CHAPTER IV
RESULTS AND DISCUSSION

4.1 Hydrogen Production Step

Hydrogen is produced from alcohol wastewater by using upflow anaerobic sludge blanket reactors (UASB). The UASB reactor was operated at a mesophilic temperature (37 °C) and a controlled pH of 5.5. The wastewater was fed with an initial feed COD of 45,000 mg/l. The liquid effluent was recycled to the reactor with a recycle ratio of 1:1. The mixed culture seed sludge was boiled at 95 °C for eliminating methane-producing bacteria that consume hydrogen. The system was operated at different COD loading rate (23, 31, 46 and 62 kg/m^{3}d). When the system was operated until reached to the steady state conditions the effect of COD loading rate on the biohydrogen production was investigated.

4.1.1 Gas Production Rate and COD Removal Efficiency

The gas production rate in terms of L/d and the COD removal efficiency under steady state conditions at different COD loading rates is presented in Figure 4.1 Both the gas production rate and COD removal efficiency increased with increasing COD loading rate from 23 to 46 kg/m^{3}d and then decreased with further increasing COD loading rate from 46 to 62 kg/m^{3}d. The maximum gas production rate and COD removal efficiency were 64 l/d and 27.3%, respectively at a COD loading rate of 46 kg/m^{3}d. The results can be explained by the fact that high amount of organic compounds in the reactor at a high COD loading rate provided higher substrates available for microbes to convert into higher quantities of gaseous products (Yang *et al.*, 2006). However at a very high COD loading rate, both gas production rate and COD removal decreased due to the microbial cells were washed out from the system as a result of the increasing toxicity from the VFA accumulation in the system which will be discussed later.

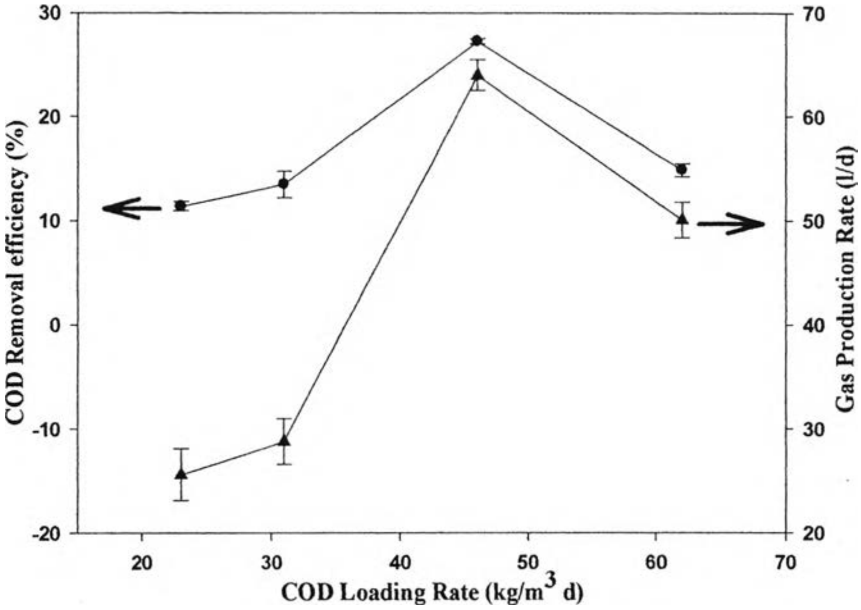

Figure 4.1 COD removal efficiency and gas production rate versus COD loading rate at 37°C and pH 5.5.

4.1.2 Hydrogen Production Rate and Gas Composition

The hydrogen production rate is calculated from the gas production rate and hydrogen composition. From Figure 4.2, the hydrogen production rate increases with increasing COD loading rate and then decreased with further increased COD loading rate. The maximum hydrogen production rate of 17.7 l/d was found at a COD loading rate of 46 kg/m^{3}d. For the gas composition mainly consisted of hydrogen and carbon dioxide. The hydrogen percentage increased with increasing COD loading rate and reached a maximum at a COD loading of 46 kg/m^{3}d. After that, the hydrogen percentage decreased to 24.8% with further increasing COD loading rate from 46 to 62 kg/m^{3}d. The results can be explained in that more microbial cells were washed out from the system as a result from the toxicity of VFA accumulation (Yang *et al.*, 2006). When the COD loading rate increased from 23 to 62 kg/m^{3}d, the carbon dioxide content showed an opposite trend to the hydrogen content. Fang found the same results that the produced gas comprised of mostly hydrogen and carbon dioxide and the carbon dioxide had an opposite trend to the hydrogen content (Fang *et al.*, 2002).

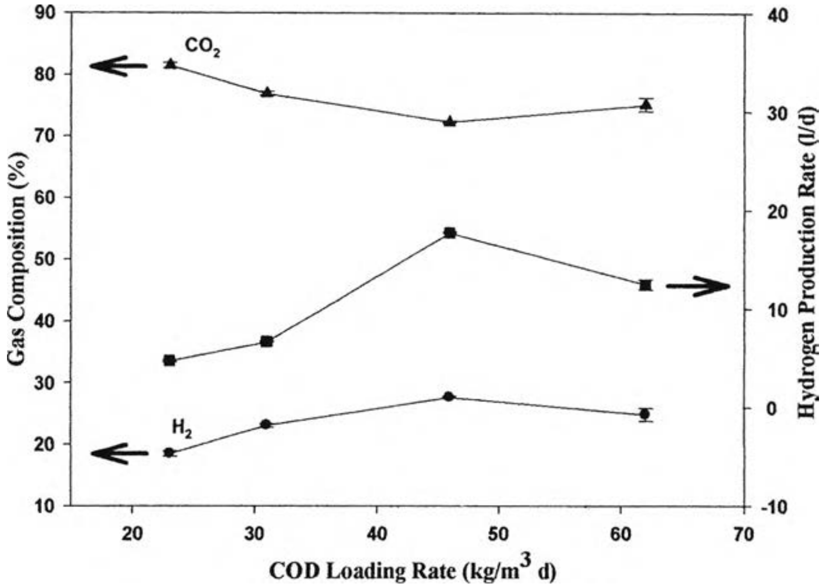

Figure 4.2 Gas composition and Hydrogen production rate versus COD loading rate at 37°C and pH 5.5.

