CHAPTER IV
RESULTS AND DISCUSSION

In this research, the effect of COD loading rate was investigated on the biohydrogen production from the alcohol distillery wastewater. Firstly, the alcohol distillery wastewater was fed into the ASBR systems at the initial feed COD of 40,000 mg/l and COD loading rate of 30 kg/m^{3}d and was allowed to accommodate for 2 months at thermophilic temperature of 55 °C and pH controlled at 5.5. The acclimatization was considered to be completed when the reactors reached steady state at this COD loading rate. After the complete acclimatization, the experiments were studied at different COD loading rates in the range of 30 to 75 kg/m^{3}d with 15 kg/m^{3}d increment at the initial feed COD value of 40,000 mg/l.

In an ASBR system; where acidogenic bacteria were dominant, COD was removed and converted to liquid intermediate products, such as acetic acid, butyric acid, propionic acid, and ethanol. In general, COD removal during fermentative hydrogen production from molasses is about 20 %, which is closely related to the H_2 and VFA productions (Ren *et al.*, 2006). Figure 4.1 shows the effect of COD loading rate on the COD removal at the initial feed COD of 40,000 mg/l. It was found that the maximum COD removal was 26 % at the COD loading rate of 75 kg/m^{3}d. The results show that the anaerobic sludge more effectively converted organic materials in the wastewater at a higher COD loading rate, resulting in high gas production rate, high hydrogen production rate, as shown next. However, a lower COD removal in this present work as compared to that in the case of a sucrose-containing wastewater (Lin and Chen 2006) was observed, possibly because this alcohol distillery wastewater contained higher-molecular-weight organic components than simple sugar (Ginkel *et al.*, 2005).

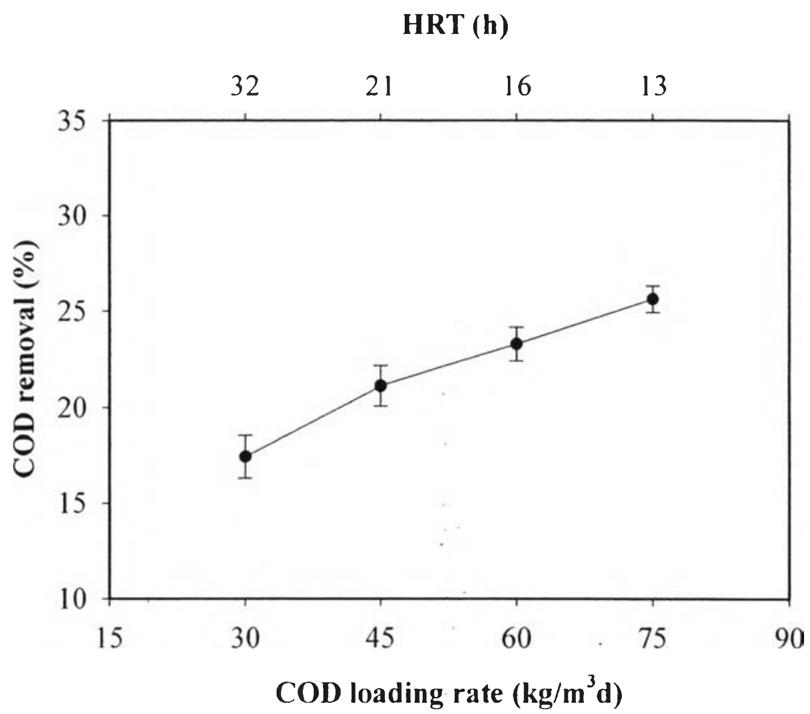

Figure 4.1 COD removal during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

The gas production rate at the initial feed COD value of 40,000 mg/l also increased with the increase in the COD loading rate. Figure 4.2 shows that the initial gas production rates at the first two COD loading rates of 30 and 45 kg/m^{3}d were not much different (0.42 and 0.48 l/h, respectively). After that, the gas production rate reached the maximum of 0.96 l/h at the COD loading rate of 60 kg/m^{3}d. Then, it decreased to 0.95 l/h with further increase in the COD loading rate to 75 kg/m^{3}d. This is possibly because a higher COD loading rate results in a larger amount of organic substrate available in the system. Hence, the microbes could consume this available organic substrate for producing more gas products (Yusoff *et al.*, 2010).

