CHAPTER IV
RESULTS AND DISCUSSION

4.1 The Effect of COD Loading Rate

The biohydrogen productivity of anaerobic sequencing batch reactor (ASBR) operated without pH control was initially examined as function of COD loading rate in the range of 10 to 40 kg m^{-3} d^{-1} with 10 kg m^{-3} d^{-1} increment. Afterwards, the obtained optimum COD loading rate was further increased to 50 kg m^{-3} d^{-1} in order to investigate the continuous production of hydrogen with pH control at 5.5, the optimum pH (Fang and Liu, 2002). Finally, the optimum COD loading rate for the biohydrogen production in ASBR with pH control was obtained. All of the experiments were studied at mesophilic temperature (37°C) and 24 h of HRT or 6 h for each operating step (cycle time).

4.1.1 Final pH

pH of the system is one of the important parameters for studying the feasibility of biohydrogen production because it directly affects the efficiency of hydrogen production.

Biohydrogen production via anaerobic fermentation was studied by varying the COD loading rate from 10 to 40 kg m^{-3} d^{-1}. Figure 4.1 shows the effect of COD loading rate on final pH after reaching the steady state condition. The final pH of system decreased drastically from 6.68 at COD loading rate of 10 kg m^{-3} d^{-1} to 5.2 at COD loading rate of 30 kg m^{-3} d^{-1}. This is due to the formation of larger amount of acids (VFA) during the fermentation because of more source of substrate to be decomposed at higher COD loading rate. However, when COD loading rate increased to 40 kg m^{-3} d^{-1}, final pH of system remained nearly constant (5.2-5.23), indicating that acid formation was inhibited at low pH. It is due to the suppression of bacteria's ability under acidic condition.

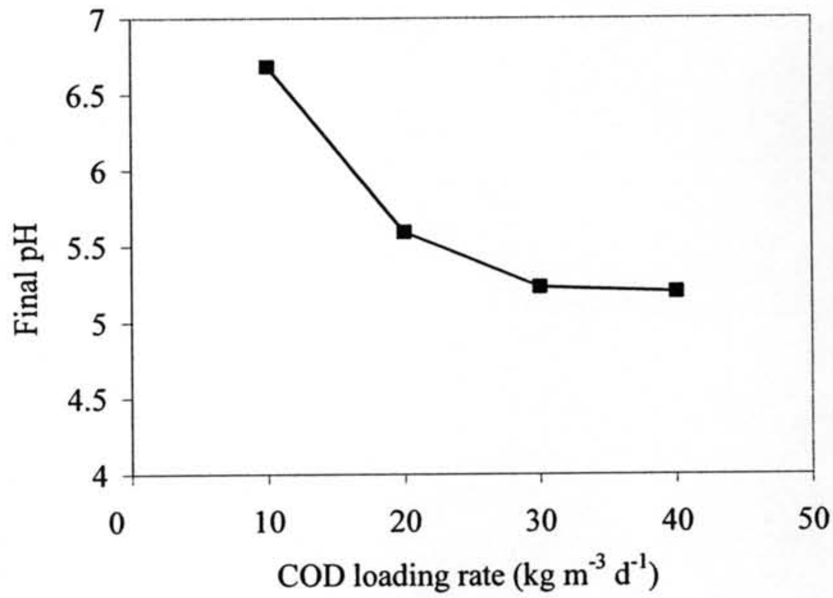

Figure 4.1 Effect of COD loading rate on the final pH of system at 37°C and 24 h of HRT.

4.1.2 Glucose Conversion

For sustainable biohydrogen production, carbohydrates are the preferred organic carbon source for biohydrogen-producing fermentations. Glucose (or its isomer, hexose) is the fermentation substrate most studied in the laboratory (Hawkes *et al.,* 2002).

Figure 4.2 depicts that degradation of glucose decreased with the increase in COD loading rate. Nevertheless, glucose was still acidified much effectively, since the percentage of glucose conversion was higher than 99% for all COD loading rates. It indicated that glucose was readily degraded under the tested conditions, and COD loading rate had little influence on the glucose degradation efficiency. This can be explained in that glucose is the smallest molecule of carbohydrate, so it can be easily degraded by microflora. This is consistent with the reported 91.5-99.0% for the acidification of carbohydrate (Yu and Fang, 2001).

