CHAPTER IV
RESULTS AND DISCUSSION

Hydrogen was produced from ethanol wastewater by using upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor was operated at a mesophilic temperature of 37 °C and a controlled pH of 5.5. The wastewater was fed with an initial feed COD of 60,000 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 effect of COD loading rate from 10 to 35 kg/m^3 d with 5 kg/m^3 d increment was studied. For each run, gas production rate, gas composition, and effluent COD were measured daily until the system reached steady state. The optimum COD loading rate was determined by considering not only the hydrogen yield and specific hydrogen production rate, but also the COD removal efficiency.

4.1 Gas Production Results

The effect of COD loading rate on the gas production rate is shown in Figure 4.1. The gas production rate increased with increasing COD loading rate from 4.7 l/d at a COD loading rate of 10 kg/m^3 d to 50.6 l/d at a COD loading rate of 35 kg/m^3 d. This result can be explained in that at a higher COD loading rate, there was a large amount of organic substrate available in the system. The microbes could therefore more effectively consume this available organic substrate for gas production and their growth, corresponding well to the increase in microbial concentration with increasing COD loading rate (Yusoff et *al.*, 2010), as shown in section 4.2.

Figure 4.2 shows the effect of COD loading rate on the produced gas composition. The produced gas mainly consisted of hydrogen, carbon dioxide, and methane. At a COD loading rate of 10 kg/m^3 d, the hydrogen percentage yielded 17.8%, and the maximum hydrogen percentage was achieved at a COD loading rate 20 kg/m^3 d. Then, the hydrogen percentage slightly decreased with further increasing COD loading rate from 25 to 35 kg/m^3 d. This might be possibly due to the higher

VFA accumulation toxicity (as shown in section 4.4), which can inhibit the activity of hydrogen-producing bacteria (Chang and Lin, 2004). However, the methane percentage rapidly decreased from 19 to 4.4% with increasing COD loading rate from 10 to 15 kg/m^3 d. After that, it was nearly constant when the COD loading rate increased from 15 to 35 kg/m^3 d. This might be due to a shorter hydraulic retention time at a higher COD loading rate, which was unfavorable condition for the growth of methane-producing bacteria (Hawkes *et al.*, 2002). The carbon dioxide percentage reached the minimum value of 58.3% at a COD loading rate of 20 kg/m^3 d. After that, the carbon dioxide percentage slightly increased with further increasing COD loading rate up to 35 kg/m^3 d, which was in the opposite trend to the hydrogen percentage.

Basically, the hydrogen production rate depends on both the gas production rate and hydrogen percentage in the produced gas. The effect of COD loading rate on the hydrogen production rate is shown in Figure 4.3. The hydrogen production rate increased from 0.8 to 14.6 l/d with increasing COD loading rate from 10 to 35 kg/m^3 d. The maximum hydrogen production rate of 14.6 l/d corresponds well to the maximum gas production rate at this COD loading rate. It was interestingly found that even though the hydrogen percentage in the produced gas reached the maximum value at the COD loading rate of 20 kg/m^3 d, a comparatively low hydrogen production rate of 5.7 l/d was observed at this COD loading rate. This is possibly because there was a high proportion of hydrogen-producing bacteria in the entire low microbial population in the bioreactor, as evidenced by the microbial concentration in section 4.2.

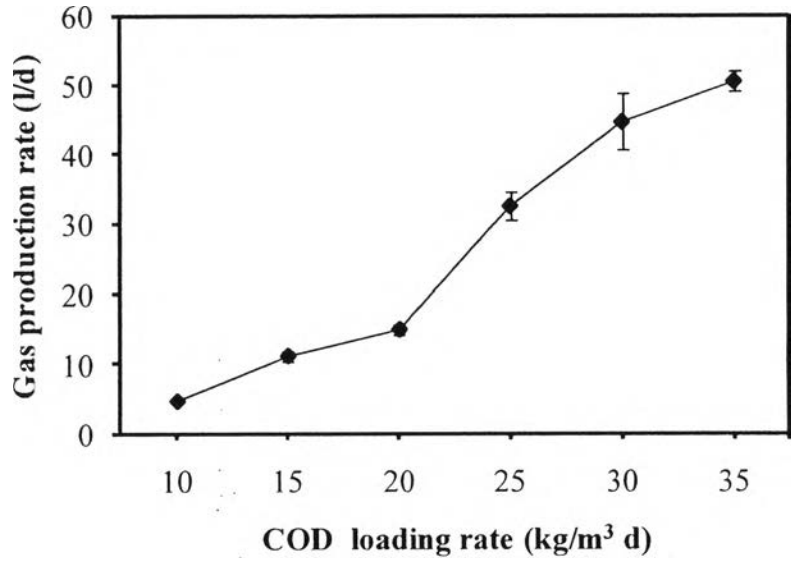

Figure 4.1 Effect of COD loading rate on gas production rate.