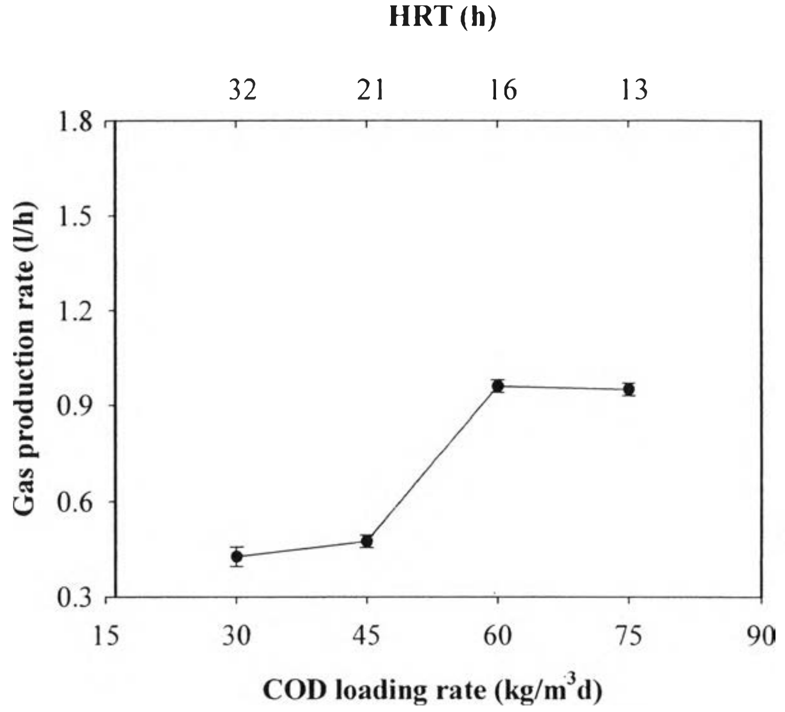

Figure 4.2 Gas production rate during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

The compositions of the produced gas at different COD loading rates are shown in Figure 4.3. The hydrogen percentage increased with the increase in the COD loading rate to reach the maximum of 19.72 % at the COD loading rate of 45 kg/m^{3}d, and then decreased to 7.96 % at the higher COD loading rate of 60 kg/m^{3}d. This might be due to the toxicity of higher VFA accumulation in the bioreactor (Argun *et al.*, 2008), which will be discussed in the part of total VFA concentration. However, the carbon dioxide percentage showed the opposite trend to the hydrogen percentage, with the minimum value of 73.82 % at the COD loading rate of 45 kg/m^{3}d, and after that it increased to 87.72% at the COD loading rate of 60 kg/m^{3}d, which was not different to the next investigated COD loading rate of 75 kg/m^{3}d. The methane percentage in the produced gas seemed to be almost unchanged, about 4-6 % is might be possibly from the effect of the methanogenic bacteria gradually adjusting themselves over a long time period at high substrate concentration. It can be clearly observed that the system operated at the initial feed COD value of 40,000 mg/l—with the COD loading rate of 45 kg/m^{3}d and a HRT of 21 h—provided the maximum hydrogen content in the produced gas.