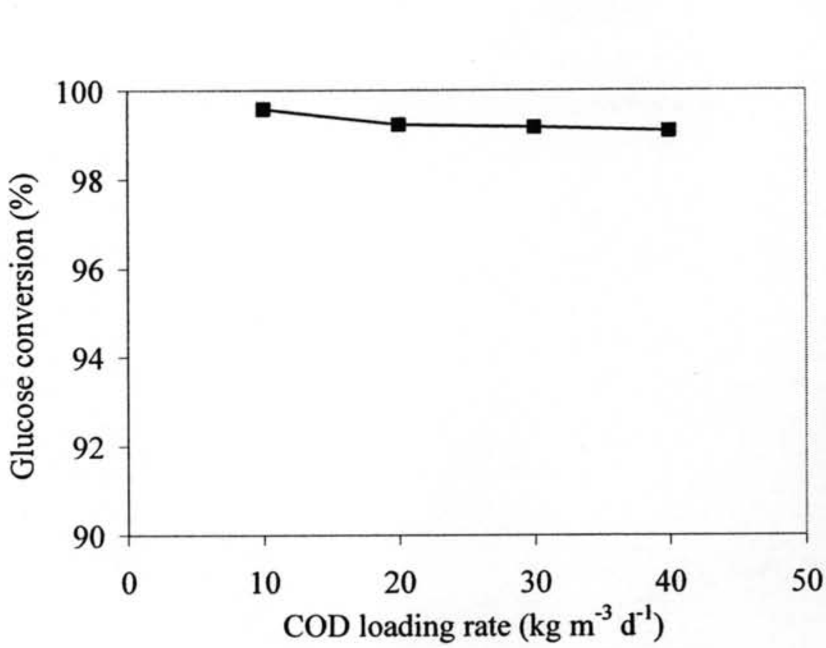

Figure 4.2 Effect of COD loading rate on glucose conversion at 37°C and 24 h of HRT.

4.1.3 Gas Production Rate

As shown in Figure 4.3, gas production rate substantially increased with increasing COD loading rate from 0.274 L h^{-1} at 10 kg COD m^{-3} d^{-1} to 2.085 L h^{-1} at 30 kg COD m^{-3} d^{-1}. Nevertheless, gas production slightly increased as COD loading rate increased from 2.085 L h^{-1} at 30 kg COD m^{-3} d^{-1} to 2.144 L h^{-1} at 40 kg COD m^{-3} d^{-1}. The increase in glucose source enhanced the production of gas by microflora, but its large increase caused the inhibition of hydrogen production.

From the analysis of the produced gas composition, Figure 4.4 illustrates that the gas comprised only hydrogen and carbon dioxide. The hydrogen content increased with COD loading rate from 20% at 10 kg COD m^{-3} d^{-1} to 38% at 40 kg COD m^{-3} d^{-1}, whereas carbon dioxide decreased from 80 to 62%, correspondingly. There was no methane gas in produced gas at all COD loading rates. This might be due to the deactivation of methanogens at low pH and high COD loading rate. pH was not controlled in these experiments since methanogens activity could have been deactivated by the decrease in pH due to the formation of acids during the fermentation (Morimoto *et al.,* 2004).

