CHAPTER IV
RESULTS AND DISCUSSION

In this work, the effects of initial feed COD and COD loading rate were investigated on the biohydrogen production from the alcohol distillery wastewater. Firstly, the anaerobic seed sludge and the alcohol distillery wastewater were together fed into the ASBR systems. The anaerobic seed sludge was allowed to acclimate with the alcohol distillery wastewater for 2-3 weeks at a mesophilic temperature of 37°C. During the acclimatization, the initial feed COD and COD loading rate were 20,000 mg/l and 15 kg/m^{3}d, respectively. The acclimatization was considered to be completed when the reactors reached steady state at this COD loading rate. After the complete acclimatization, three series of the experiments were studied at different COD loading rates in the ranges of 15 to 37.5 kg/m^{3}d with 7.5 kg/m^{3}d increment, 30 to 90 kg/m^{3}d with 15 kg/m^{3}d increment, and 45 to 112.5 kg/m^{3}d with 22.5 kg/m^{3}d increment at different initial feed COD values of 20,000, 40,000, and 60,000 mg/l, respectively.

In an ASBR system, where acidogenic bacteria were dominant, COD was removed and converted to liquid intermediate products, such as acetate, butyrate, propionate, 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 4.1a shows the effect of COD loading rate on the COD removal efficiency at different initial feed COD values. It was found that the maximum COD removal efficiencies at the initial feed COD values of 20,000, 40,000, and 60,000 mg/l were 27, 30, and 32% at the COD loading rates of 37.5, 60, and 112.5 kg/m^{3}d, respectively. The results indicate that the anaerobic sludge more effectively converted organic materials in the wastewater at higher initial feed COD value and higher COD loading rate, resulting in high hydrogen percentage, high gas production rate, high hydrogen production rate, high specific hydrogen production rate, and high hydrogen yield, as shown next. However, a lower COD removal efficiency 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).

The gas production rates at initial feed COD values of 20,000, 40,000, and 60,000 mg/l are also shown in Figure 4.1. For the initial feed COD value of 20,000 mg/l (Figure 4.1b), the gas production rate dramatically increased with increasing COD loading rate from 0.11 l/h at 15 kg/m^{3}d to 1.09 l/h at 37.5 kg/m^{3}d. For the initial feed COD value of 40,000 mg/l, (Figure 4.1c) the gas production rate increased with increasing COD loading rate and reached the maximum of 1.59 l/h at a COD loading rate of 60 kg/m^{3}d. Then, it decreased to 1.35 l/h with further increasing COD loading rate to 75 kg/m^{3}d. Further increase in COD loading rate at this initial feed COD value resulted in a lower gas production rate, as well as a lower hydrogen percentage in the produced gas, probably due to a higher VFA accumulation (Argun *et al.*, 2008), which will be discussed in the part of total VFA concentration. For the initial feed COD value of 60,000 mg/l (Figure 4.1d), the gas production rate remained nearly constant at about 0.75 l/h at the COD loading rates in the range of 45 to 90 kg/m^{3}d, but it increased to 1.49 l/h at a COD loading rate of 112.5 kg/m^{3}d. The comparative results at different COD loading rates for the initial feed COD values of 20,000, 40,000, and 60,000 mg/l showed that the gas production rate for the initial feed COD value of 40,000 mg/l—with a COD loading rate of 60 kg/m^{3}d and a hydraulic retention time (HRT) of 16 h—was much higher than those for the initial feed COD values of 20,000 and 60,000 mg/l. This is possibly due to a lower bacterial growth at these two initial feed COD values, as well as a negative effect of high VFA production at too high initial feed COD value with high COD loading rates, as shown later in the part of total VFA concentration.