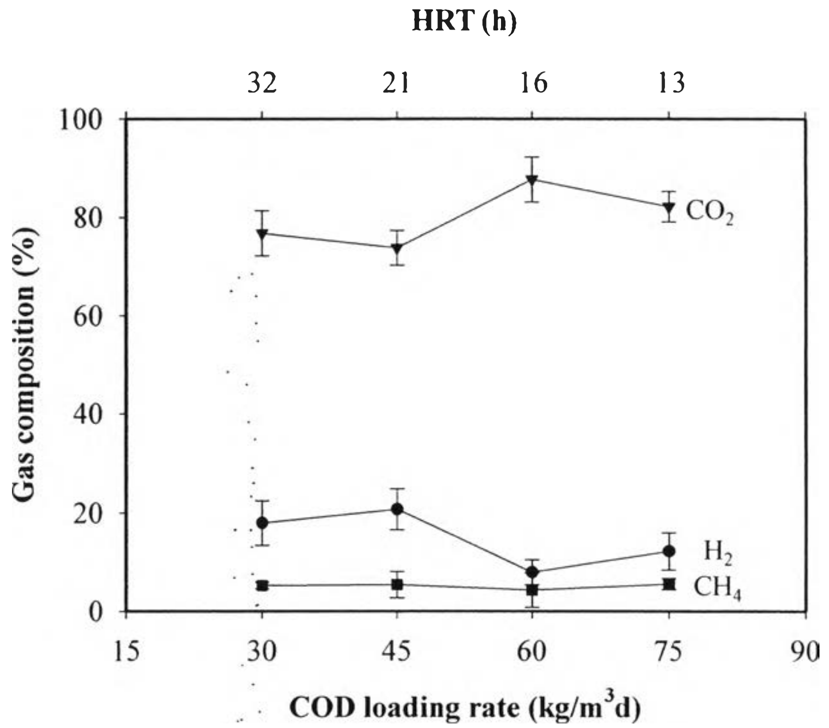

Figure 4.3 Gas composition during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

Figure 4.4 shows the bacterial concentration in the bioreactor in terms of MLVSS. The MLVSS increased rapidly when the COD loading rate was increased from 30 to 60 kg/m^{3}d. Again, this is possibly because the methanogenic bacteria could adjust themselves over a long time period, as mentioned before. After that, the MLVSS tended to decrease with further increase in the COD loading rate to 75 kg/m^{3}d. The further decrease may be because the methanogenic bacteria could not survive at a too high COD loading rate under a short hydraulic retention time (HRT) and a high VFA concentration (low-pH operation), resulting in the increase in the microbial washout from the bioreactor (Hawkes *et al.*, 2002). This is indicated by the increase in the bacterial concentration in the effluent in terms of TSS, as shown next in Figure 4.5.

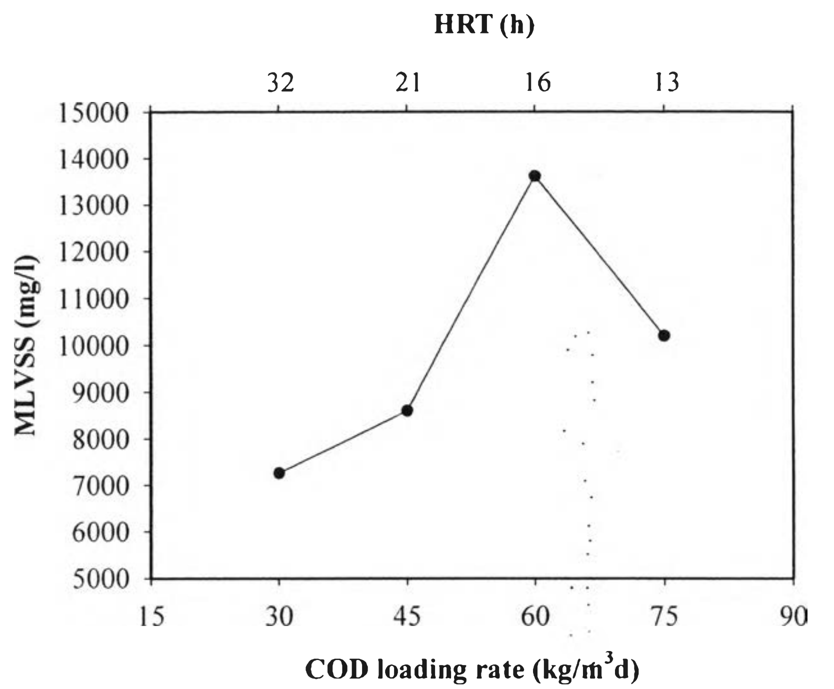

Figure 4.4 MLVSS during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

Figure 4.5 shows the microbial washout from the bioreactor in terms of effluent TSS. At the COD loading rate of 45 kg/m^{3}d, the effluent TSS decreased to the lowest value of 917 mg/l. It indicates that this condition was the most appropriate for the growth of hydrogen-producing bacteria in this work. resulting in the highest hydrogen percentage in the produced gas, as previously described.