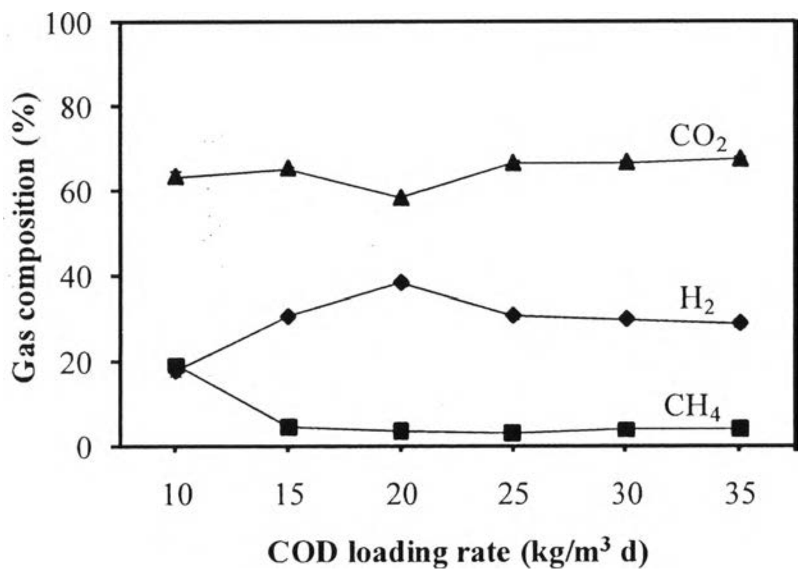

Figure 4.2 Effect of COD loading rate on gas composition.

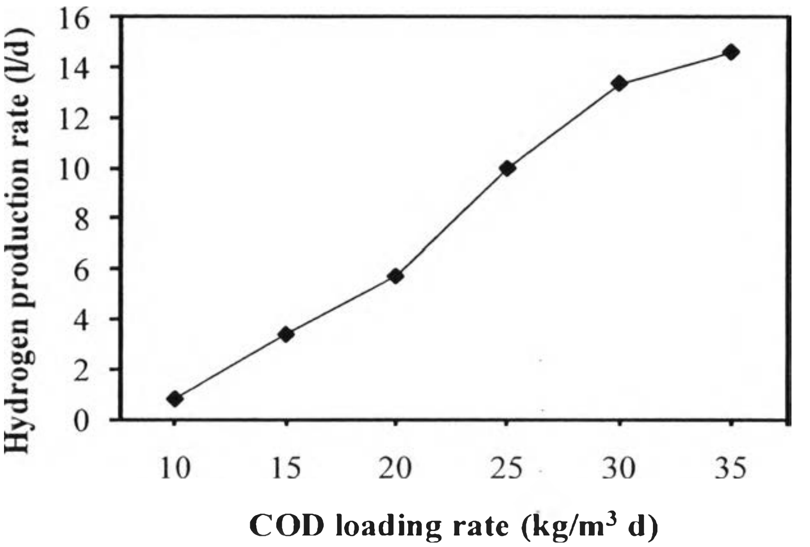

Figure 4.3 Effect of COD loading rate on hydrogen production rate.

Hydrogen yield is a measurement of the capability of the microbes to convert organic substrate (in terms of COD) to hydrogen gas. It 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, as well as to the amount of initial organic substrate in the unit of ml/g COD applied. Figure 4.4 shows the effect of the COD loading rate on the hydrogen yield. The hydrogen yield in terms of ml H_2/g COD removed increased with increasing COD loading rate from 10 to 35 kg/m^3 d, and the maximum hydrogen yield of 114.5 ml H_2/g COD removed was observed at a COD loading rate of 35 kg/m^3 d, at which the maximum hydrogen production rate was achieved (Figure 4.3). This implies a higher efficiency of the microbes to convert the available organic substrate to hydrogen gas at a higher COD loading rate. For the hydrogen yield in terms of ml H_2/g COD applied, it increased with increasing COD loading rate from 10 to 30 kg/m^3 d and reached the maximum value of 18.5 ml/g COD applied at a COD loading rate of 30 kg/m^3 d. Then, it slightly decreased with further increasing COD loading rate to 35 kg/m^3 d.