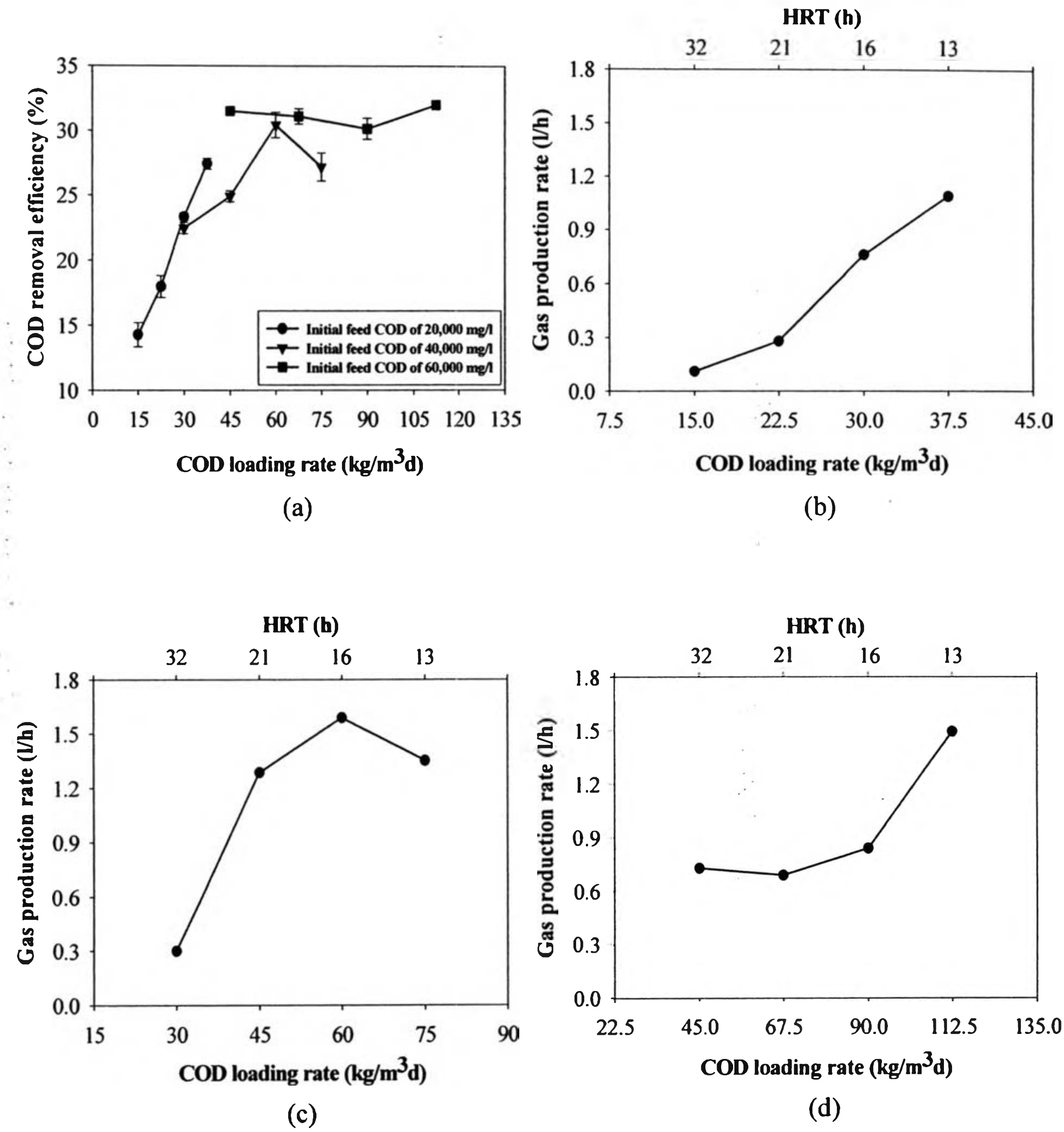


Figure 4.1 COD removal efficiency (a) and gas production rate at initial feed COD values of 20,000 mg/l (b), 40,000 mg/l (c), and 60,000 mg/l (d).