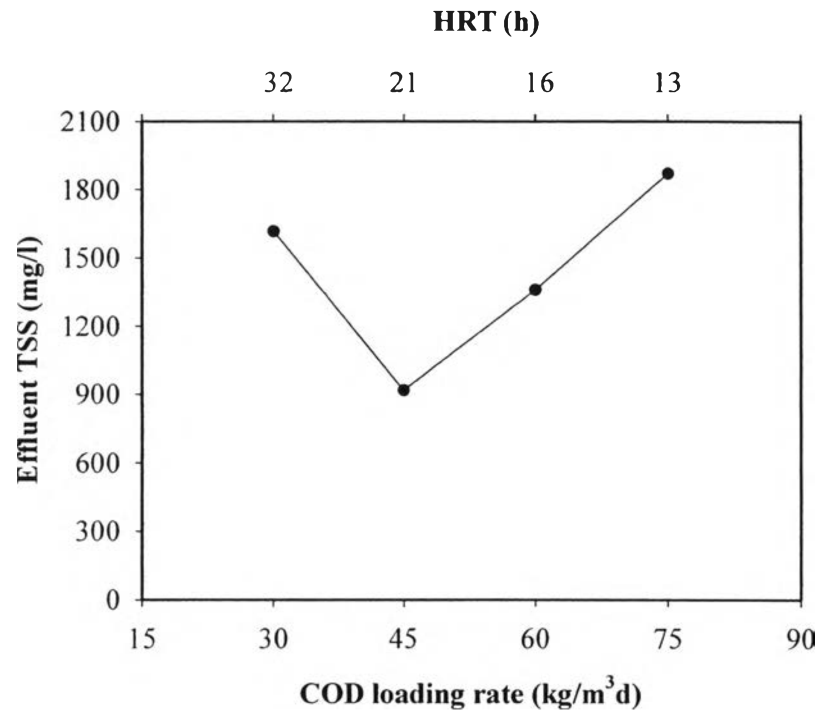

Figure 4.5 Effluent TSS during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

An anaerobic system not only deals with the degradation of the organic materials in the wastewater to hydrogen, carbon dioxide, and methane, but also contributes to the production of volatile fatty acids (VFA). The total VFA concentrations at various COD loading rates are shown in Figure 4.6. The results show that the total VFA concentration decreased from 4,360 mg/l to the lowest value of 3,680 mg/l when increasing COD loading rate from 30 to 45 kg/m^{3}d, which resulted in the maximum hydrogen percentage in the produced gas (Figure 4.3). The total VFA concentration then increased with further increasing COD loading rate to reach the maximum value of 4,530 mg/l at the COD loading rate of 75 kg/m^{3}d. This reveals that a further increase in the COD loading rate negatively affected the hydrogen content in the produced gas, possibly due to the production 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 rather than the hydrogen production.

Accordingly, 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 efficiency (Fan *et al.*, 2006).

