CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of Number of Cycles per Day

From the literature, the cyclic duration during the operating cycle can play an important role on biohydrogen production (Chen *et al.*, 2008). As aforementioned, the principle of operating the ASBR is based on the four sequential steps, which are continuously repeated in each cycle. The number of operational cycles can be adjusted according to the demand of the operation. Therefore, the effect of number of cycles per day on the biohydrogen production under a mesophilic temperature of 37°C, a controlled pH of 5.5, and a constant HRT of 24 h was first investigated in order to determine the optimum COD loading rate and number of cycles per day for the highest efficiency in hydrogen production.

Figure 4.1(a) depicts the variation of COD removal efficiency with the number of cycles per day. COD removal is normally related to the H_2 and VFA production. The COD removal efficiency reached the maximum of 67% at COD loading rates of 20 and 30 kg/m^{3}d for 4 and 6 cycles per day, respectively. The results indicate that the anaerobic sludge could effectively degrade organic materials in the wastewater to the hydrogen gas, resulting in high hydrogen production rate, high specific hydrogen production rate, and high hydrogen yield as illustrated latter. At lower or higher COD loading rates for both numbers of cycles per day, the COD removal efficiency tended to decrease. This change might be due to the fact that a high VFA concentration was produced in the system, as shown next in Figures 4.2. The gas production rate at different COD loading rates for both 4 and 6 cycles/d is depicted in Figure 4.1(b). For the system with 4 cycles per day, the gas production rate was nearly constant when COD loading rate increased from 10 to 20 kg/m^{3}d. After that, it rapidly decreased with increasing COD loading rate from 20 to 25 kg/m^{3}d. When the system was operated at 6 cycles per day, the gas production rate rapidly increased with increasing COD loading rate from 15 to 30 kg/m^{3}d, but it slightly decreased at a COD loading rate of 37.5 kg/m^{3}d. The decrease in gas production rate at high COD loading rates was observed in both the cases of 4 and 6 cycles per day. The possible reason is the same as previously described for the produced gas composition. The comparative results at different COD loading rates for 4 and 6 cycles per day showed that the gas production rate for the system with 6 cycles per day was much higher than that for the system with 4 cycles per day. This is possibly due to the higher the number of number of cycles per day, the higher the uniformity of organic concentration in the reactor, leading to the higher the gas production rate. Figure 4.1(c) shows the composition of produced gas between 4 and 6 cycles per day at different COD loading rates. For the system with 4 cycles per day, the percentage of hydrogen in produced gas increased with increasing COD loading rate from 0.4% at a COD loading rate of 10 kg/m^{3}d to 37.3% at a COD loading rate of 20 kg/m^{3}d. After that, it dropped to 30.8% at a COD loading rate of 25 kg/m^{3}d because of the negative effect of toxicity from volatile fatty acid (VFA) accumulation. Methane was detected at low COD loading rates in the range of 10-15 kg/m^{3}d for 4 cycles per day since the methanogens in the reactor could utilize both hydrogen and organic acids to form methane with the toxicity of VFA accumulation at a COD loading rate lower than 15 kg/m^{3}d and the methane content decreased dramatically with increasing COD loading rate. It approached zero when the COD loading rate increased or higher than 20 kg/m^{3}d. This is because the system contained a higher VFA concentration which will be further discussed latter. The percentage of carbon dioxide slightly varied in the range from 62.7 to 68.4%. At 6 cycles per day, the percentages of hydrogen and methane show the same trend as those for the system with 4 cycles per day. The maximum hydrogen percentage was obtained at a COD loading rate of 30 kg/m^{3}d. These are in the same trend as the results of Chen *et al.* (2008). They found that the highest hydrogen percentage sucrose fermentation using an ASBR was achieved when operating with 4-h duration, which corresponded to 6 cycles per day. The percentage of carbon dioxide was in the opposite trend to the hydrogen percentage, and it reached the minimum of 58.6% at the same COD loading rate of 30 kg/m^{3}d, as experimentally found in this research. In a comparison between the two different cycles per day, the operation time of 6 cycles per day provided a higher optimum COD loading rate than the lower cycles per day. Figure 4.1(d) shows the microbial concentration in the bioreactor in terms of MLVSS for both 4 and 6 cycles per day operation. The MLVSS decreased with increasing COD loading rate at both 4 and 6 cycle per day. The reason might be that some methanogen would not survive in the ASBR system at high COD loading rate and would be washed out with the liquid effluent.