The compositions of the produced gas at initial feed COD values of 20,000, 40,000, and 60,000 mg/l are shown in Figure 4.2. For the initial feed COD value of 20,000 mg/l (Figure 4.2a), the hydrogen percentage increased with increasing COD loading rate and reached the maximum of 29.6% at a COD loading rate of 37.5 kg/m^{3}d. However, the carbon dioxide and methane percentages showed the opposite trend to the hydrogen percentage, with the minimum values of 63.9% and 6.5%, respectively, at a COD loading rate of 37.5 kg/m^{3}d. For the initial feed COD value of 40,000 mg/l (Figure 4.2b), the hydrogen percentage increased from 7.0 to 34.7% with increasing COD loading rate from 30 to 60 kg/m^{3}d and then decreased to 28.0% at a higher COD loading rate of 75 kg/m^{3}d. This might be due to the higher VFA accumulation, as explained above for the gas production rate. The carbon dioxide percentage also showed the opposite trend to the hydrogen percentage. It decreased from 81.3 to 65.3% when increasing COD loading rate to 60 kg/m^{3}d, and after that it increased to 72.0% at a COD loading rate of 75 kg/m^{3}d. However, the methane percentage in the produced gas decreased dramatically with increasing COD loading rate, and no methane was detected at a COD loading rate higher than 60 kg/m^{3}d. This is possibly because the system was in a high acidic environment (high VFA concentration), which was unfavorable condition for the methane production (Hawkes *et al.,* 2002). For the initial feed COD value of 60,000 mg/l (Figure 4.2c), the hydrogen percentage became almost unchanged at 24.0% when increasing COD loading rate from 45 to 90 kg/m^{3}d, and then it increased to 33.7% at a COD loading rate of 112.5 kg/m^{3}d. There was no methane detected at this initial feed COD value. It can be clearly observed that the system operated at the initial feed COD value of 40,000 mg/l—with a COD loading rate of 60 kg/m^{3}d and a HRT of 16 h—provided the maximum hydrogen content in the produced gas.