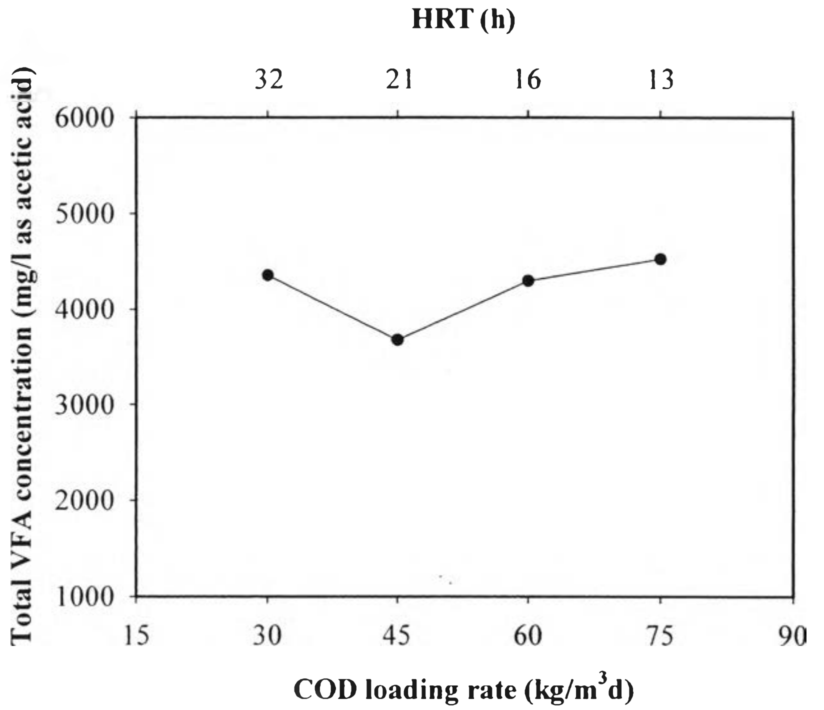

Figure 4.6 Total VFA concentration during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

The concentrations of VFA and ethanol in the effluent at different COD loading rates were also analyzed. It can be noticed that the main VFA components were acetic acid, propionic acid, butyric acid, and valeric acid with a low concentration of ethanol. Although the butyric and acetic acid fermentations in an anaerobic system have been found to be favorable metabolic pathways of acidogenic bacteria for the hydrogen production, the acetic acid production may be also found to inhibit the hydrogen production, as shown in Equation (4.1) (Luo *et al.*, 2010). The results further show that at the COD loading rate of 45 kg/m^{3}d, the amount of acetic acid was the lowest, which resulted in the highest hydrogen percentage.

Acetic acid production: $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ (4.1)

The propionic acid and ethanol fermentations have been found to be the metabolic pathway for the consumption of produced hydrogen (Hawkes *et al.,* 2002). According to the Equations (4.2) and (4.3), the low propionic acid and ethanol concentrations were obtained with the high hydrogen composition, as shown in the Figure 4.7, which presents the VFA and ethanol concentrations at different COD loading rates. The results show that at the COD loading rate of 45 kg/m^3d, the lowest VFA and ethanol concentrations were obtained. In the meantime, this COD loading rate also provided the highest hydrogen percentage in the produced gas (Figure 4.3), as well as the lowest bacterial washout (Figure 4.5). Therefore, this COD loading rate was the most suitable for operating the ASBR system.

Propionic acid production: $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$ (4.2)

Ethanol production: $CH_3COOH + H_2 \rightarrow CH_3CH_2OH + H_2O$ (4.3)