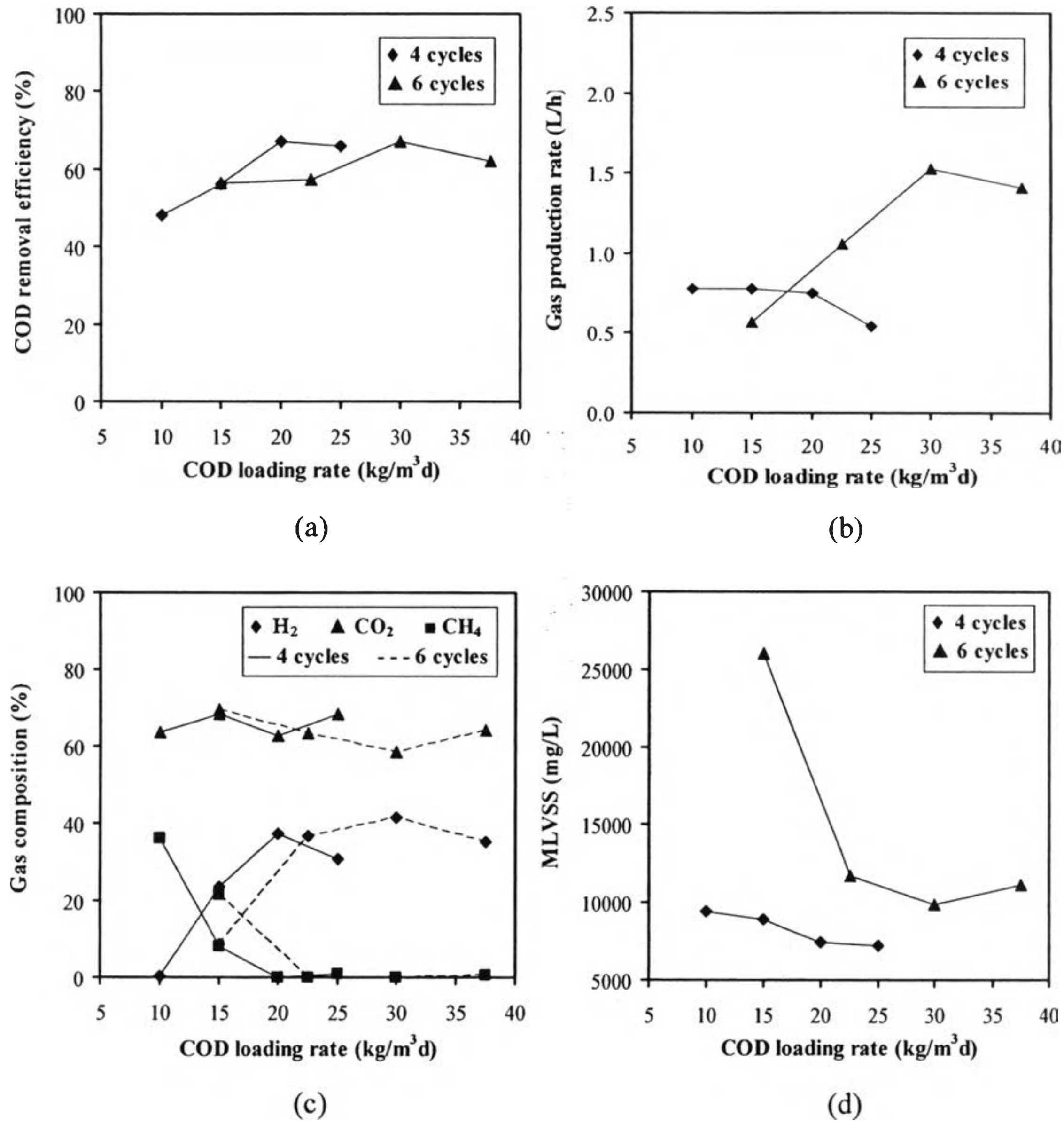
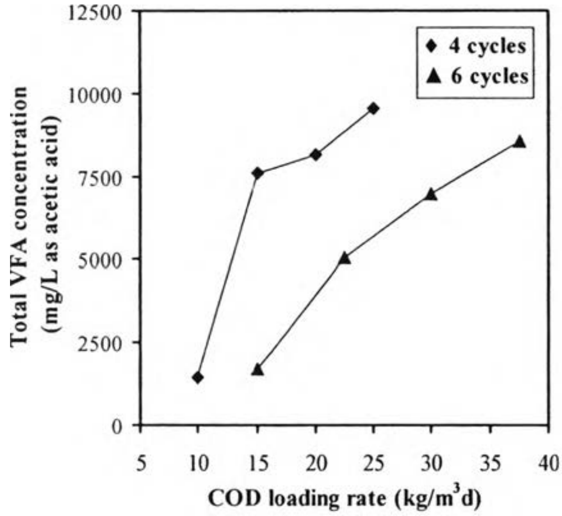
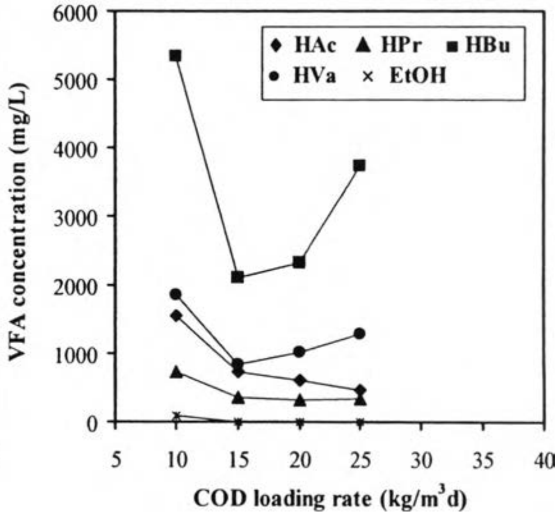


Figure 4.1 Effect of number of cycles per day on (a) COD removal efficiency, (b) gas production rate, (c) gas composition, and (d) MLVSS at different COD loading rate.