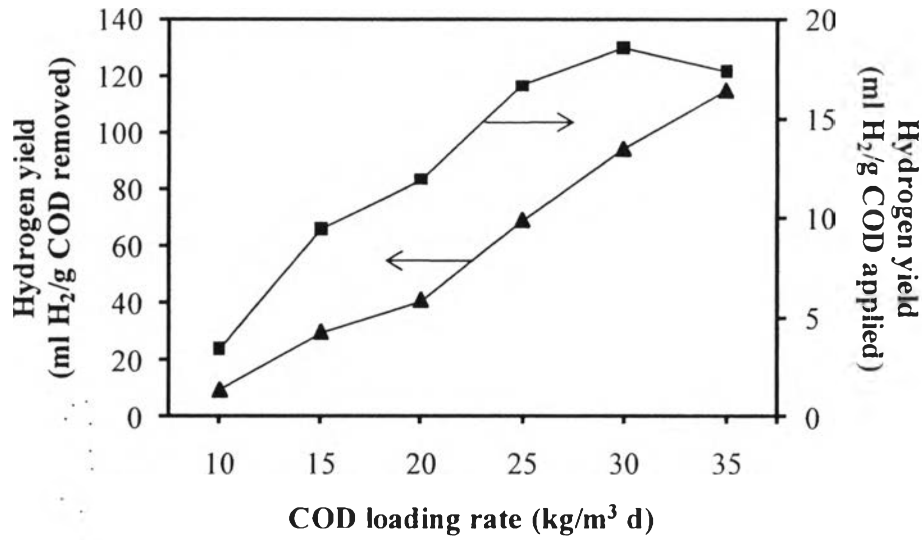

Figure 4.4 Effect of COD loading rate on hydrogen yield.

Specific hydrogen production rate (SHPR) is used to express the hydrogen production ability of the microbes. It is defined in the units of both ml/g MLVSS d and ml/l d. The results of the SHPR as a function of COD loading rate are shown in Figure 4.5. At the increase in COD loading rate, the SHPR increased and reached the maximum value of 46.1 ml H_2/g MLVSS d (or 607.9 ml H_2/l d) at a COD loading rate 35 kg/m^3 d, corresponding well to the maximum hydrogen yield (Figure 4.4). This indicates that at a higher COD loading rate, the microbes consumed the sufficiently high organic substrate for hydrogen gas production more preferably to their growth.