4.1.3 Specific Hydrogen Production Rate

Specific hydrogen production rate (SHPR) is defined as the hydrogen production rate per unit weight of the microbial cells in the bioreactor. Figure 4.3 shows the specific hydrogen production rate at different COD loading rate. The results showed that with increasing COD loading rate, the SHPR increased and reached a maximum value of 1,076.5 ml H_2/g MLVSS d or 4,430 ml H_2/ L d corresponding to the highest microbial concentration in the system which will be discussed later. When the system was operated at a higher COD loading rate higher than 46 kg/m^{3}d, the SHPR decreased markedly. The decrease in SHPR with further increasing COD loading rate results from the toxicity from VFA accumulation in the bioreactor, which can inhibit the growth of hydrogen-producing bacteria (Chang and Lin, 2004)..

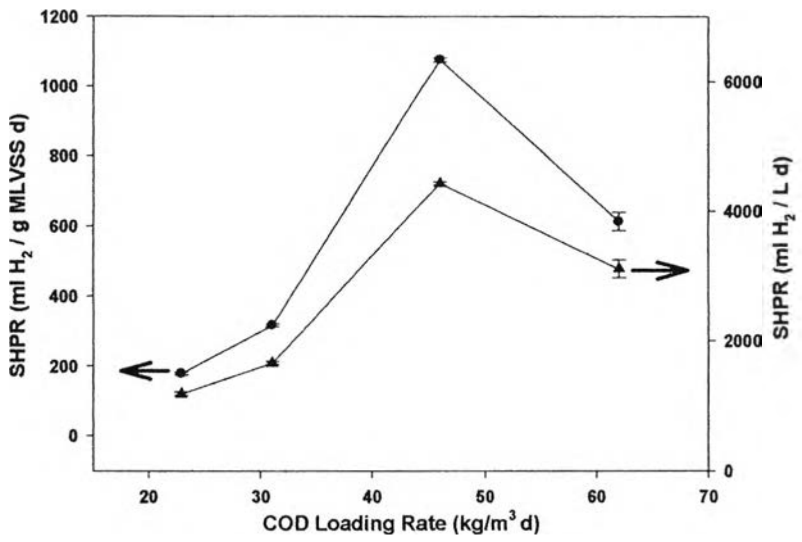

Figure 4.3 Specific hydrogen production rate (SPHR) versus COD loading rate at 37°C and pH 5.5.

4.1.4 Hydrogen Yield

Hydrogen yield is defined as the ratio of the amount of produced hydrogen to the amount of organic substrate consumed in the unit of ml/g COD removed. Figure 4.4 shows the hydrogen yield at different COD loading rate. The hydrogen yield increased with increasing COD loading rate and reached the maximum hydrogen yield of 125.1 ml H_2/g COD removed. However, at a COD loading rate of 62 kg/m^{3}d, the yield of hydrogen decreased to 58.9 ml H_2/g COD removed because the high concentration of volatile fatty acids in the reactor, resulting in the high toxicity to the microbes.

4.1.5 Microbial Concentration

The microbial concentration (MLVSS) and the microbial wash-out (VSS) in bioreactor are shown in Figure 4.5. The MLVSS decreased with increasing COD loading rate while VSS increased with increasing COD loading rate from 23 to 46 kg/m^{3}d. With further increasing COD loading rate, the MLVSS slightly increased up to 5,000 mg/l while the VSS decreased to 2,400 mg/l. The results suggest that both MLVSS and VSS indicating microbial concentration and microbial wash-out can affect hydrogen production performance.

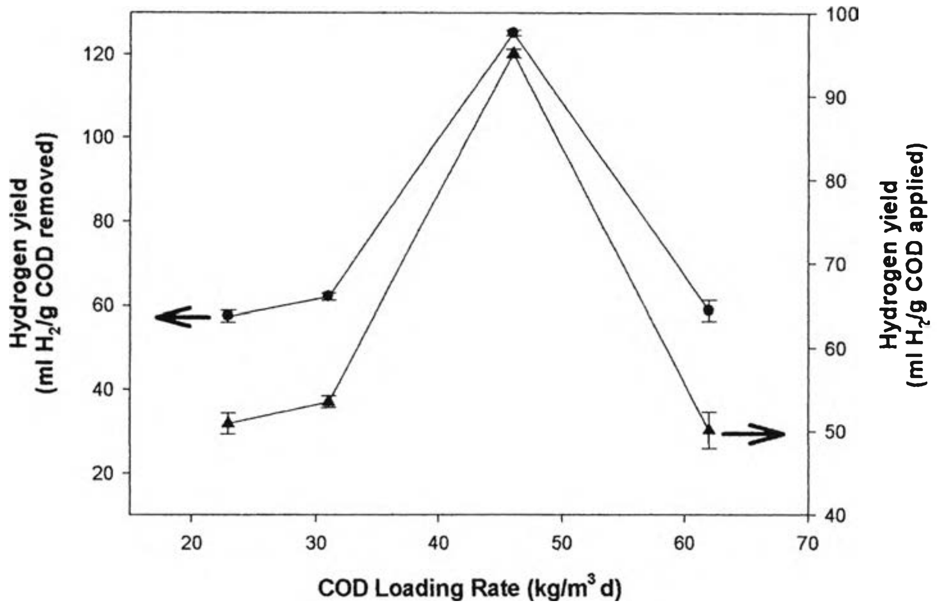

Figure 4.4 Hydrogen yield versus COD loading rate at 37°C and pH 5.5.