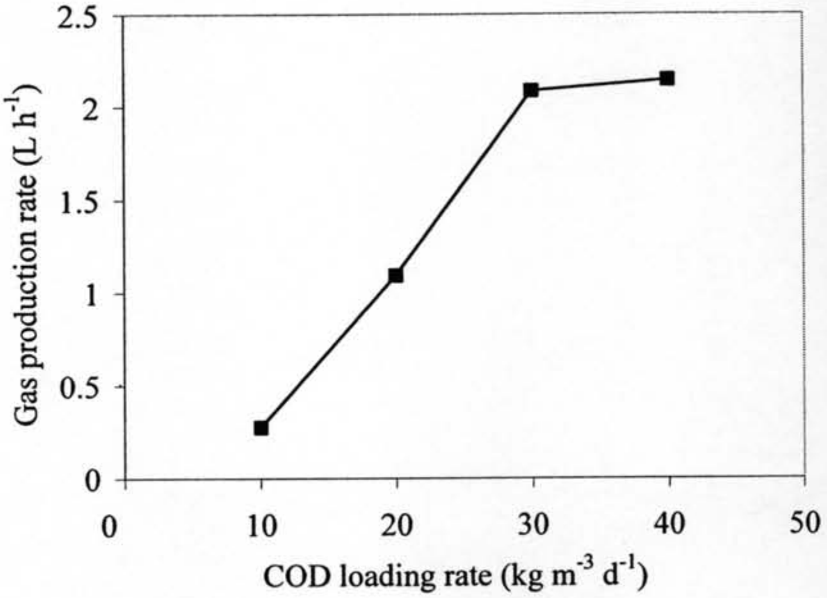

Figure 4.3 Effect of COD loading rate on gas production rate at 37°C and 24 h of HRT.

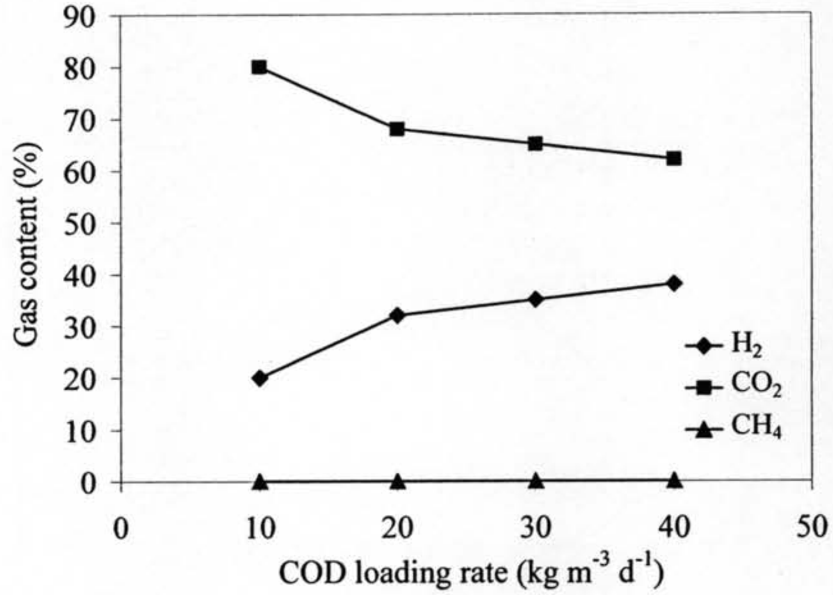

Figure 4.4 Effect of COD loading rate on gas content at 37°C and 24 h of HRT.

The hydrogen production rate increased readily from 0.055 L h^{-1} at 10 kg COD m^{-3} d^{-1} to 0.780 L h^{-1} at 30 kg COD m^{-3} d^{-1}, in correspondence to the glucose degradation. Further increase in COD loading rate to 40 kg m^{-3} d^{-1} only slightly increased the hydrogen production rate due to the decreased bioactivity of hydrogenic bacteria under acidic condition, as shown in Figure 4.5.

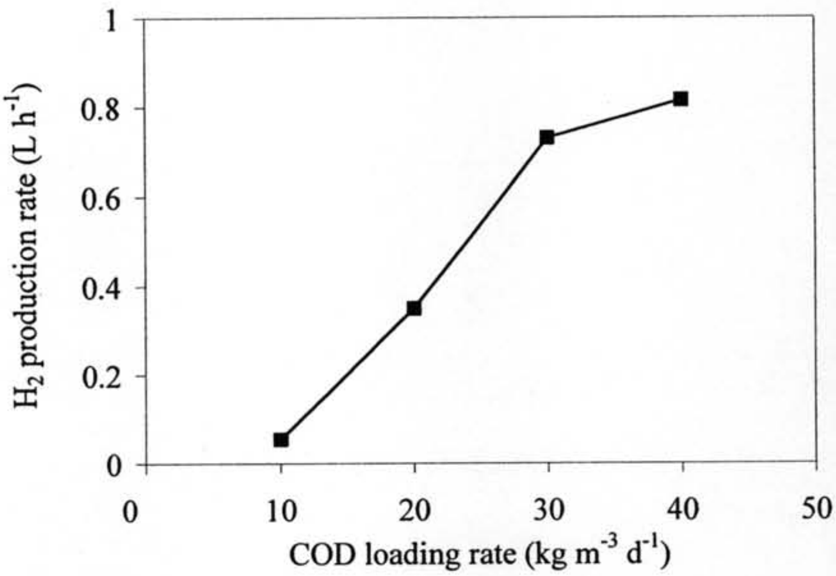

Figure 4.5 Effect of COD loading rate on H_2 production rate at 37°C and 24 h of HRT.