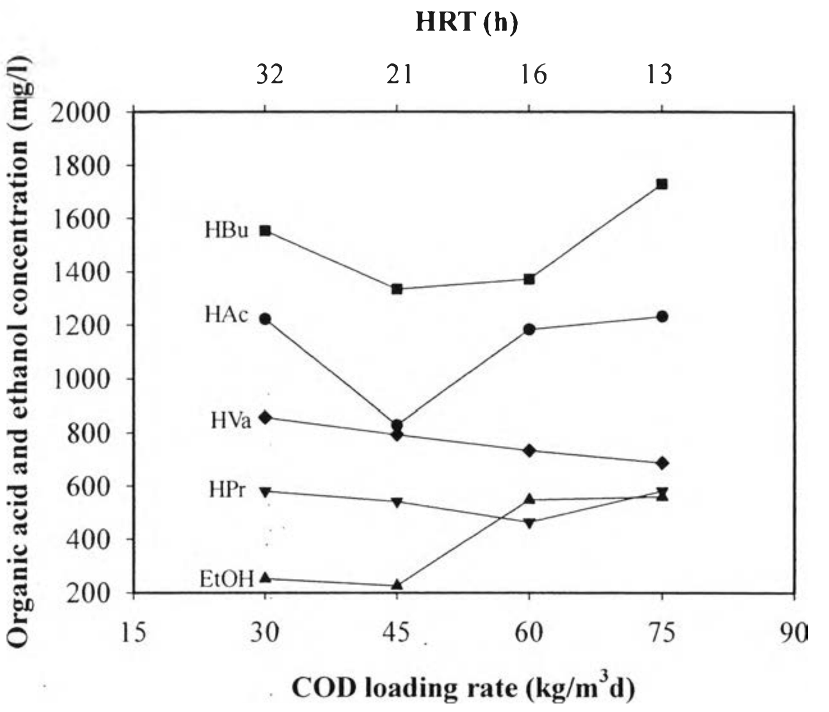

Figure 4.7 VFA and ethanol concentrations during the hydrogen production in the ASBR system the thermophilic condition (55 °C) and pH 5.5.

The hydrogen production rate depends on both the gas production rate and the gas composition, and the results of hydrogen production rate are shown in Figure 4.8. It was found that the hydrogen production rate was in the range of 0.08-0.12 l/h with the COD loading rate between 30 and 75 kg/m^{3}d.

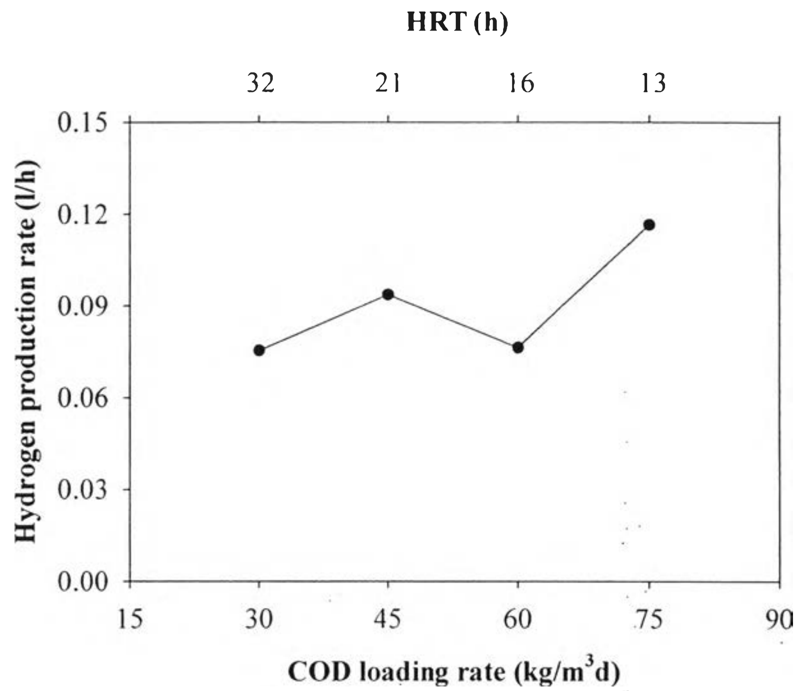

Figure 4.8 Hydrogen production rate during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

The specific hydrogen production rate (SHPR) can also be used to identify the ability of bacteria to produce hydrogen in the bioreactor, and the results are shown in Figure 4.9. Because the degradation of the organic materials in the wastewater to produce gaseous products and VFA affected the hydrogen production in the ASBR system, the optimum condition for the SHPR could be realized. Although the COD loading rate of 75 kg/m^{3}d provide the maximum SHPR of 68.6 ml H_2/g MLVSS d (or 699 ml H_2/l d), the VFA and alcohol concentrations were on the high side with low hydrogen percentage in the produced gas. For the COD loading rate of 45 kg/m^{3}d, the maximum hydrogen percentage in the produced gas and the minimum VFA and alcohol concentrations was obtained. Moreover, the SHPR at this COD loading rate was also acceptably of high, 65.3 ml H_2/g MLVSS d (or 562 ml H_2/l d), being only lower than the maximum value. Therefore, the optimum condition of the SHPR for the hydrogen production in the ASBR system was at the COD loading rate of 45 kg/m^{3}d.