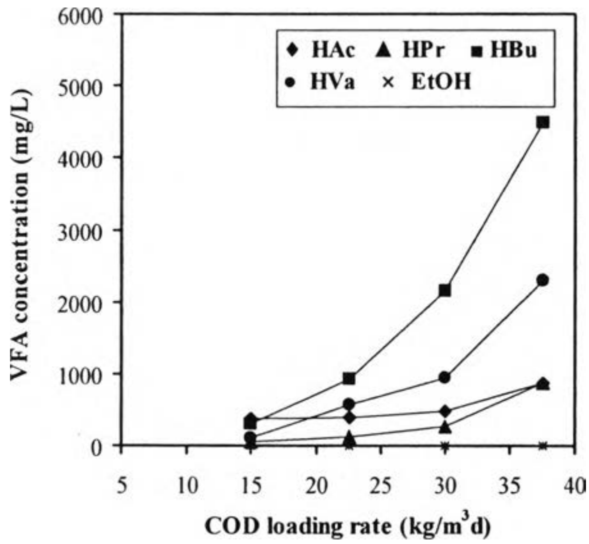
The production of VFA can be quantified approximately as acetic acid by using the distillation-titration method as a standard method (Greenberg *et al.*, 1992). The biohydrogen production from organic waste always accompanies with the VFA production. Figure 4.2(a) shows the dependence of the total VFA concentration in the effluent on the number of cycles per day. The VFA concentration in the effluent increased with increasing COD loading rate for both 4 and 6 cycles per day. The highest VFA concentrations were obtained at COD loading rates of 25 and 37.5 kg/m^3d for the 4 and 6 cycles per day, respectively. These results indicated that too much VFA may negatively lead to a lower hydrogen production, as shown in Figures 4.3. The composition of VFA in effluent liquid was analyzed by a gas chromatograph equipped with a flame ionization detector. Figure 4.2(b) and (c) summarizes the effect of number of cycles per day on the composition of VFA. It can be noticed that the distributions of VFA varied markedly with an increase in COD loading rate for both 4 and 6 cycles per day. Propionic acid (HPr) was also observed throughout the experiments. The HPr concentration decreased from 730 mg/L at a COD loading rate of 10 kg/m^3d to 360 mg/L at a COD loading rate of 15 kg/m^3d for the 4 cycles per day operation. After that, it was nearly constant when COD loading rate increased from 15 to 25 kg/m^3d; whereas, it increased with increasing COD loading rate for the 6 cycles per day operation. This suggested that propionate fermentation was carried out either by hydrogen-producing bacteria or by other microbial populations (Hwang *et al.*, 2004 and Hallenbeck 2005). The valeric acid (HVa) concentration rapidly decreased from 1,850 mg/L at a COD loading rate of 10 kg/m^3d to 840 mg/L at a COD loading rate of 15 kg/m^3d, and afterwards gradually increased to 1,280 mg/L at a COD loading rate of 25 kg/m^3d for the 4 cycles per day. For the system with 6 cycles per day, the HVa concentration had the same trend as HPr and acetic acid (HAc) concentrations. The amount of HAc produced increased from 380 to 870 mg/L for the 6 cycles per day. However, it decreased from 1,550 to 470 mg/L for the 4 cycles per day. For both 4 and 6 cycles per day, butyric acid (HBu) concentration changed remarkably. For the 4 cycles per day, an initial decrease of HBu concentration from 5,330 mg/L at a COD loading rate of 10 kg/m^3d to 2,110 mg/L at a COD loading rate of 15 kg/m^3d was observed, followed by a rapid increase to 3,730 mg/L at a COD loading rate of 25 kg/m^3d; however, it significantly increased with increasing COD loading rate for the 6 cycles per day. The results showed that HBu was the major product for the system with 4 and 6 cycles per day. In addition, it has been reported that the presence of some VFA with high concentrations might result in the production of alcohols, especially ethanol (EtOH), which could also be found throughout the experiments, and the EtOH concentration decreased with increasing number of cycles per day. It may indicate that the metabolism of ethanol production shifts to the formation of VFA because of an adaptive response of the microbe (Yang *et al.*, 2006).



(a)



(b)



(c)

Figure 4.2 Effect of number of cycles per day on (a) total VFA concentration, (b) VFA composition at 4 cycles per day, and (c) VFA composition at 6 cycles per day at different COD loading rates.