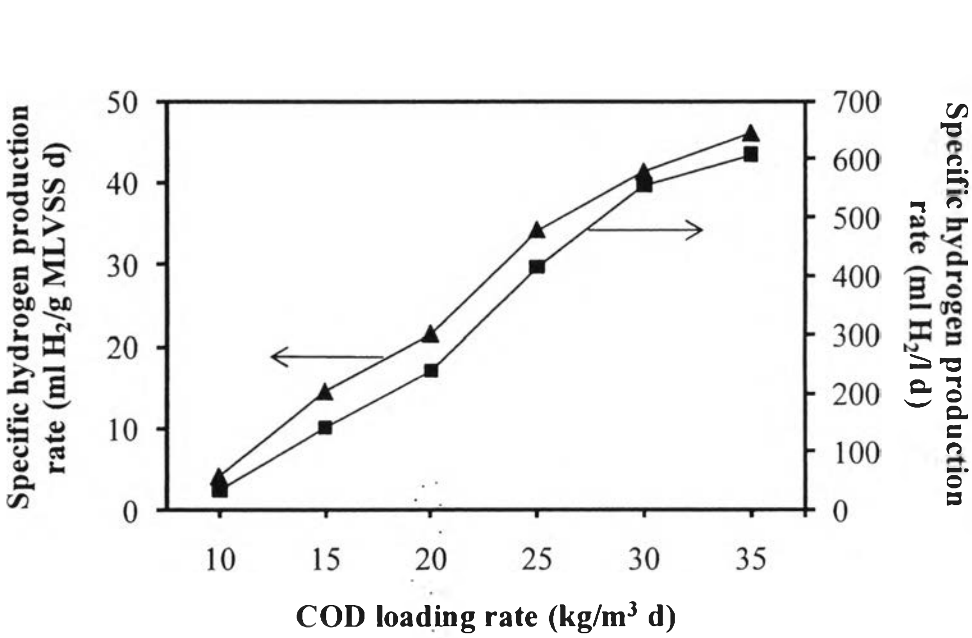

Figure 4.5 Effect of COD loading rate on specific hydrogen production rate.

4.2 Microbial Concentration Results

The microbial concentration in the bioreactor and microbial washout are defined in terms of mixed liquor volatile suspended solids (MLVSS) and total suspend solids (TSS), respectively. The microbial concentration is an important parameter for determining the capability of microbial growth and degradation of organic substrate in the bioreactor. In this research, under any given operation conditions, the microbial concentration measured at the steady state is reported. Figure 4.6 shows the effect of COD loading rate on the MLVSS. At the increase in COD loading rate, the MLVSS was increased and reached its maximum value of 13,440 mg/l at 30 kg/m^3 d. This indicates that a fewer MLVSS was found at a lower COD loading rate. This might be due to the higher VFA accumulation toxicity at a lower COD loading rate (as evidenced by the total VFA concentration in section 4.4), by which the excess VFA accumulation can inhibit the microbial cell activity as well as cell growth (Zhang *et al.*, 2006). However, the MLVSS slightly decreased with further increasing COD loading rate from 30 to 35 kg/m^3 d. This is possibly because a too high feed flow rate at a very high COD loading rate resulted in the microbial washout from the bioreactor (Sema, 2008), corresponding to the observed highest effluent TSS at a COD loading rate of 35 kg/m^3 d, as shown in Figure 4.7.

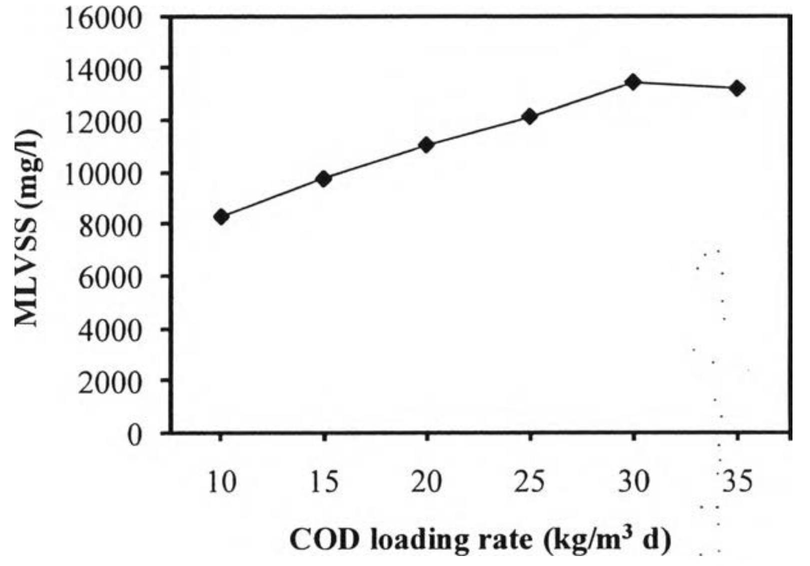

Figure 4.6 Effect of COD loading rate on microbial concentration in terms of MLVSS.