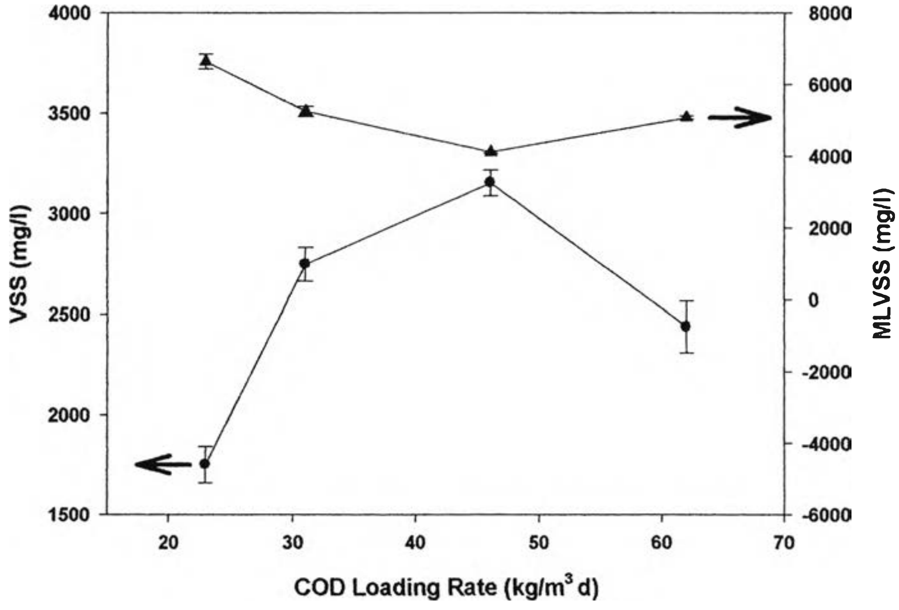

Figure 4.5 MLVSS and effluent VSS versus COD loading rate at 37°C and pH 5.5.

4.1.6 The Amount of Volatile Fatty Acid (VFA)

The amount of VFA was quantified approximately as acetic acid by using a distillation-titration method as a standard method (Eaton *et al.*, 2005). Figure 4.6 shows the effect of COD loading rate on amount of VFA. The total VFA concentration increased remarkably with increasing COD loading rate. The maximum total VFA concentration was greater than 10,000 mg/l as acetic acid at a higher COD loading rate (>46 kg/m^{3}d) shows the toxicity to the microbes which is the cause of the decrease in hydrogen production performance.

From the results can be explained that a further increase in the COD loading rate negatively affected the hydrogen production performance because of too high total VFA concentration. This also indicates that higher total VFA concentrations were produced at a higher COD loading rate, since the bacteria would shift its metabolism towards the VFA production greater than the hydrogen production. So, it could be concluded that too much total VFA produced at a very high COD loading rate may inhibit the growth of hydrogen-producing bacteria and also reduce the hydrogen production performance (Fan *et al.*, 2006).

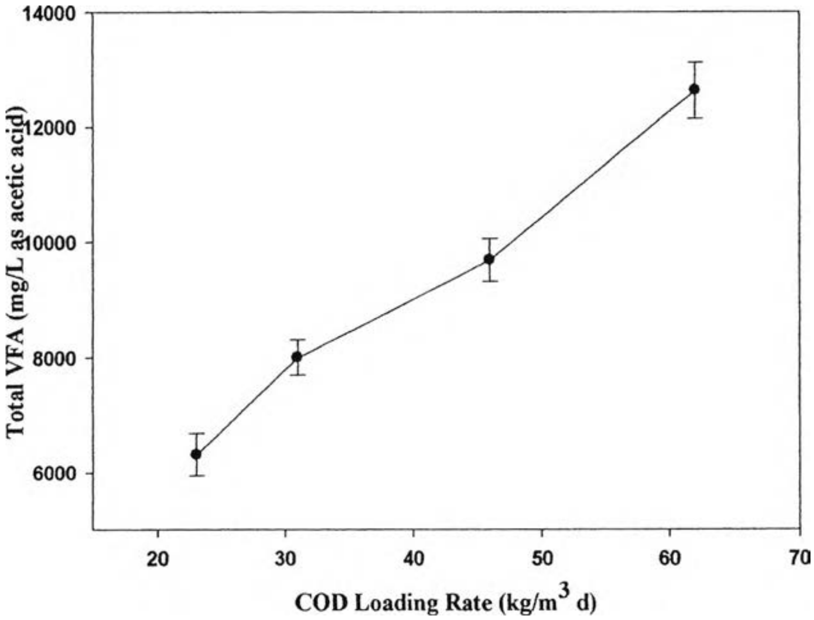

Figure 4.6 The amount of volatile fatty acid as a function of COD loading rate at 37°C and pH 5.5.

Aside from hydrogen and carbon-dioxide, the main liquid products were VFA, which useful parameters for monitoring hydrogen production (Yang *et al.*, 2006). The main components of VFA were acetic acid, propionic acid, butyric acid, and valeric acid. Figure. 4.7 shows the concentration of VFA and ethanol, the results show that ethanol concentration increased with increasing COD loading rate from 23 to 62 kg/m^3 d. At a COD loading rate 62 kg/m^3 d, the highest ethanol concentration of 2,300 mg/l was archived by the highest hydrogen production rate. From the result can be explained that the produced ethanol can reduce the acidity of wastewater and accordingly improve the efficiency of hydrogen production, according to Equation 4.1 .