4.1.4 Yield of Hydrogen Production

The yield of hydrogen production as function of COD loading is illustrated in Figure 4.6. The yield of hydrogen increased from 0.259 mol-H_2/mol-glucose consumed at 10 kg COD m^{-3} d^{-1} to 1.156 mol-H_2/mol-glucose consumed at 30 kg COD m^{-3} d^{-1}, the highest yield of hydrogen in this study. After that, it slightly declined to 0.968 mol-H_2/mol-glucose consumed at 40 kg COD m^{-3} d^{-1}. The low hydrogen yield at higher COD loading rate is likely due to the high production of VFA, affecting on the system's low pH. It is a result of the high initial concentration of glucose in the wastewater.

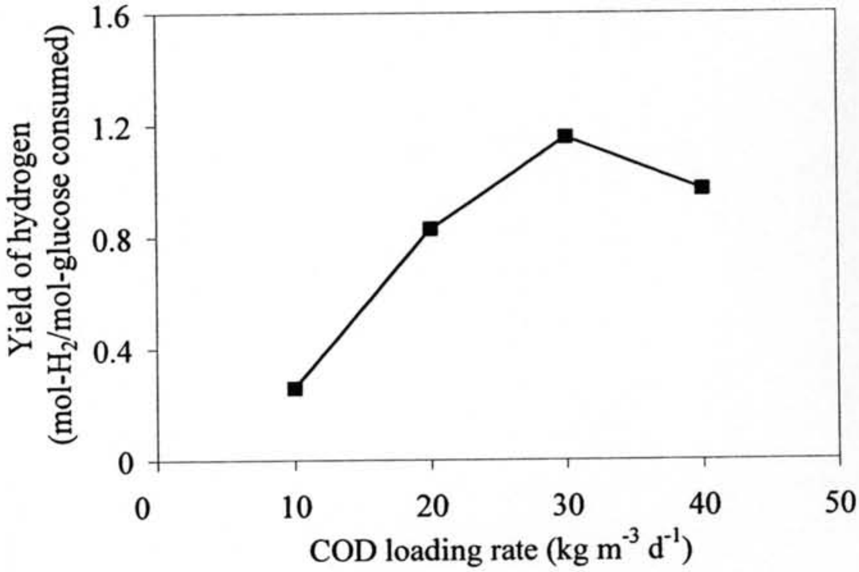

Figure 4.6 Effect of COD loading rate on yield of hydrogen at 37°C and 24 h of HRT.

4.1.5 Production of VFA

The biohydrogen production from organic waste always accompanies with a VFA production. Figure 4.7 illustrated that the VFA concentrations in the effluent increased from 7,614 mg as acetic acid L^{-1} at 10 kg COD m^{-3} d^{-1} to 34,607 mg as acetic acid L^{-1} at 40 kg COD m^{-3} d^{-1}. These results demonstrate that VFA production increased with glucose consumption, which exactly corresponds to the glucose conversion shown in Figure 4.2.

