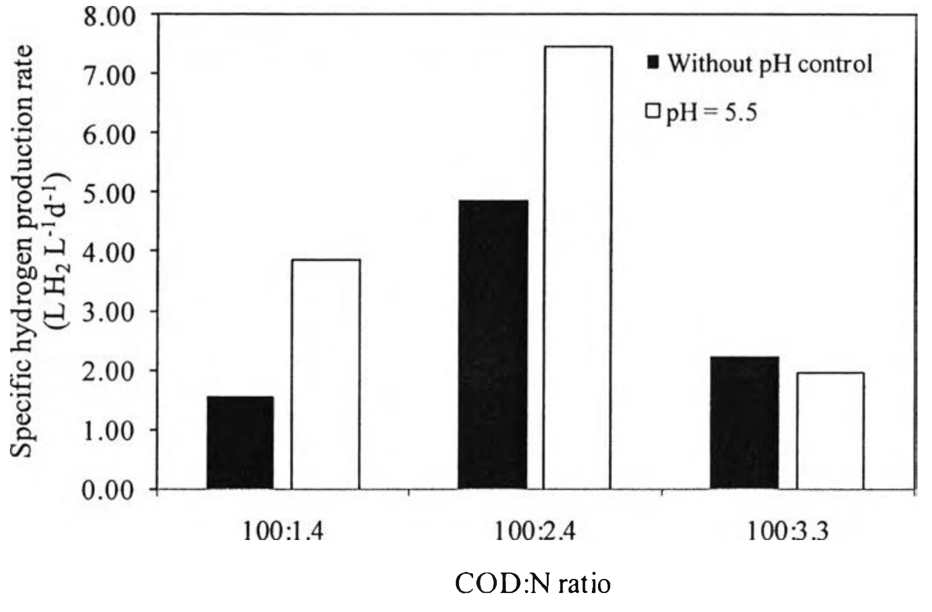


Figure 4.15 Effect of pH and COD:N ratio on specific hydrogen production rate at 37°C and 24 h HRT.

4.2.5 Glucose Conversion

The experimental results of glucose conversion are shown in Figure 4.16. In all operating conditions, glucose was degraded more than 98% and converted to hydrogen, carbon dioxide, VFA, alcohol, and biomass. The highest glucose conversion of 99.65% was achieved at COD loading rate of 40 kg m^{-3} d^{-1} and COD:N ratio of 100:2.4 under the system with pH control. The decrease in glucose conversion at COD:N ratio of 100:1.4 under the system without pH control might result from the insufficiency of essential nutrient for bacterial metabolism.

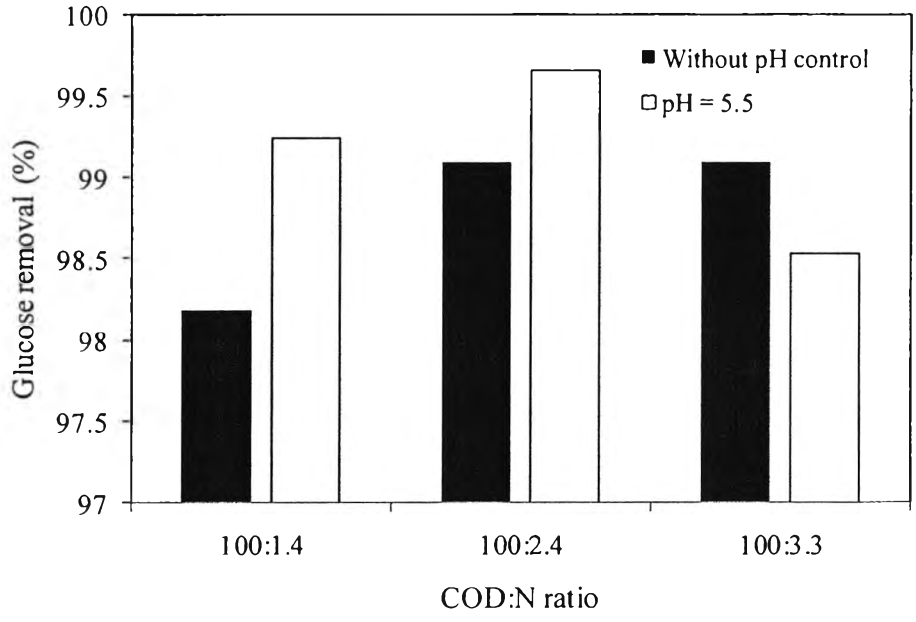
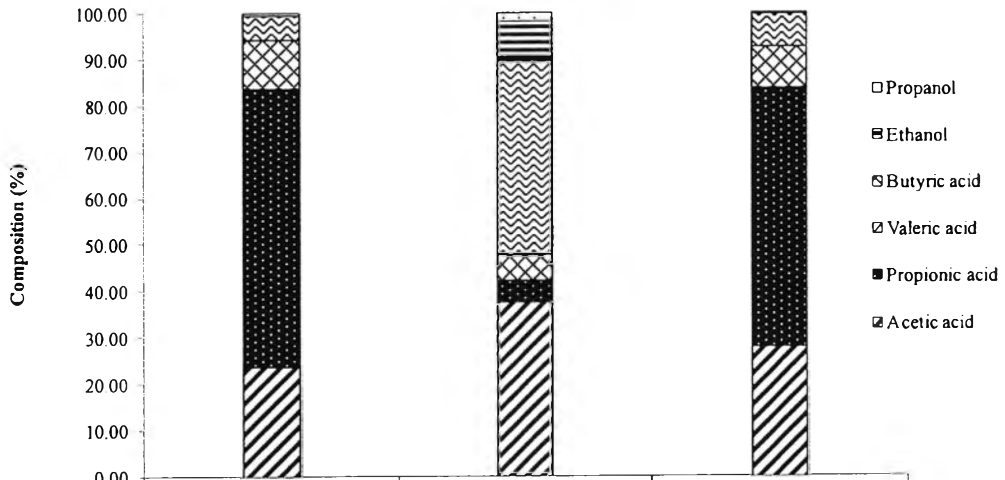