Ethanol production: $C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2H_2 + 2CO_2$ (4.1)

Butyric acid and valeric acid drastically increased with increasing COD loading rate higher than 46 kg/m^{3}d. From the results, butyric acid was the highest among four kinds of acids which had the same trend with Zhao. In 2008, Zhao and co-workers studied the optimization of hydrogen production by UASB and found that, the fraction of VFA remained in the order: butyric acid > acetic acid> propionic acid (Zhao *et al.*, 2008). As known, the higher amount of butyric acid and acetic acid can lead to higher hydrogen production according to the Equation 4.2 and 4.3.

Acetic acid production: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (4.2)

Butyric acid production: $C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2CO_2 + 2H_2$ (4.3)

From the results, both acetic acid and propionic acid slightly increased with increasing COD loading rate. The propionic acid concentration negatively affected to hydrogen production because of the metabolic pathway for the consumption of produced hydrogen according to Equation 4.4. Therefore, to maximize the hydrogen production rate, the propionic acid production should be avoided by operating under optimum condition (Sreethawong *et al.*, 2010).

Propionic acid production: $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (4.4)

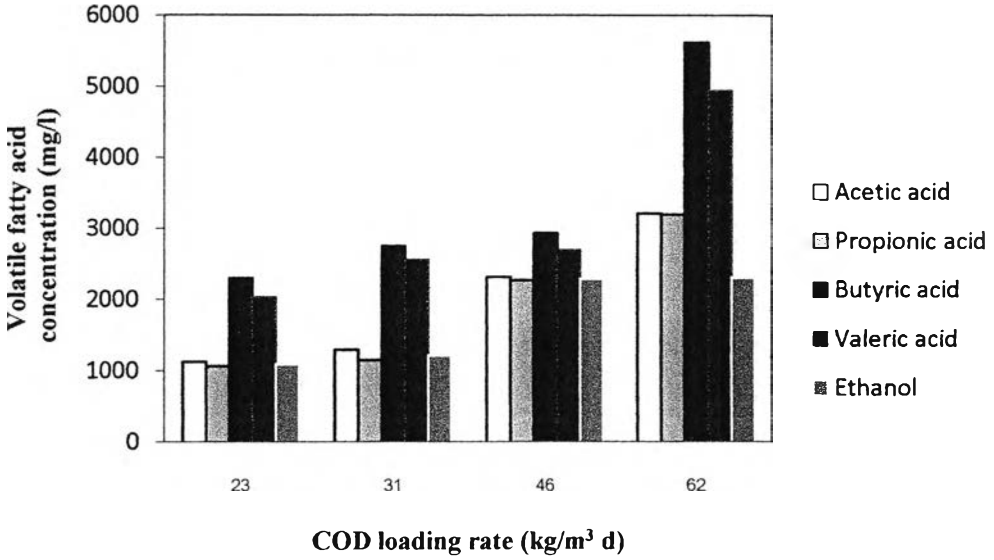

Figure 4.7 The volatile fatty acid concentration as a function of COD loading rate at 37°C and pH 5.5.

4.1.7 The Nitrogen and Phosphorus Removal Efficiency

As known, Nitrogen and Phosphorus were the nutrient of bacteria. Figure 4.8, shown the effect of COD loading rate on nitrogen and phosphorus removal efficiency. Both nitrogen and phosphorus removal efficiency decreased with increasing COD loading rate and then increased with further increased COD loading rate. The lowest nitrogen and phosphorus removal efficiency was found at a COD loading rate of 46 kg/m^3d. From the results can explain that at a COD loading rate of 46 kg/m^3d was the maximum hydrogen production rate. The results can be explained by the fact that high amount of organic compounds in the reactor at a high COD loading rate provided higher substrates available for microbes to convert into higher quantities of gaseous products (Yang *et al.*, 2006).

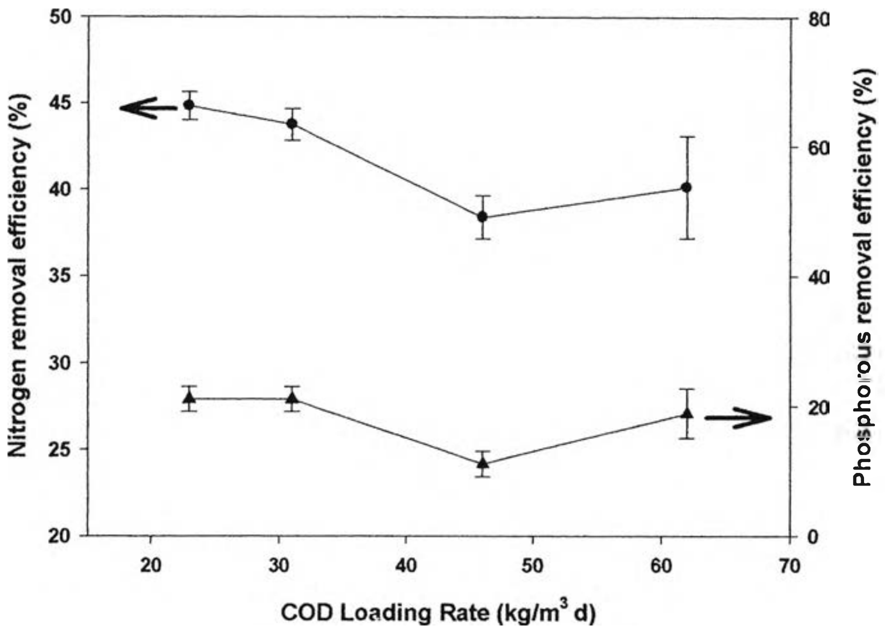

Figure 4.8 Nitrogen and phosphorus removal efficiency versus COD loading rate at 37°C and pH 5.5.