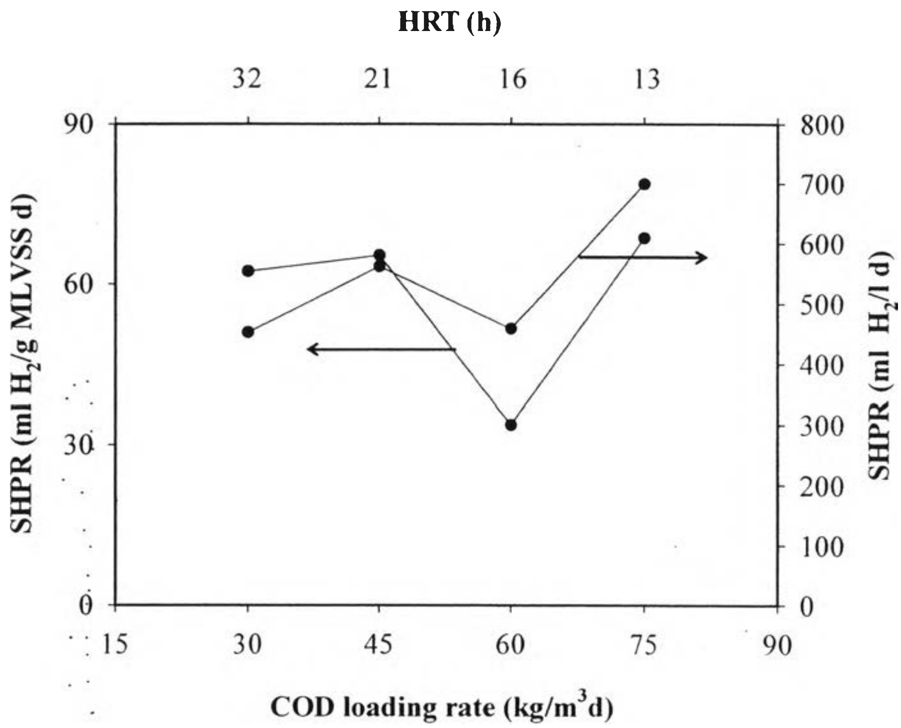

Figure 4.9 Specific hydrogen production rate (SHPR) during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

The hydrogen yield was calculated from the hydrogen production rate and COD removal results, as shown in Figure 4.10. Similar to the SHPR, the degradation of the organic materials in the wastewater to produce various gaseous and liquid products also affected the hydrogen yield. Although the highest hydrogen yield was obtained at the COD loading rate of 30 kg/m^{3}d (82.1 ml H_2/g COD removed), the low hydrogen percentage in the produced gas, as well as high VFA and alcohol concentrations, were observed at this COD loading rate. At the COD loading rate of 45 kg/m^{3}d, the hydrogen yield was the second highest of approximately 56.1 ml H_2/g COD removed, with the highest hydrogen percentage in the produced gas and the lowest VFA and alcohol concentrations. Accordingly, the optimum condition of the hydrogen yield from the hydrogen production in ASBR system was also considered to be at the COD loading rate value of 45 kg/m^{3}d.