Figure 4.16 Effect of pH and COD:N ratio on glucose removal at 37°C and 24 h HRT.

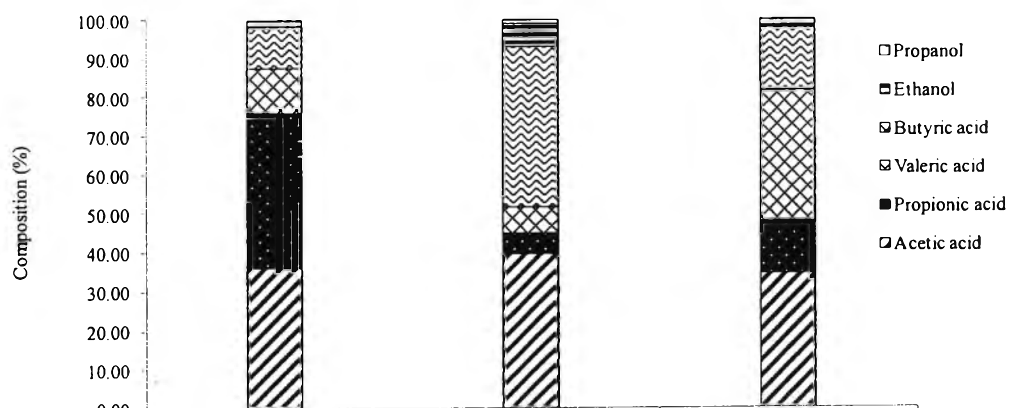
4.2.6 VFA Composition

The results of VFA composition from systems without and with pH control are shown in Figures 4.17 and 4.18, respectively. For the system without pH control, propionic acid was the main product of the anaerobic fermentation at COD:N ratios of 100:1.4 and 100:3.3. These results well corresponded to the decrease in specific hydrogen production rate because hydrogen was utilized to form propionic acid according to Equation 3. The lowest propionic acid production was found at COD:N of 100:2.4, at which the maximum specific hydrogen production rate of 7.44 L h^{-1} was obtained (Figure 4.15). The main components of liquid product were propionic and acetic acids under COD:N ratio of 100:1.4, and acetic and valeric acids under COD:N ratio of 100:3.3. The production of propionic and valeric acids was a plausible reason of the decrease in hydrogen production at too low and excess N content. The butyric acid/acetic acid ratio (B/A) shown in Figure 4.19 shows the same trend as the hydrogen production. The B/A ratio of the system with pH control was slightly higher than that the system without pH control, and the maximum B/A ratio was achieved under COD:N ratio of 100:2.4 in both systems.



	100:1.4	100:2.4	100:3.3
Propanol	0.00	2.00	0.00
Ethanol	0.54	8.32	0.19
Butyric acid	5.44	41.93	7.39
Valeric acid	10.42	5.73	8.90
Propionic acid	59.87	4.59	55.66
Acetic acid	23.74	37.42	27.86

Figure 4.17 Effect of COD:N ratio on VFA composition at 37°C and 24 h HRT under the system without pH control.