From the overall experimental results on hydrogen production step, the optimum COD loading rate was suggested at 46 kg/m^{3}d. Under this condition, the highest hydrogen content (27%), hydrogen yield (125.1 ml H_2/g COD removed and 95.1 ml H_2/g COD applied), hydrogen production rate (18 l/d), specific hydrogen production rate (1080 ml H_2/g MLVSS d and 1430 ml H_2/ L d), and COD removal (24%) were obtained. the COD loading rate of 46 kg/m^3 was chosen as the optimum condition because it provided the maximum hydrogen content, hydrogen yield, COD removal efficiency and microbial concentration which can be indicates that such COD loading rate was suitable for microbial growth.

4.2 Methane Production Step

For methane production step, the effluent from the hydrogen production step operated (at the optimum conditions) was fed into the UASB system with an initial feed COD of 31,000 mg/l. The system was operated at different COD loading rates

(4.5, 6.2, 8.8 and 11.6 kg/m^{3}d) without pH control and without recycling in order to obtain a maximum methane production.

4.2.1 Gas Production Rate and COD Removal Efficiency

The gas production rate on methane production step in terms of L/d and the COD removal efficiency under steady state conditions at different COD loading rates is presented in Figure 4.9. Both the gas production rate and COD removal efficiency increased with increasing COD loading rate from 4.5 to 8.8 kg/m^{3}d and then decreased with further increasing COD loading rate from 8.8 to 11.6 kg/m^{3}d. The maximum gas production rate and COD removal efficiency were 60.6 l/d and 50.4%, respectively at a COD loading rate of 8.8 kg/m^{3}d.

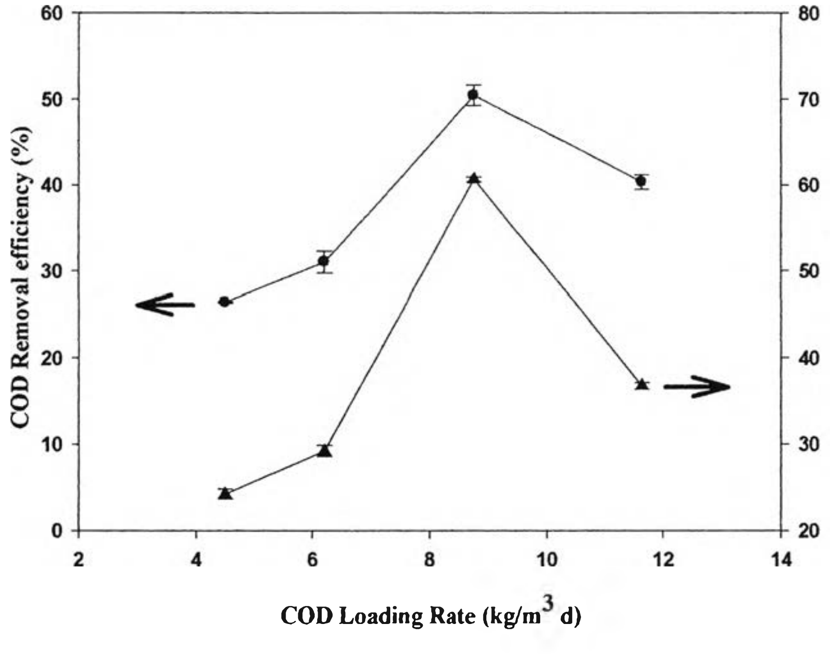

Figure 4.9 COD removal efficiency and gas production rate versus COD loading rate at 37°C.

4.2.2 Methane Production Rate and Gas Composition

The methane production rate is calculated from the gas production rate and hydrogen composition. From Figure 4.10, the methane production rate increases with increasing COD loading rate and then decreased with further increased COD loading rate. The maximum methane production rate of 20.37 l/d was found at a COD loading rate of 8.8 kg/m^{3}d. For the gas composition mainly consisted of methane and carbon dioxide. The methane percentage increased with increasing COD loading rate and reached a maximum methane percentage of 66.41% at a COD loading of 8.8 kg/m^{3}d. After that, the methane percentage decreased to 64.48% with further increasing COD loading rate from 8.8 to 11.6 kg/m^{3}d. The methane production was relatively on hydrogen and VFA generated from hydrogen production step. The lower in CH_4 produced in this study might be that the extraction of hydrogen gas formed during acidogenic process (in the hydrogen production step) which is essential for CH_4 formation.