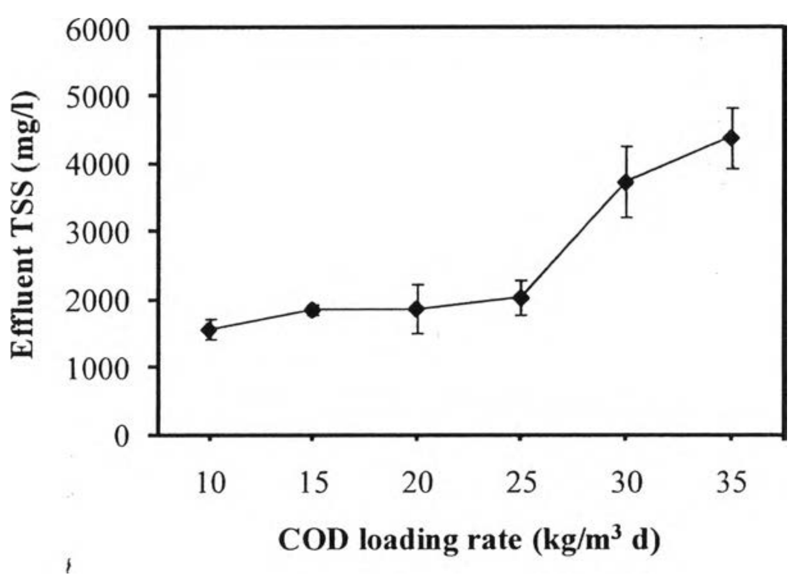

Figure 4.7 Effect of COD loading rate on microbial washout in terms of effluent TSS.

4.3 COD Removal Results

Figure 4.8 shows the effect of COD loading rate on the COD removal. The COD removal decreased from 37.0 to 15.2% with increasing COD loading rate from 10 to 35 kg/m^3 d. This is possibly because at a higher COD loading rate, the microbes required a longer time for degrading a larger quantity of available substrate; whereas at a lower COD loading rate, the holding time of wastewater was sufficiently high for the microbes to degrade the substrate, resulting in a higher COD removal at a lower COD loading rate. The decrease in COD removal with increasing COD loading rate was also observed by Mohan *et al.* (2007). However, it was interestingly observed that the higher COD removal was not accompanied by the high hydrogen production rate at a lower COD loading rate (Figure 4.3). This is possibly because the metabolic pathway of microbes for the formation of organic acids is more dominant than that for the hydrogen production, as evidenced by a comparatively high total VFA concentration shown in section 4.4.

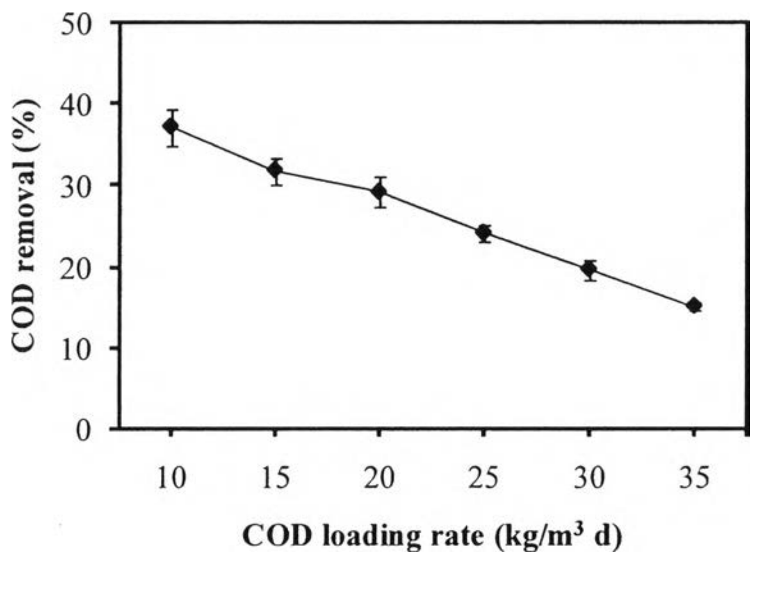

Figure 4.8 Effect of COD loading rate on COD removal.

4.4 Volatile Fatty Acids (VFA) and Alcohol Production Results

The amount of VFA in the effluent wastewater was analyzed after the system reached steady state. 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 4.9 shows the effect of COD loading rate on the total VFA concentration. The total VFA concentration decreased from 13,040 to 12,480 mg/l with increasing COD loading rate from 10 to 25 kg/m^3 d. After that, the total VFA concentration increased with further increasing COD loading rate up to 35 kg/m^3 d. The maximum total VFA concentration was 13,040 mg/l at a COD loading rate of 10 kg/m^3 d, corresponding to the highest COD removal at this COD loading rate. This is possibly due to the dominant metabolic pathway of microbes for more organic acid formation, as mentioned above. However, the increase in the total VFA concentration at a COD loading rate higher than 25 kg/m^3 d is possibly because there was a large amount of organic substrate fed into the bioreactor. Therefore, the acidogenic bacteria (VFA-producing bacteria) greatly consumed this available substrate for the hydrogen production and their growth (as clearly seen by the hydrogen production rate and MLVSS in Figures 4.3 and 4.6, respectively), accordingly resulting in the high VFA production.