	100:1.4	100:2.4	100:3.3
Propanol	0.00	1.22	0.00
Ethanol	1.68	5.80	2.33
Butyric acid	10.66	41.47	16.18
Valeric acid	11.82	6.71	33.79
Propionic acid	39.81	5.15	13.41
Acetic acid	36.04	39.64	34.29

Figure 4.18 Effect of COD:N ratio on VFA composition at 37°C and 24 h HRT under the system with pH control.

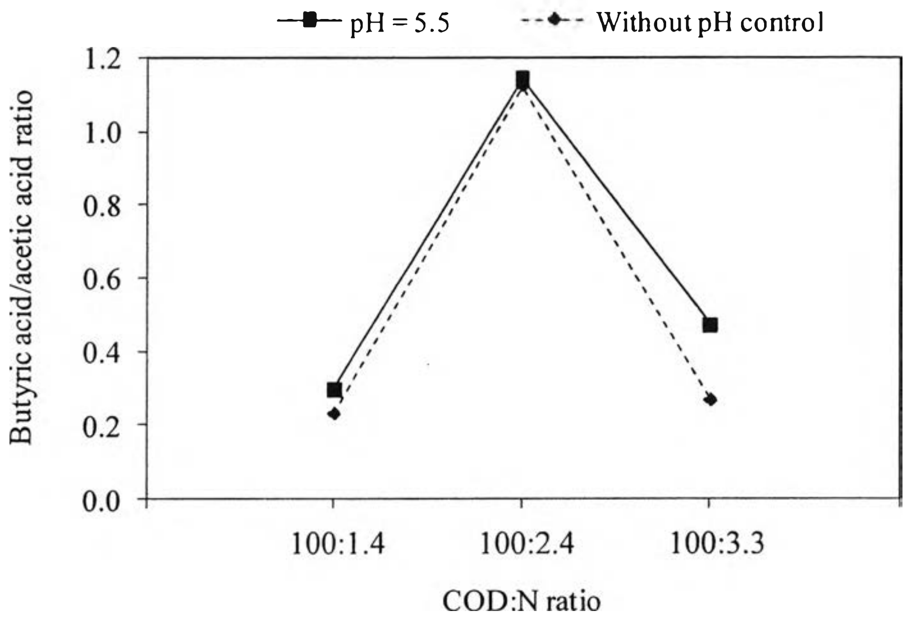


Figure 4.19 Effect of pH and COD:N ratio on butyric acid/acetic acid ratio at 37°C and 24 h HRT.

4.2.7 Yield of Hydrogen Production

The comparative results of yield of hydrogen production between systems without and with pH control at 5.5 are shown in Figure 4.20. For the system with pH control, the highest yield of hydrogen of 1.46 mol H_2/mol glucose consumed was found at COD:N ratio of 100:2.4. The comparative results between yield of hydrogen production under various COD:N ratios and yield of hydrogen production under various COD loading rates show that the yield under various COD loading rates (previous part) was higher than that under various COD:N ratios (this part) at almost all COD loading rates, except COD loading rate of 10 kg m^{-3} d^{-1} (Figure 4.9 and Figure 4.20). When the COD:N ratio was changed from 100:2.4 to 100:3.3 under system with pH control, the yield of hydrogen production rapidly decreased from 1.46 to 0.39 mol H_2/mol glucose consumed. But, when COD loading rate was changed from 40 to 20 kg m^{-3} d^{-1}, the yield of hydrogen production only decreased from 1.46 to 0.95 mol H_2/mol glucose consumed. This might be because nutrient supplementation has more effect on biohydrogen production than COD loading rate. The decreases in the yield of hydrogen production at COD:N ratios of 100:1.4 and

100:3.3 are in conjunction with the increase in the production of valeric and propionic acids (Figure 4.17) because hydrogen was utilized to form propionic acid according to Equation 3. The optimum hydrogen yield should be achieved with acetate as the fermentation end product, not propionic and valeric acids. The decrease in hydrogen yield at deficient and excess N content agrees well with a literature, which reported that high nitrogen and phosphorous concentrations inhibited hydrogen formation by dark fermentation probably by shifting the metabolic pathway (Argun et al., 2008).