The hydrogen production rate was calculated from the gas production rate and the gas composition and is shown in Figure 4.3(a). For both 4 and 6 cycles per day, the hydrogen production rate was low at low COD loading rate due to the low hydrogen percentage in the produced gas. It increased with increasing COD loading rate, reached a maximum, and afterwards decreased at high COD loading rate due to high VFA accumulation (Figure 4.2(a)). At 6 cycles per day, the hydrogen production rate reached as high as 0.63 L/h at a COD loading rate of 30 kg/m^3d. It can be rapidly increased by 56% when compared with the highest hydrogen production rate of 0.28 L/h at a COD loading rate of 20 kg/m^3d for the system with 4 cycles per day. As discussed earlier, the number of cycles per day has a strong impact on the hydrogen production performance from the ASBR. The greater number of cycles per day could increase the production of hydrogen. The reasons for the increase in hydrogen production are still not clear. One explanation could be the microbial population shift, which facilitates the growth of hydrogen-producing bacteria in the mixed culture (Chen *et al.*, 2008), as also shown later by the large increase in MLVSS for the 6 cycles per day operation compared with that for the 4 cycles per day operation. In this study, the change in COD loading rate for the different numbers of cycles per day could provide an opportunity to select hydrogen-producing microbial populations in the bioreactor in order to obtain higher hydrogen production. It was interestingly found that a higher optimum COD loading rate is required for a higher number of cycles per day. The specific hydrogen production rate is directly related to the hydrogen production ability of the bacteria and the bioreactor performance. Figure 4.3(b) shows the effect of number of cycles per day on the specific hydrogen production rate. The optimum specific hydrogen production rate of 388 mL H_2/g VSS d (3,800 mL H_2/L d) was obtained at a COD loading rate of 30 kg/m^3d for the 6 cycles per day operation since the maximum gas production rate and hydrogen percentage in the produced gas were achieved at this conditions (Figures 4.1(b) and (c)). The hydrogen yield is a measure of converting degraded starch into hydrogen gas. The effect of number of cycles per day on hydrogen yield is shown in Figure 4.3 (c) and (d). For both 4 and 6 cycles per day, there was a similar trend of the hydrogen yield. Firstly, it increased when the COD loading rate was varied from 10 to 15 kg/m^3d and 15 to 22.5 kg/m^3d, and then decreased as the COD loading rate was over 20 and 30 kg/m^3d for the system with 4 and 6 cycles per day, respectively. By comparing the results between 4 and 6 cycles/d, the optimum hydrogen yield (186 mL H_2/g COD removed or 125 mL H_2/g COD applied) at a COD loading rate of 30 kg/m^3d for the 6 cycles per day operation was higher than that at a COD loading rate of 15 kg/m^3d for the 4 cycles per day operation (170 mL H_2/g COD removed or 95 mL H_2/g COD applied). Furthermore, a COD loading rate of 30 kg/m^{3}d for the 6 cycles per day operation was the optimum value for hydrogen production since the maximum hydrogen production rate and the maximum specific hydrogen production rate were obtained.