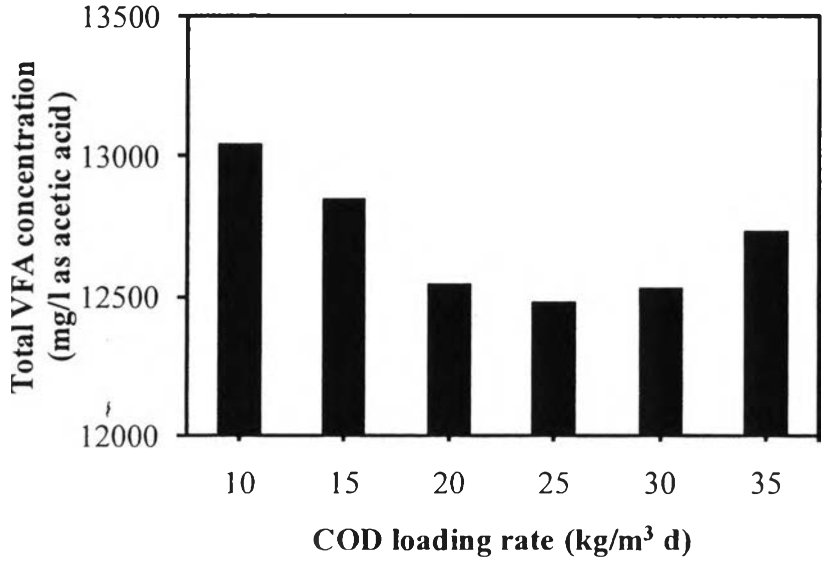

Figure 4.9 Effect of COD loading rate on total VFA concentration.

In general, the VFA composition can directly indicate the performance of hydrogen production, where the acetic and butyric acid fermentations are favorable metabolic pathways of acidogenic bacteria for the hydrogen production, according to Equations 4.1 and 4.2 (Wu *et al.,* 2008). In this research, the VFA composition mainly consisted of acetic acid, propionic acid, butyric acid, and valeric acid. Figure 4.10 shows the effect of COD loading rate on the VFA concentration. The acetic acid concentration significantly increased from 370 mg/l at a COD loading rate of 10 kg/m^3 d to 3,990 mg/l at a COD loading rate of 20 kg/m^3 d, which was the maximum acetic acid concentration in this research. It was clearly observed that the highest hydrogen percentage in the produced gas was also obtained at this COD loading rate (Figure 4.2). With further increasing COD loading rate, the acetic acid concentration decreased to 2,520 mg/l at a COD loading rate of 35 kg/m^3 d. The butyric acid concentration also rapidly increased from 800 mg/l at a COD loading rate of 10 kg/m^3 d to 2,730 mg/l at a COD loading rate of 20 kg/m^3 d, which is in the same manner as the acetic acid concentration. After that, it was slightly varied in the range from 2,250 to 2,590 mg/l when the COD loading rate increased from 25 to 35 kg/m^3 d. In general, the highest acetic and butyric acid concentrations can lead to the highest hydrogen yield and SHPR; however, the hydrogen yield and SHPR results in this research (Figures 4.4 and 4.5) did not correspond to the general finding. This can be possibly due to the influence of other factors, particularly ethanol production, which will be discussed later.

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

Butyric acid production: $C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2CO_2 + 2H_2$ (4.2)

The propionic acid concentration sharply decreased from 4,080 mg/l at a COD loading rate of 10 kg/m^3 d to 1,520 mg/l at a COD loading rate of 20 kg/m^3 d. Then, it slightly increased when the COD loading rate increased from 25 to 35 kg /m^3 d. At a COD loading rate 10 kg/m^3 d, the lowest hydrogen percentage in the produced gas and the lowest hydrogen production rate were obtained (Figures 4.2 and 4.3), and this can be explained in that the highest propionic acid concentration was formed via the metabolic pathway for the consumption of produced hydrogen, according to Equation 4.3 (Zhang *et al.*, 2006). 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: $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (4.3)

The valeric acid concentration also rapidly decreased from 4,440 mg/l at a COD loading rate of 10 kg/m^3 d to 1,500 mg/l at a COD loading rate of 20 kg/m^3 d, and afterwards it gradually increased to 1,960 mg/l at a COD loading rate of 35 kg/m^3 d. This might be due to the overloading of the organic substrate, which could cause the increase in valeric acid accumulation at a higher COD loading rate (Han *et al.*, 2005).