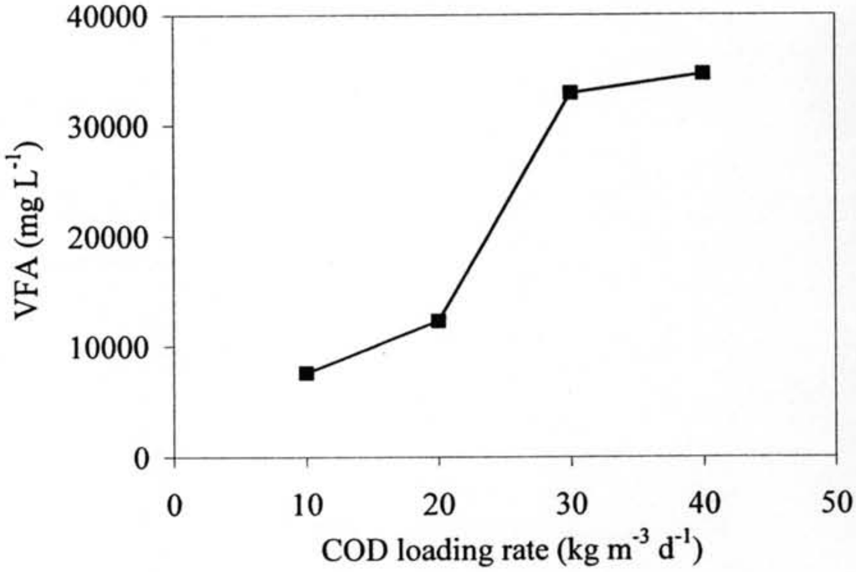

Figure 4.7 Effect of COD loading rate on VFA production at 37°C and 24 h of HRT.

4.1.6 Biomass Production

The high level of biomass concentration is one of the expected consequences of ASBR process since one step of its operation is settle period. Carbon in the influent was mainly converted to biomass, carbon dioxide, and VFA in the effluent. It is clearly seem from Figure 4.8 that COD loading rate affected the biomass concentration in terms of volatile suspended solids (VSS). Two ranges of biomass production were found with increasing COD loading rate as follows: 725 to 796 mg VSS L^{-1} at 10 to 30 kg COD m^{-3} d^{-1}, and 796 to 781 mg VSS L^{-1} at 30 to 40 kg COD m^{-3} d^{-1}. In the first range, the biomass concentration increased with the increase in COD loading rate. According to this result, it can be concluded that the increase in biomass concentration was due to high concentration of glucose in the solution. However, in the second range, the biomass concentration decreased with the further increase in COD loading rate because of acidic conditions of the system, which can suppress the growth of gas-producing bacteria.

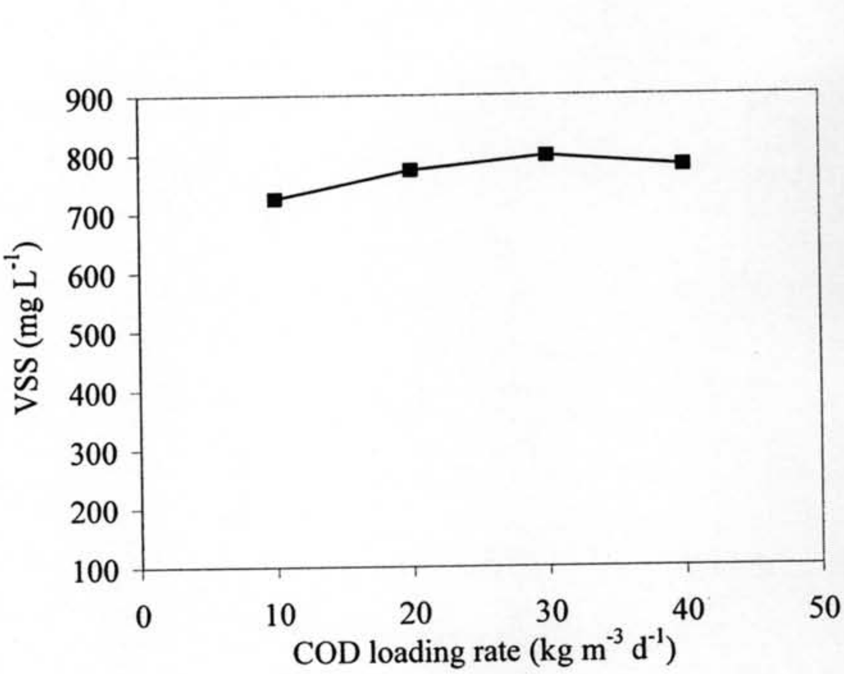

Figure 4.8 Effect of COD loading on biomass concentration (VSS) at 37°C and 24 h of HRT.