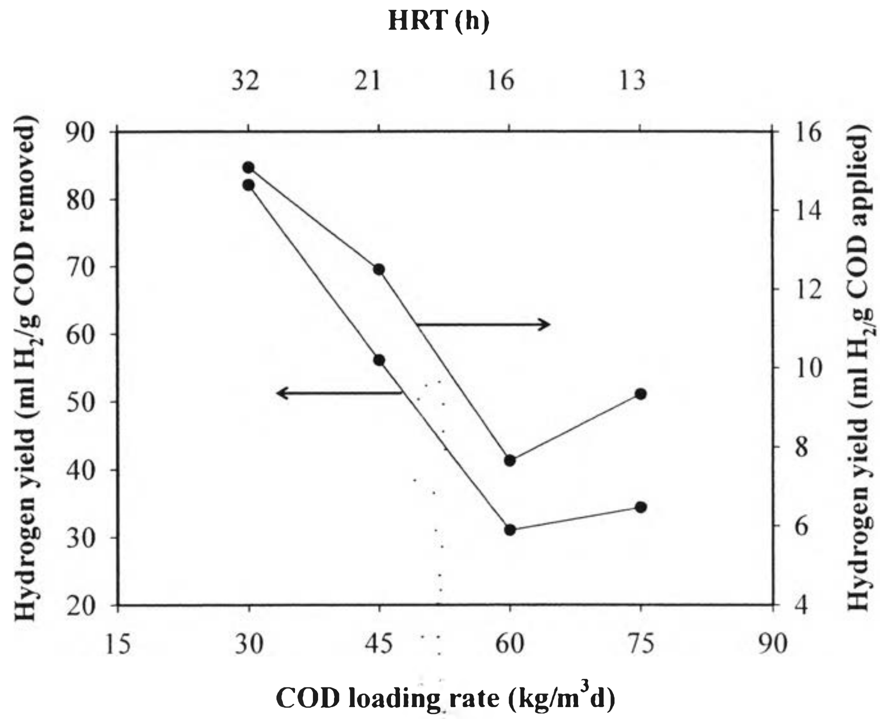

Figure 4.10 Hydrogen yield during the hydrogen production in the ASBR system at the thermophilic condition (55 °C) and pH 5.5.

Other two factors that may inhibit the hydrogen production is the toxicity of potassium and sulphate. For the toxicity of potassium, the alcohol distillery wastewater used in this work contained a high concentration of potassium (about 9,000 mg/l in the as-received wastewater with a COD value of 120,000 mg/l) of 3,000 mg/l in the initial feed COD value of 40,000 mg/l. Parkin and Owen (1986) work reported that the potassium concentration in the range of 2,500–4,500 mg/l could moderately inhibit hydrogen production (Table 4.1), which can be toxic to acedogenic bacteria in an anaerobic digestion. Similarly, the toxicity of sulphate, about 5,200 mg/l in the as-received wastewater with a COD value of 120,000 mg/l and 1,700 mg/l in alcohol distillery wastewater at initial feed COD value of 40,000 mg/l, also inhibited the hydrogen production. This might be a reason why the hydrogen production rate at the initial feed COD value to 40,000 mg/l resulted in the low hydrogen production. However, this work only focused on the feasibility study of hydrogen production from the alcohol distillery wastewater without any pretreatment step. Therefore, an interesting perspective for the future work is to reduce the toxicity of potassium and sulphate to enhance the hydrogen production efficiency.

Table 4.1 Potassium toxicity level for hydrogen production (Parkin and Owen, 1986)

Toxicity level	Potassium concentration (mg/l)
Stimulatory	200–400
Moderately inhibitory	2,500–4,500
Strongly inhibitory	12,000

Searmsirimongkol (2010) studied the hydrogen production from alcohol distillery wastewater using an ASBR under mesophilic temperature or 37 °C with the COD loading rates of 30, 45, 60, and 75 kg/m^{3}d at the initial feed COD value of 40,000 mg/l. Comparison to the present work shows that at the COD loading rate of 30 kg/m^{3}d, the hydrogen production was more efficient when the temperature was increased from 37 to 55°C. However, at the COD loading rate between 45 and 75 kg/m^{3}d, the efficiency of the hydrogen production was not much different. Unlike Searmsirimongkol's work, some methane was obtained along with hydrogen. This might be because the methanogen cells may gradually adjust themselves over a long time period, and the organic acid composition, used as substrate for the methane production, in this research was less than the previous work. Moreover, the different operating temperatures may affect the methane production. Even though there was no work to confirm this hypothesis, Dugba and Zhang (1998), who studied the effect of temperature for methane production in two-stage anaerobic sequencing batch reactor systems, reported that the thermophilc-mesophilic system produced more methane than the mesophilic-mesophilic system, which can be deduced that methane production was favorable in the thermophilic condition.