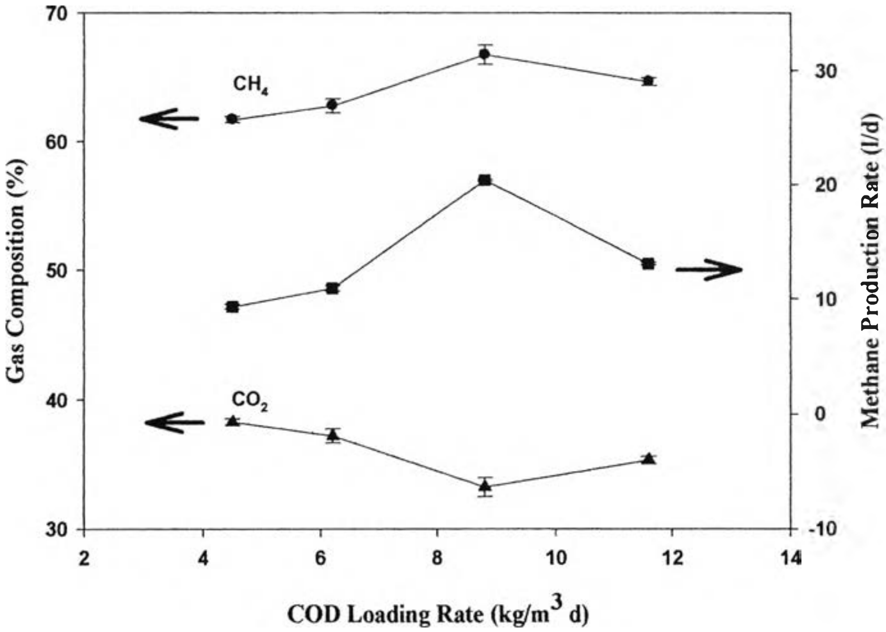

Figure 4.10 Gas composition and Methane production rate versus COD loading rate at 37°C.

4.2.3 <u>Specific Methane Production Rate</u>

Specific methane production rate is defined as the methane production rate per unit weight of the microbial cells in the bioreactor. Figure 4.11 shows the specific methane production rate at different COD loading rate. The results showed that with increasing COD loading rate, the specific methane production rate increased and reached a maximum value of 1,720 ml CH_4/g MLVSS d or 5,091 ml CH_4/ L d corresponding to the highest microbial concentration in the system which will be discussed later. When the system was operated at a higher COD loading rate higher than 8.8 kg/m^{3}d, the specific methane production rate was decreased.

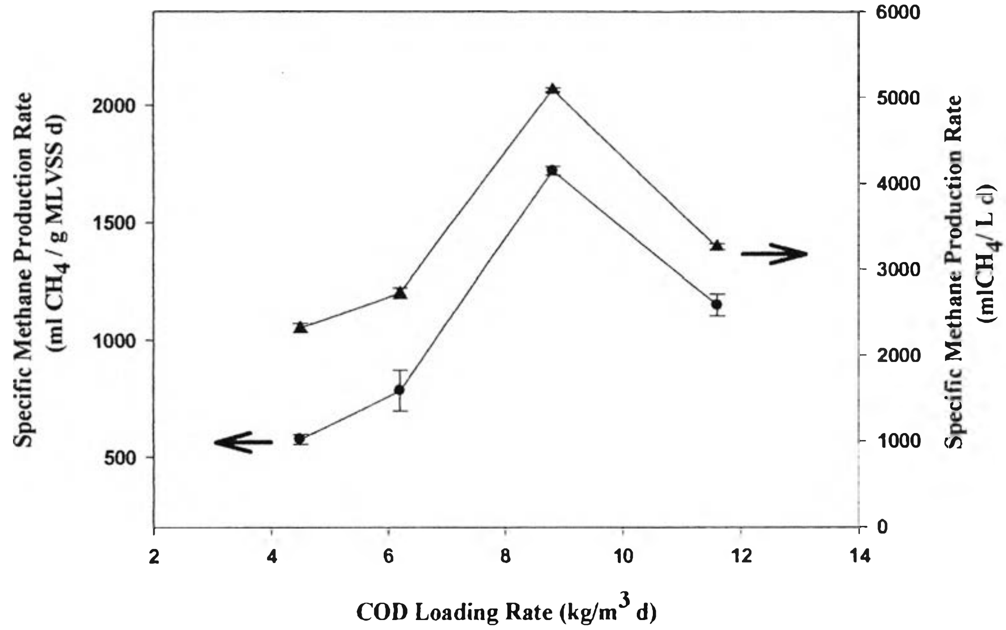

Figure 4.11 Specific methane production rate versus COD loading rate at 37°C.

4.2.4 Methane Yield

Methane yield is defined as the ratio of the amount of produced methane to the amount of organic substrate consumed in the unit of ml/g COD removed. Figure 4.12 shows the methane yield at different COD loading rate. The methane yield increased with increasing COD loading rate and reached the maximum methane yield of 1,172.96 ml CH$_4$/g COD removed. However, at a COD loading rate of 11.6 kg/m^{3}d, the yield of methane decreased to 470.44 ml CH$_4$/g COD removed.

4.2.5 Microbial Concentration

The microbial concentration (MLVSS) and the microbial wash-out (VSS) in bioreactor are shown in Figure 4.13. The MLVSS decreased with increasing COD loading rate while VSS increased with increasing COD loading rate. With further increasing COD loading rate(>8.8 kg/m^{3}d), VSS slightly decreased to 2,860 mg/l. The results suggest that both MLVSS and VSS indicating microbial concentration and microbial wash-out can affect methane production performance.