4.1.7 COD Removal

As shown in Figure 4.9, the percentage of COD removal increased with increasing the COD loading rate, from 54.37% at 10 kg COD m^{-3} d^{-1} to 69.23% at 20 kg COD m^{-3} d^{-1}. After that, it was nearly constant at 68.44% when the COD loading rate increased to 30 kg COD m^{-3} d^{-1}. This result indicated that glucose in synthetic wastewater was mainly degraded to gas (hydrogen and carbon dioxide) higher than converted to VFA. With further increase in the COD loading rate to 40 kg COD m^{-3} d^{-1}, the percentage of COD removal decreased substantially to 48.58% because glucose was converted mainly to VFA, as confirmed by Figure 4.3 and 4.7.

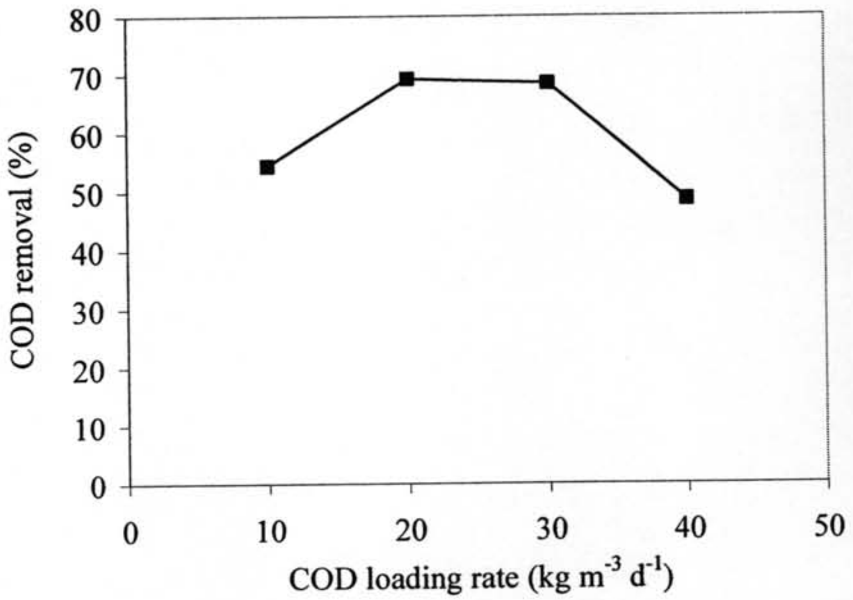

Figure 4.9 Effect of COD loading on COD removal at 37°C and 24 h of HRT.

From the results of the effect of COD loading rate on the biohydrogen production in the ASBR system without pH control, there was no formation of methane in the evolved gas, and only hydrogen and carbon dioxide were formed. The produced hydrogen increased with an increase in COD loading rate. The results show that the yield of hydrogen reached the maximum value at 1.156 mol-H_2/mol-glucose consumed, at the COD loading rate of 30 kg m^{-3} d^{-1}. After that, it decreased to 0.968 mol-H_2/mol-glucose consumed with further increasing the COD loading rate. The percentage of hydrogen was 35% at this COD loading rate. It can be concluded that the optimum COD loading rate is 30 kg m^{-3} d^{-1} for the system without pH control.

Even though the hydrogen yield at the COD loading rate higher than the optimum one decreased doe to the absence of pH control, it is strongly believed that higher hydrogen yield can be obtained at this high COD loading rate of 40 kg m^{-3} d^{-1} if pH of the system is controlled not to be too acidic. Therefore, this high COD loading rate was used as a starting value for further experiments with pH control in order to find the optimum condition for biohydrogen production.

4.2 The Effect of COD Loading Rate at Optimum pH (pH = 5.5)

The pH of the fermentation system is a crucial factor for biohydrogen production since it was reported that the optimum pH was 5.5, and pH below 5.0 inhibited biohydrogen productions for CSTR (Fang and Liu, 2002). Therefore, this work was also performed to find the maximum COD loading rate, which can be applied to produce biohydrogen effectively, at an optimum pH of 5.5. In these experiments, the COD loading rate was started at 40 kg COD m^{-3} d^{-1}, at which the hydrogen productivity from the experiments without pH control was suppressed because of too acidic condition.