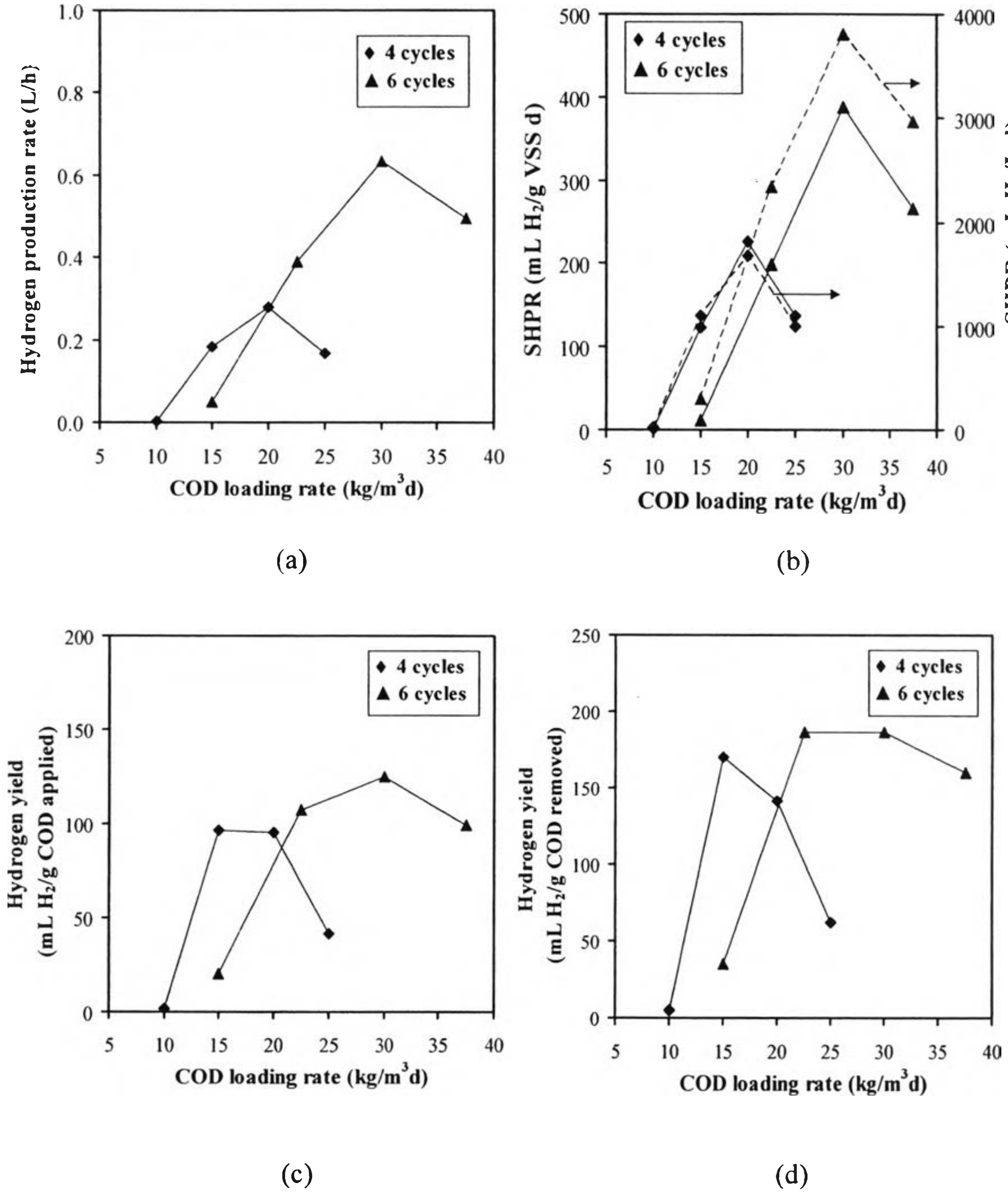


Figure 4.3 Effect of number of cycles per day on (a) hydrogen production rate, (b) SHPR (mL H_2/g VSS d), (c) SHPR (mL H_2/L d), and (d) hydrogen yield at different COD loading rates.

4.2 Effect of Nutrient Supplementation

The COD:N ratio is an important parameter in operating the biological process. Microflora requires a proper nitrogen supplementation for metabolism during fermentation. A proper COD:N ratio value for mixed microflora is necessary to optimize anaerobic hydrogen production from organic wastewater (Lin and Lay, 2004a). Therefore, the effect of COD:N ratio was further investigated in this study in order to optimize the biohydrogen hydrogen production from the cassava wastewater. The optimum conditions obtained in the previous part, which were a COD loading rate of 30 kg/m^{3}d and a number of cycle per day of 6 cycles/d, were used in this part. The raw cassava wastewater was nutrient-supplemented by adding NH_4HCO_3 as the nitrogen source at the COD:N ratios of 100:2.2 (theoretically optimum nitrogen concentration), 100:3.3 (50% excess of nitrogen concentration), and 100:4.4 (100% excess of nitrogen concentration). The system pH was controlled at 5.5. The operational temperature was also under mesophilic condition of 37°C.

Figure 4.4(a) shows the effect of nitrogen supplementation on the COD removal efficiency. The COD removal efficiency slightly decreased with increasing COD:N ratio. The possible reason might be that the bacteria in the bioreactor mainly converted organic wastewater to VFA, as shown later in Figure 4.5, which was also measured as a part of total COD. The maximum COD removal efficiency of 54% was obtained at a COD:N ratio of 100:2.2. It was much less than the system without nutrient supplementation (67%) because the system with nutrient supplementation had a significant high dissolution of carbonate, which was resulted from high NH_4HCO_3 concentration. This was also unavoidably to be measured and included in total COD. This increased the carbon dioxide concentration in the produced gas, as previously mentioned. For the system with nutrient supplementation, the gas production rate slightly decreased with increasing nitrogen concentration from 2.3 L/h at a COD:N ratio of 100:2.2 to 2.1 L/h at a COD:N ratio of 100:4.4, as shown in Figure 4.4(b). The possible reason might be resulted from the accumulation of excess ammonium