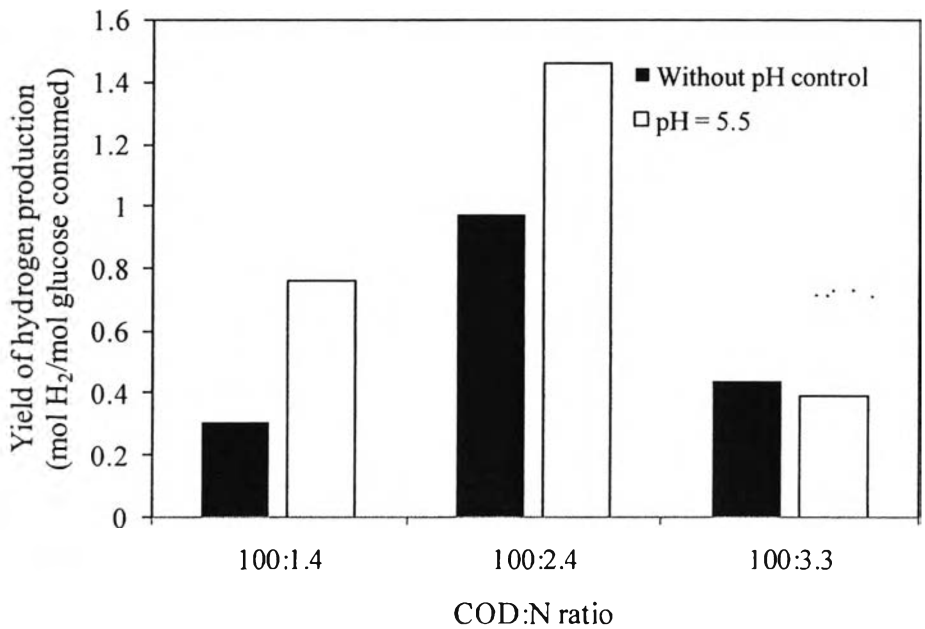


Figure 4.20 Effect of pH and COD:N ratio on yield of hydrogen production at 37°C and 24 h HRT.

The comparative results between this work and other works are shown in Table 4.1. For the maximum hydrogen yield, this work gave a low yield if compared with other studies. This is because the experiment was operated at high HRT. At high HRT, the gas production decreased because the glucose was converted to VFA more than H_2. When considering the specific hydrogen production rate (SHPR), its comparatively high value was achieved in this work compared with the other works, meaning that the ASBR could provide a high specific H_2 production rate compared with other types of reactor. Moreover, the ASBR could be operated at high COD loading with smaller size of reactor compared with other types of reactor due to the high SHPR.

Table 4.1 Comparative results between this work and other works

Substrate	Reactor type	Maximum H_2 yield[a]	HRT (h)	SHPR[b]	Reference
Sugar wastewater	CSTR	2.59	12	0.69	Ueno *et al.*, 1996
Winery wastewater	UASB	2.14	2	3.81	Yu *et al.*, 2002
Sucrose	ASBR	2.6	8	10.08	Lin *et al.*, 2003
Glucose	-	2.1	12	3.60	Morimoto *et al.*, 2004
Glucose	ASBR	1.46	24	7.44	This work

[a]Maximum H_2 yield (mol H_2 mol hexose consumed^{-1})

[b]SHPR = Specific Hydrogen Production Rate (L H_2 L^{-1} d^{-1})

4.2.8 Biomass Production (VSS)

Figure 4.21 shows the experimental data of biomass production at various COD:N ratios. For the system without pH control, the biomass concentration in terms of VSS slightly increased with increasing COD:N ratio. For the system with pH control, VSS significantly increased with adjusting COD:N ratio from 100:1.4 to 100:2.4 and then decreased with further adjusting COD:N ratio to 100:3.3. The comparative results of the systems without and with pH control show that the biomass concentration increased when the pH was controlled. This is reasonable because pH of 5.5 is suitable for the growth of hydrogen-producing bacteria.

Notice: The amount of VSS shown in Figure 4.21 was measured during the decant step, so it was low if compared with other studies.