4.2.1 Glucose Conversion

Studies in the laboratory have concentrated on pure organic substrates, including glucose. This study also used glucose as organic carbon source for hydrogen-producing fermentations. From the experiments without pH control, the results show that most of glucose was easily converted to H_2, CO_2, VFA, alcohols, and biomass (more than 99%), due to the smallest molecule of glucose ($C_6H_{12}O_6$). In the same way, the results from the system with pH control at 5.5 show that at COD loading rate of 40 kg m^{-3} d^{-1}, glucose was consumed by acidogens effectively (99.65%), exhibiting the highest of glucose consumption, as shown in Table 4.1.

Table 4.1 Comparison of percentage of glucose conversion between systems without and with pH control

COD loading rate (kg m^{-3} d^{-1})	Glucose conversion (%)	
	Un-controlled pH	pH = 5.5
10	99.58	-
20	99.23	-
30	99.17	-
40	99.09	99.65
50	-	81.46

However, when the COD loading rate was increased to 50 kg m^{-3} d^{-1}, the percentage of glucose conversion significantly decreased to 81.46%. It is clear that although the system was controlled at optimum pH of 5.5 as reported in many literature, high concentration of glucose had negative effect on glucose conversion. This might be due to from too high COD loading rate for hydrogen production.

4.2.2 Gas Production rate

The results of comparative experiments between systems without pH control and with pH control at 5.5 show that gas production rate increased from 2.14 to 2.88 L h^{-1} at the same COD loading rate of 40 kg m^{-3} d^{-1}. This evidence suggests that the system with pH control at optimum conditions can provide higher gas production than that without pH control, as shown in Table 4.2. On the other hand, when the system was performed with higher COD loading rate at 50 kg m^{-3} d^{-1}, gas production rate decreased dramatically to 1.33 L h^{-1}. The possible reasons might be the toxicity both from too high of glucose in wastewater and from too high amount of HCl and NaOH used for pH adjustment.

Table 4.2 Comparison of gas production rate between systems without and with pH control

COD loading rate (kg m^{-3} d^{-1})	Gas production rate (L h^{-1})	
	Un-controlled pH	pH = 5.5
10	0.27	-
20	1.09	-
30	2.09	-
40	2.14	2.88
50	-	1.33

In the same way, the percentage of hydrogen in produced gas reached the maximum of 43% at COD loading rate of 40 kg m^{-3} d^{-1} and decreased to 30% at COD loading rate of 50 kg m^{-3} d^{-1}, as summarized in Table 4.3. The reasons are the same as previously explained. Nevertheless, there was still no methane detected in all experiments.

Table 4.3 Comparison of percentage of produced gas composition between systems without and with pH control

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	Un controlled pH			pH = 5.5		
	%H_2	%CO_2	%CH_4	%H_2	%CO_2	%CH_4
10	20	80	0	-	-	-
20	32	68	0	-	-	-
30	35	65	0	-	-	-
40	38	62	0	43	57	0
50	-	-	-	30	70	0

The hydrogen production rate was calculated as shown in Table 4.4. The results show that the hydrogen production rate reached the maximum of 1.237 L h^{-1} at COD loading rate of 40 $kg\ m^{-3}\ d^{-1}$ due to high percentage of hydrogen content. It is clearly seem that the gas production rate at this condition directly affected to the hydrogen production rate.

Table 4.4 Comparison of gas production rate between systems without and with pH control

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	Hydrogen production rate ($L\ h^{-1}$)	
	Un-controlled pH	pH = 5.5
10	0.05	-
20	0.35	-
30	0.73	-
40	0.81	1.24
50	-	0.40

4.2.3 Yield of Hydrogen Production

Yield of hydrogen production or hydrogen productivity was defined as ratio of mole of produced hydrogen to mole of glucose consumed. As shown in Table 4.5, the hydrogen production yield of the system with pH control at 5.5 increased to 1.46 mol-H_2/mol-glucose consumed at COD loading rate of 40 kg m^{-3} d^{-1}, while the hydrogen production yield of the system without pH control reached the maximum value of 1.16 mol-H_2/mol-glucose consumed at 30 kg COD m^{-3} d^{-1}. This might be concluded that pH control induced the system to produce hydrogen more effective than that pH un-controlled system.