used as the nitrogen source in wastewater. High ammonium concentration caused toxicity problem (Lin and Lay, 2004b). The maximum gas production rate was also found at a COD:N ratio of 100:2.2. As compared to the system without nutrient supplementation, the gas production rate could be increased by 52%. This clearly indicates that the nutrient supplementation was necessary for anaerobic fermentation since the addition of nitrogen in wastewater can enhance the hydrogen production by stimulating the microflora in the wastewater and improving their degradation activity (Lee *et al.*, 1993). Figure 4.4(c) shows the effect of nitrogen supplementation on produced gas composition. From the results, hydrogen and carbon dioxide were also the main composition in the produced gas. Methane was detected with only a few amounts, which could be neglected. For the system with nutrient supplementation, the hydrogen percentage decreased with increasing COD:N ratio from 40.6% at a COD:N ratio of 100:2.2 to 27.2% at a COD:N ratio of 100:4.4 since the excess amount of nitrogen in wastewater could drive the metabolic pathway to VFA production instead of hydrogen production (Liu and Shen, 2004 and Argun *et al.*, 2008). Moreover, NH_4HCO_3 used as the nitrogen source has a carbonate group in the molecule, which can be correlated with carbon dioxide content in the produced gas. When NH_4HCO_3 concentration increased, carbonate dissolution increased, consequently resulting in high carbonate concentration. This accordingly increased the carbon dioxide fraction, and therefore decreased the hydrogen fraction in the produced gas (Lin and Lay, 2004b). The maximum hydrogen percentage was obtained at a COD:N ratio of 100:2.2, which was almost the same as the system without nutrient supplementation (raw cassava wastewater). Figure 4.4(d) shows the microbial concentration in the bioreactor in terms of MLVSS at different COD:N ratios. The MLVSS declined rapidly from 10,840 mg/L at a COD:N ratio of 100:2.2 to 7,860 mg/L at a COD:N ratio of 3.3. This is reasonable because a COD:N ratio of 100:2.2 is suitable for the growth of hydrogen-producing bacteria. After that, the MLVSS increased rapidly to 11,600 mg/L at a COD:N ratio of 100:4.4 since excess nitrogen concentration in the media may shift the metabolism to the cell growth rather than the gas production. This negatively affected the hydrogen production, as shown in Figure 4.6.