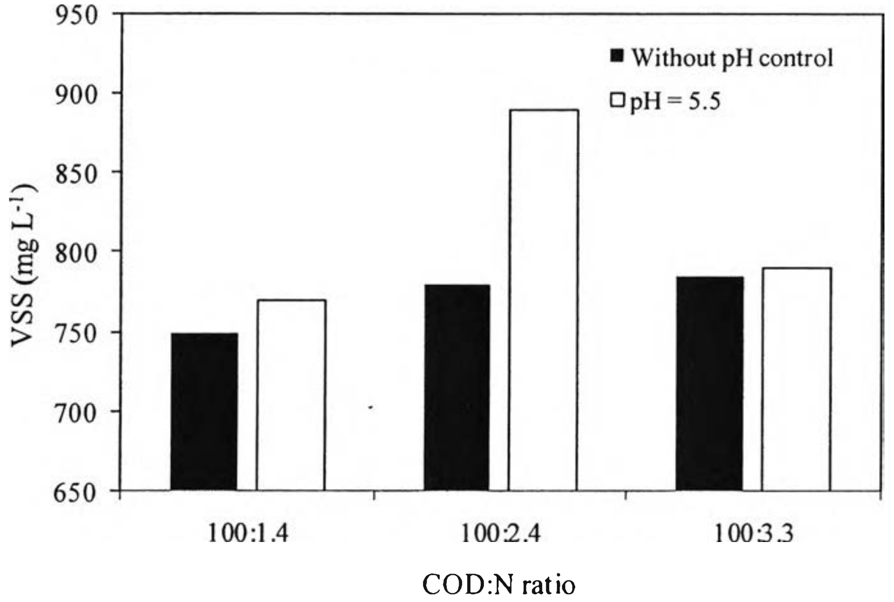


Figure 4.21 Effect of pH and COD:N ratio on VSS at 37°C and 24 h HRT.

4.2.9 COD Removal

The comparative results of COD removal between the systems without and with pH control at 5.5 are shown in Figure 4.22. From the results, the percentage of COD removal for the system without pH control rapidly decreased from 48.58 to 31.27% when changing COD:N ratio from 100:2.4 to 100:1.3. This trend also occurred in the system with pH control, of which the maximum percentage of COD removal of 80.24% was obtained at COD:N ratio of 100:2.4. A possible explanation is the insufficiency of nutrient for bacterial metabolism because nitrogen is classified as a macronutrient, which is one of necessary nutrients for bacterial growth, such as C, N, S, P, K, Mg, Ca, Fe, Na, and Cl (McCarty et al., 2001). At the COD:N ratio of 100:3.3 (excess nitrogen content in feed solution), percentage of COD removal decreased to 42.73 and 40.53% in the systems without and with pH control, respectively. The optimum COD:N ratio for COD removal was obtained by moderate addition of NH_4HCO_3 at COD:N ratio of 100:2.4, which is quite close to the theoretical ratio of 100:2.2. Because the yield of hydrogen production reached the maximum at COD:N ratio of 100:2.4, this corresponded to the low production amount of organic compounds (Figure 4.13) and the high COD removal (Figure 4.22). The increase in percentage of COD removal when the pH was controlled can be explained by the same reason that the microflora converted glucose to gases more selectively than VFA due to better activity at the optimum conditions (pH = 5.5).

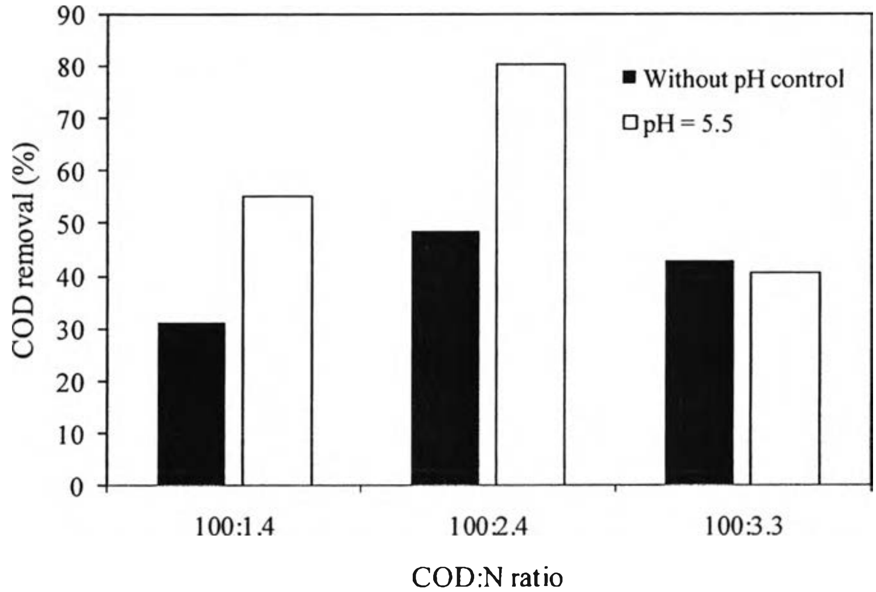


Figure 4.22 Effect of pH and COD:N ratio on COD removal at 37°C and 24 h HRT.