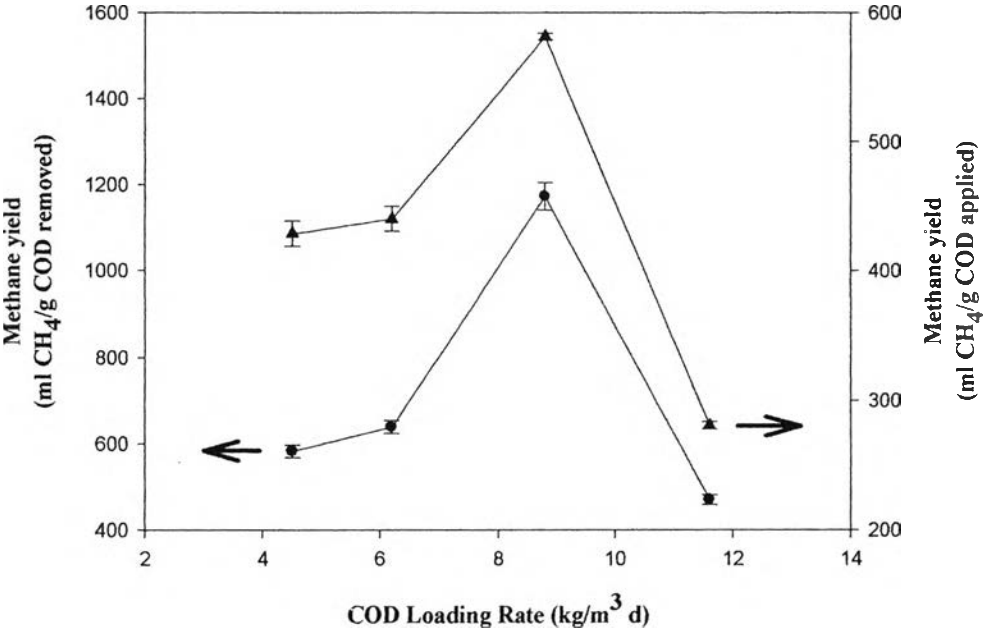

Figure 4.12 Methane yield versus COD loading rate at 37°C.

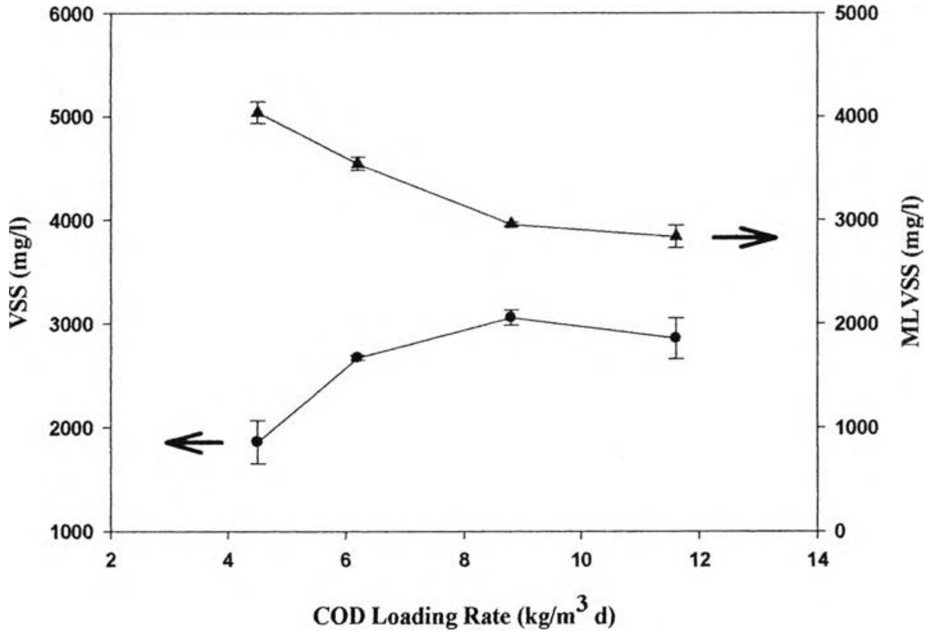

Figure 4.13 MLVSS and effluent VSS versus COD loading rate at 37°C.

4.2.6 The Nitrogen and Phosphorus Removal Efficiency

As mentioned before in the hydrogen production step, nitrogen and phosphorus were the nutrient of bacteria. Figure 4.14, shows the effect of COD loading rate on nitrogen and phosphorus removal efficiency on methane production. Both nitrogen and phosphorus removal efficiency show the same trend with hydrogen production step, nitrogen and phosphorus removal efficiency decrease with increasing COD loading rate and then increased with further increased COD loading rate. The lowest nitrogen and phosphorus removal efficiency was found at a COD loading rate of 8.8 kg/m^{3}d.

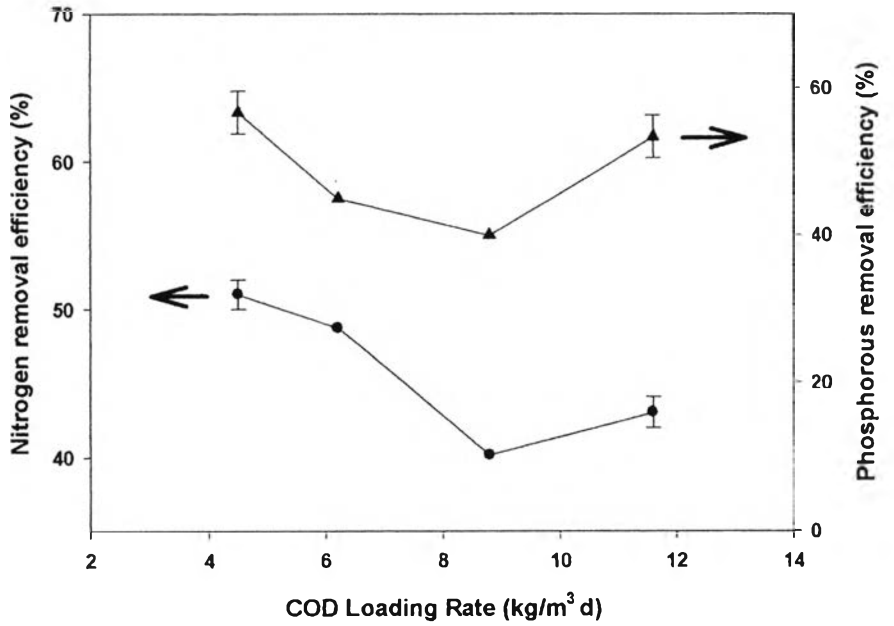

Figure 4.14 Nitrogen and phosphorus removal efficiency versus COD loading rate at 37°C.