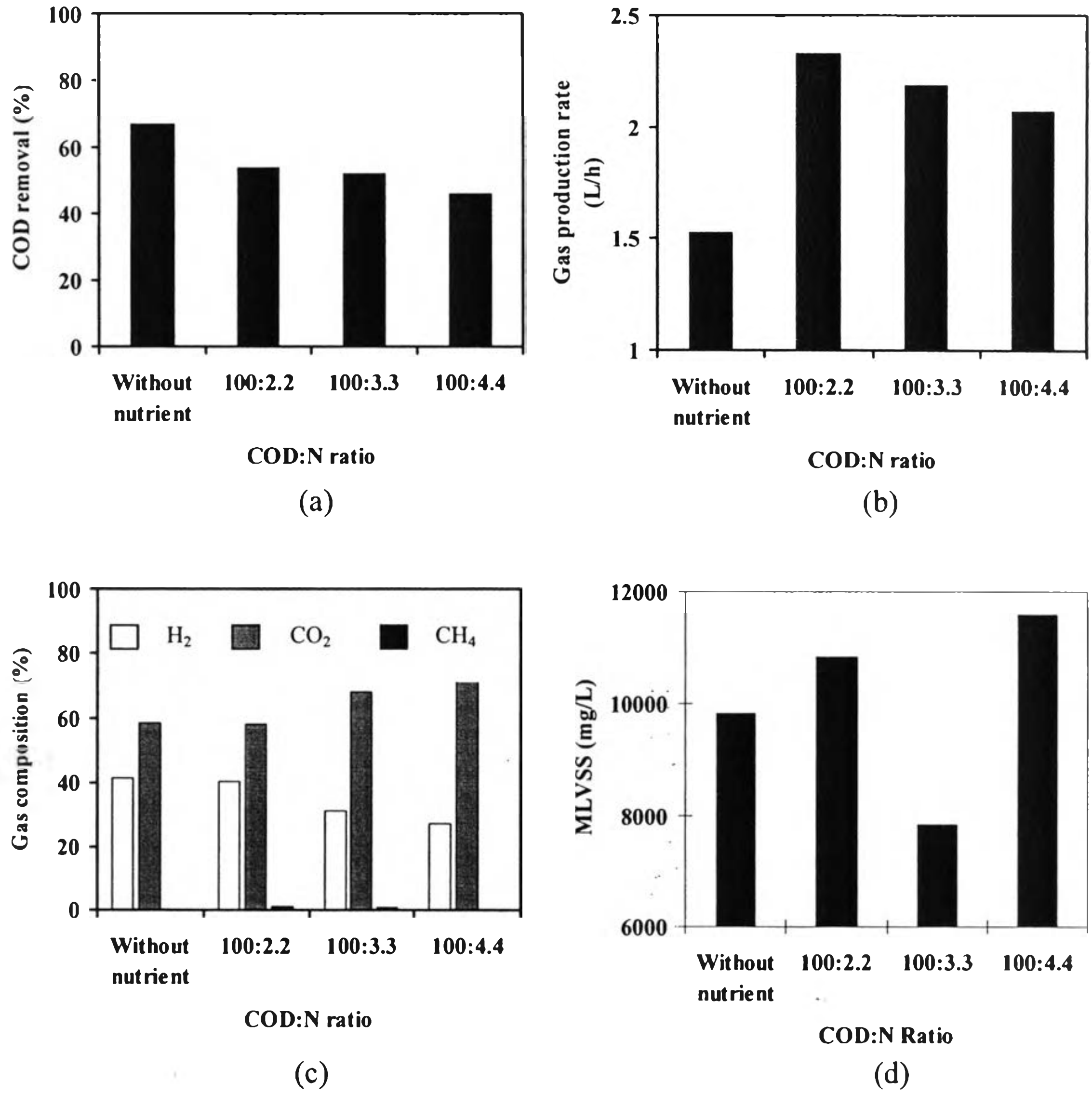


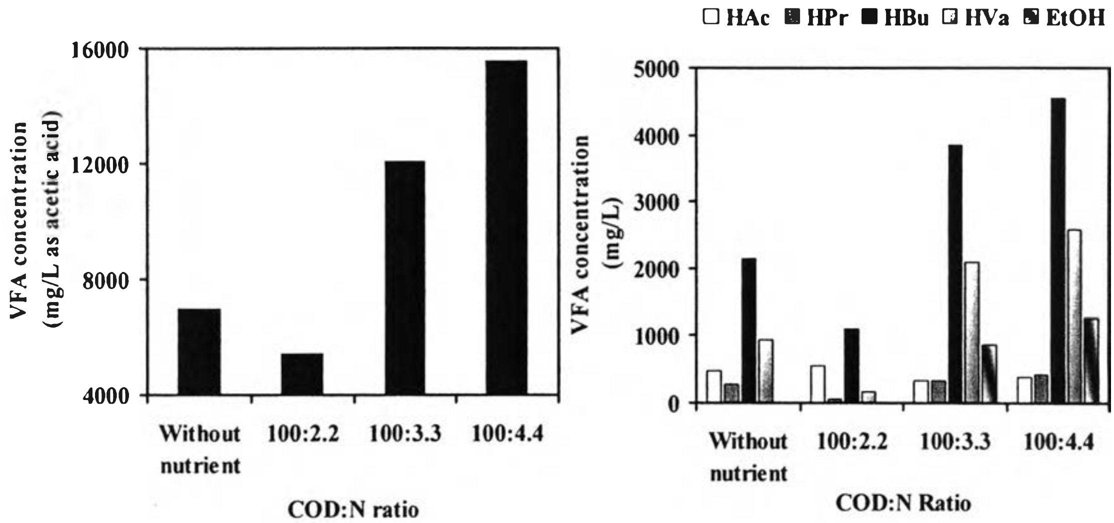
Figure 4.4 Effect of nitrogen supplementation on (a) COD removal efficiency, (b) gas production rate, (c) gas composition, and (d) MLVSS.

Figure 4.5(a) depicts the relationship between the total VFA concentration and the COD:N ratio. The VFA concentration significantly increased with increasing COD:N ratio from 5,450 mg/L at a COD:N ratio of 100:2.2 to 15,570 mg/L at a COD:N ratio of 100:4.4. These obtained results were the same as the results of Liu and Shen (2004). They reported that the increase of nitrogen concentration caused the increase in VFA production in wastewater. The increase in the VFA concentration directly leads to low hydrogen production, as shown in Figures 4.6.

The VFA concentration at the optimum COD:N ratio of 100:2.2 was less than that for the system without nutrient supplementation. Therefore, the nitrogen supplementation is essential to simultaneously achieve efficient hydrogen production rate and hydrogen yield (Figures 4.6). Figure 4.5(b) depicts the effect of nitrogen supplementation on VFA composition. When the COD:N ratio increased, the HBu and HVa concentrations increased. The HBu was the predominant VFA product (1,100-3,850 mg/L), followed by HVa (170-2,580 mg/L), HAc (329-544 mg/L), and HPr (46-420 mg/L). The increase in HBu implied that the acetate fermentation gradually shifted to the HBu fermentation, as clearly observed by the decrease in HAc. The HPr concentration increased with increasing COD:N ratio. These results well corresponded to the decrease in SHPR and hydrogen yield (Figures 4.6) because hydrogen is consumed during the production of HPr via the following equation:

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + 2H_2 \rightarrow \underset{\text{(Propionic acid)}}{2CH_3CH_2COOH} + 2H_2O \qquad (4.1)$$

Thus for biohydrogen production, the production of propionic acid should be avoided (Hawkes *et al.*, 2002). At the COD:N ratio of 100:2.2, the lowest HPr concentration was experimentally found, accompanying with the maximum SHPR of 524 mL H_2/g VSS d (5,676 mL H_2/L d) and the highest hydrogen yield of 438 mL H_2/g COD removed (Figures 4.6). The EtOH concentration increased with increasing COD:N ratio. This negatively affected the hydrogen production since alcohols, especially EtOH, is a kind of more reduced products, thereby consuming a large amount of free electrons that can be used to form hydrogen (Su *et al.*, 2009).



(a) (b)

Figure 4.5 Effect of nitrogen supplementation on (a) total VFA concentration and (b) VFA composition.