Alcohol concentration in the effluent wastewater was also analyzed. It was found that ethanol was the only detectable alcohol. Figure 4.10 also shows the effect of COD loading rate on ethanol concentration. The results show that ethanol concentration increased with increasing COD loading rate from 10 to 35 kg/m^3 d. At a COD loading rate 35 kg/m^3 d, the highest ethanol concentration of 3,140 mg/l was accompanied by the highest hydrogen production rate, the highest hydrogen yield, and the highest SHPR (Figures 4.3, 4.4, and 4.5). Guo *et al.* (2008) also reported that a high level of hydrogen yield was associated with a high ethanol production, according to Equation 4.4. The observed results can then be explained in that the ethanol production could reduce the acidity of wastewater in the bioreactor and accordingly improve the efficiency of hydrogen production.

Ethanol production: $C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2H_2 + 2CO_2$ (4.4)

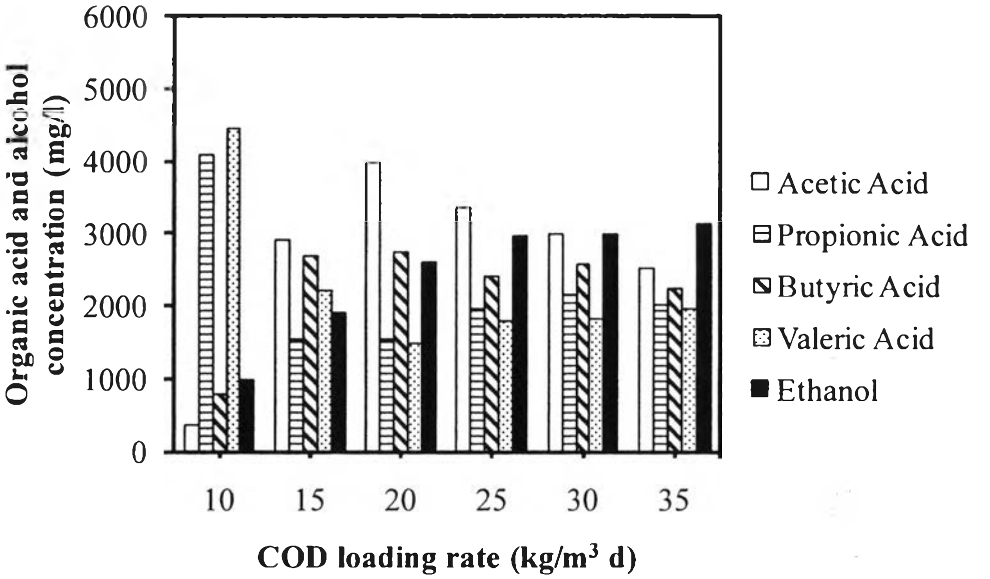

Figure 4.10 Effect of COD loading rate on organic acid and alcohol concentration.

To determine the optimum COD loading rate, the hydrogen production rate, hydrogen yield, specific hydrogen production rate, COD removal efficiency, and microbial concentration were simultaneously considered. From the overall experimental results, the optimum COD loading rate was suggested at 30 kg/m^3 d. Under this condition, the acceptably high hydrogen production rate of 13.3 l/d, hydrogen yield of 93.9 ml H_2/g COD removed, specific hydrogen production rate of 41.4 ml H_2/g MLVSS d, and COD removal efficiency of 19.7% were achieved. Moreover, the highest microbial concentration of 13,440 mg/l was observed. Even though the hydrogen production rate, hydrogen yield, and specific hydrogen production rate at the COD loading rate of 35 kg/m^3 d were slightly higher than those at the COD loading rate of 30 kg/m^3 d, the COD loading rate of 30 kg/m^3 was chosen as the optimum condition because it provided a higher COD removal efficiency. In addition, the highest microbial concentration obtained at this COD loading rate, indicates that such COD loading rate was suitable for microbial growth in the studied bioreactor.