4.2.7 The Amount of Volatile Fatty Acid (VFA)

The amount of VFA was quantified approximately as acetic acid by using a distillation-titration method as a standard method (Eaton *et al.*, 2005). Figure 4.15 shows the effect of COD loading rate on amount of VFA. The total VFA concentration decreased remarkably with increasing COD loading rate. As known, methane was produced in methanogenesis step which consumes hydrogen and VFA generated from the primary acidogenesis step, according to the Equation 4.4-4.7. (Mohan *et al.*, 2008).

$CH_3COOH \rightarrow 2H_2 + CO_2$ (4.4)

$CH_3CH_2\ CH_2COOH + 2H_2O \leftrightarrow 2CH_3COOH + 2H_2$ (4.5)

$CH_3CH_2CH_2\ CH_2COOH + H_2O \leftrightarrow CH_3\ CH_2COOH^- + CH_3COOH^- + 2H_2$ (4.6)

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (4.7)

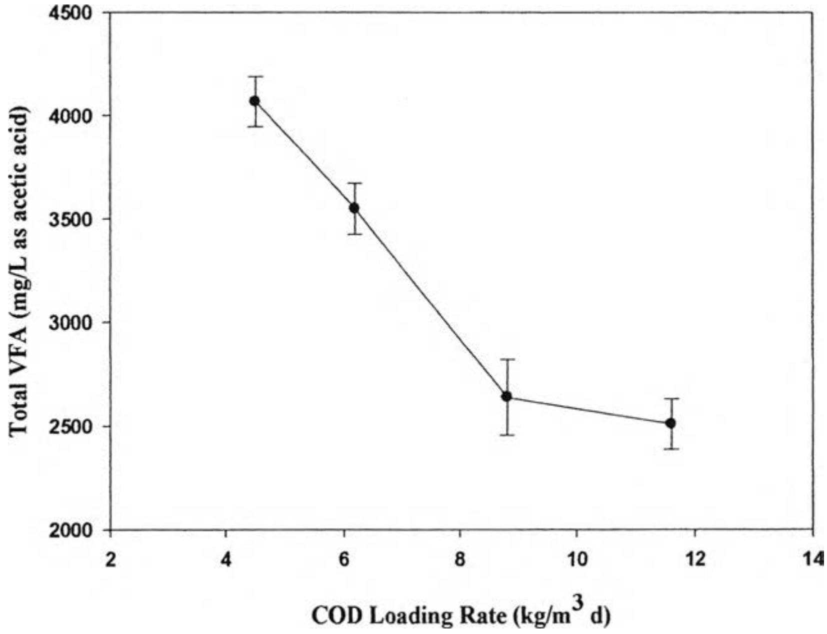

Figure 4.15 Total volatile fatty acid (VFA) versus COD loading rate at 37°C.

Figure. 4.16 show the concentration of VFA and ethanol concentration. Both ethanol and VFA concentration decreased with increasing COD loading rate from 4.5 to 8.8 kg/m^{3}d and then increased with further increasing COD loading rate from 8.8 to 11.6 kg/m^{3}d. The lowest VFA and ethanol concentration were at COD loading rate of 8.8 kg/m^{3}d which can be produced the highest methane production rate. The result shows that VFA and ethanol produced from hydrogen production step can be recovered to produce methane.

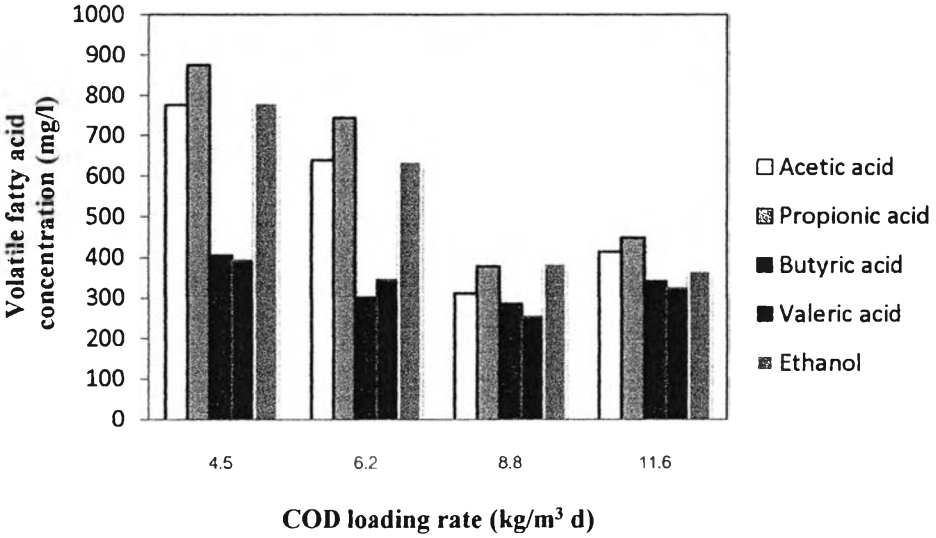

Figure 4.16 The volatile fatty acid concentration as a function of COD loading rate at 37°C.

From the overall experimental results on methane production step, the optimum COD loading rate was suggested at 8.8 kg/m^{3}d. Under this condition, the highest methane content (66.41%), methane yield (1,172.96 ml CH_4/g COD removed and 581.43 ml CH_4/g COD applied), methane production rate (20.37 l/d), specific methane production rate (1,720.32 ml CH_4/g MLVSS d and 5,091.91 ml CH_4/ L d), and COD removal (50.41%) were obtained. the COD loading rate of 8.8 kg/m^3 was chosen as the optimum condition because it achieved a maximum methane production, COD removal efficiency and microbial concentration which can be indicates that it is possible to recover the unused wastewater from acidogenic process to produce methane.