Figure 4.6(a) depicts the variation of hydrogen production rate at different COD:N ratios. At a COD:N ratio of 100:2.2, the highest hydrogen production rate of 0.95 L/h was achieved since the highest gas production rate was obtained (Figure 4.4(b)). When the COD:N ratio increased, the hydrogen production rate decreased. The possible reason is the same as explained previously in the produced gas composition and the gas production rate sections. As compared to the system without nutrient supplementation, the hydrogen production rate could also be enhanced since the nutrient supplementation increased the bacterial diversity in the reactor and promoted in particular the growth of hydrogen-producing bacteria (O-Thong *et al.*, 2007). The effect of nitrogen supplementation on the specific hydrogen production rate is shown in Figure 4.6(b). The COD:N ratio had a significant influence on the SHPR. The maximum SHPR of 524 mL H_2/g VSS d (568 mL H_2/L d) was obtained at a COD:N ratio of 100:2.2 because of the high hydrogen production rate (Figure 4.6(a)). The nitrogen content also had a strong effect on the hydrogen yield. The hydrogen yield increased from 186 mL H_2/g COD removed (125 mL H2/g COD applied) for the system without nitrogen supplementation to 438 mL H_2/g COD removed (235 mL H2/g COD applied) for the system with nitrogen supplementation at a COD:N ratio of 100:2.2, as shown in Figure 4.6(c) and (d), and this was the maximum hydrogen yield obtained in this study. The further increase in the COD:N ratio above 100:2.2 resulted in the decrease in the hydrogen yield because of the negative effect of excess nitrogen concentration.

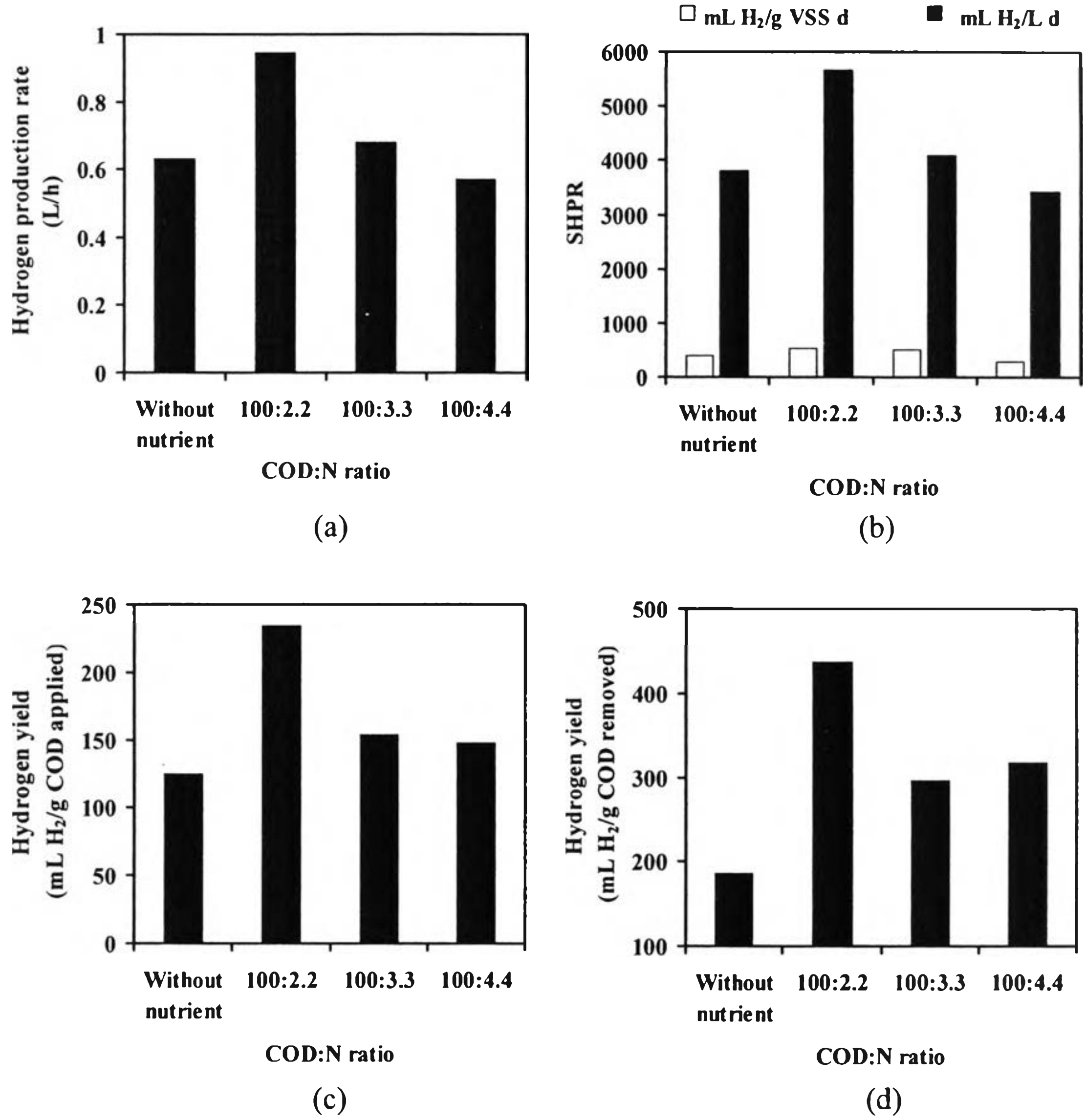


Figure 4.12 Effect of nitrogen supplementation on (a) hydrogen production rate, (b) SHPR, (c) hydrogen yield (mL H_2/g COD applied), and (d) hydrogen yield (mL H_2/g COD applied).