Table 4.5 Comparison of yield of hydrogen production between systems without and with pH control

COD loading rate (kg m^{-3} d^{-1})	Yield of hydrogen production (mol H_2/mol glucose consumed)	
	Un-controlled pH	pH = 5.5
10	0.26	-
20	0.83	-
30	1.16	-
40	0.97	1.46
50	-	0.41

4.2.4 Production of VFA

Production of VFA can be quantified approximately as acetic acids by using distillation-titration method as a standard method (Greenberg *et al.,* 1992). Table 4.6 summarizes the experimental results of comparative production of production of VFA between systems without and with pH control when the system reached steady state. For the system without pH control, the VFA production increased with an increase in COD loading rate from 7,611 mg L^{-1} at 10 kg m^{-3} d^{-1} to 34,607 mg L^{-1} at 40 kg m^{-3} d^{-1}. But, for the system with pH control (pH = 5.5) at COD loading rate of 40 kg m^{-3} d^{-1}, the VFA production was 17,304 mg L^{-1}, which was lower than that without pH control at the same COD loading rate. It then decreased with further increase in COD loading rate to 5,768 mg L^{-1} at 50 kg m^{-3} d^{-1}. This is because most of glucose was converted to gas more preferablely to VFA, as shown in Table 4.2 for gas production, resulting in lower VFA production.

Table 4.6 Comparison of VFA production between systems without and with pH control

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	Production of VFA (mg as acetic acid L^{-1})	
	Un-controlled pH	pH = 5.5
10	7,611	-
20	12,286	-
30	32,877	-
40	34,607	17,304
50	-	5,768

4.2.5 Biomass Production (VSS)

Hydrogen-producing bacteria can be quantified by VSS method as a standard method (Greenberg *et al.,* 1992). Table 4.7 shows the experimental data of biomass production for two systems (without and with pH control).

The results show that the biomass concentration in terms of VSS increased with increasing COD loading rate (glucose concentration). When COD loading rate was comparatively high, the biomass concentration in the system with pH control was not much different from that at maximum value for the system without pH control. This is consistent with the results from fermentative biohydrogen production by a pure culture (Oh *et al.,* 2003). The concentration of microflora reported in terms of VSS at COD loading rate of 40 kg m^{-3} d^{-1} for the system without and with pH control were 780 and 890 mg L^{-1}, respectively. For the system with pH control, the biomass concentration was higher than that without pH control. This is because these conditions were suitable for bacteria ability to convert glucose in wastewater. These results correspond to the yield of hydrogen production and gas production rate, as shown in Table 4.5 and Table 4.2, respectively. However, when the COD loading rate was further increased up to 50 kg COD m^{-3} d^{-1}, the biomass concentration decreased to 810 mg L^{-1} due to overloading of glucose in feed solution, which considerably affected the hydrogen-producing bacteria. Furthermore, since the pH of the system with pH control needs to be adjusted at the value of 5.5 by injecting acid and base solution into the fermentor, this can negatively affect to the hydrogen-producing bacteria due to salt formation.

Table 4.7 Comparison of biomass production between systems without and with pH control

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	Biomass production ($mg\ L^{-1}$)	
	Un-controlled pH	pH = 5.5
10	725	-
20	773	-
30	797	-
40	780	890
50	-	818

4.2.6 COD removal

The percentage of COD removal of the system with pH control at value of 5.5 was the highest at COD loading rate of 40 kg m^{-3} d^{-1}, corresponding to the results of VFA concentration in this study. The results of COD removal at 40 kg COD m^{-3} d^{-1} show that the system with pH control gave the higher COD removal as compared to that without pH control. The explanation is that glucose could be converted to gas more selectively than to VFA due to the better microflora activity at the optimum conditions (pH = 5.5), as summarized in Table 4.8, resulting in gas production rate (Table 4.2),

On the other hand, the COD removal drastically decreased to 42.056% at 50 kg m^{-3} d^{-1} of COD loading rate. This is due to the formation of biomass and bacteria ability, resulting from the higher glucose concentration and high concentration of acids produced during the fermentation.

Table 4.8 Comparison of COD removal between systems without and with pH control

COD loading rate ($kg\ m^{-3}\ d^{-1}$)	COD removal (%)	
	Un-controlled pH	pH = 5.5
10	54.38	-
20	69.23	-
30	68.44	-
40	48.58	80.24
50	-	42.06