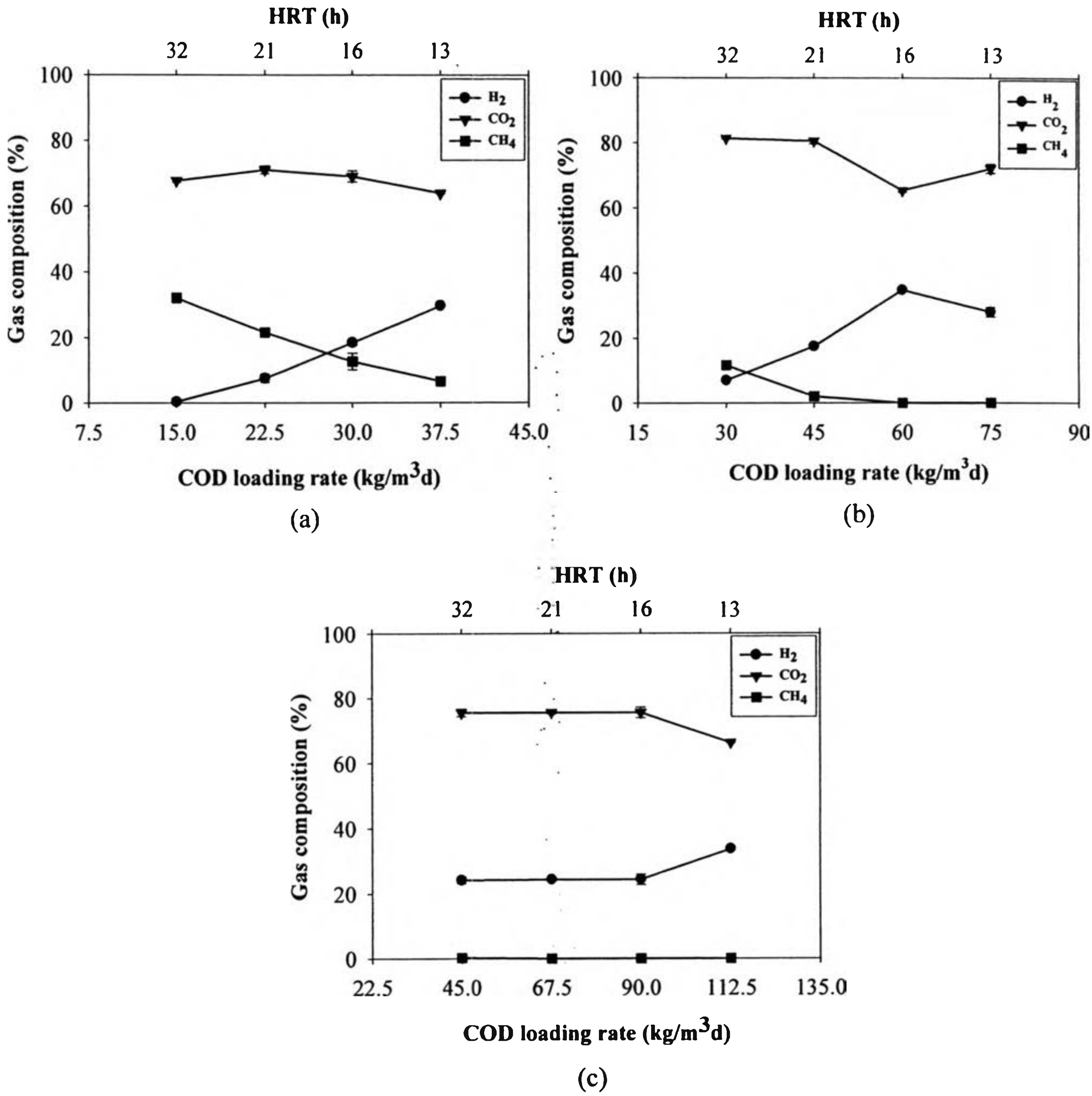
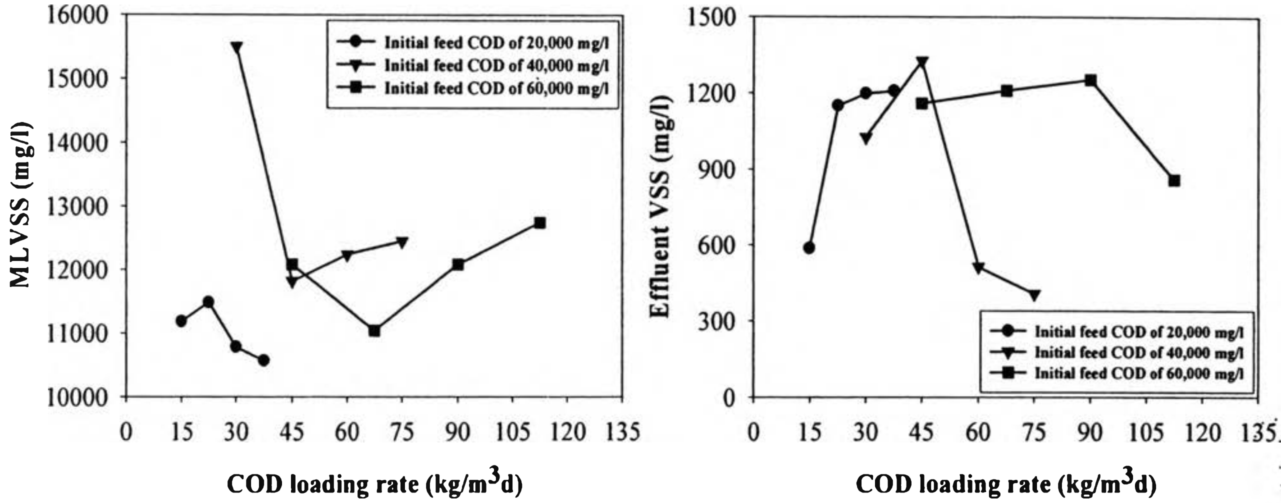


Figure 4.2 Gas composition at initial feed COD values of 20,000 mg/l (a), 40,000 mg/l (b), and 60,000 mg/l (c).

Figure 4.3a shows the bacterial concentration in bioreactor in terms of MLVSS. At the initial feed COD value of 20,000 mg/l, the MLVSS tended to decrease with increasing COD loading rate. This can be explained that methanogenic bacteria could not survive at higher COD loading rate, under a short hydraulic retention time (HRT) and a higher VFA concentration (low-pH operation), resulting in the increase of microbial washout from the bioreactor (Hawkes *et al.,* 2002). This is indicated by the increase in bacterial concentration in the effluent in terms of VSS, as shown in Figure 4.3b. This agrees well with the decrease in the produced methane with increasing COD loading rate (Figure 4.2a) The amount of the MLVSS in this system was in the range of 10,570 to 11,480 mg/l. At the initial feed COD value of 40,000 mg/l, the MLVSS decreased rapidly when increasing COD loading rate from 30 to 45 kg/m^{3}d, since some methanogens could not survive in the ASBR system. Afterwards, the bacterial cells may gradually adjust themselves at high substrate concentration, causing the gradual increase of the MLVSS with increasing COD loading rate. For the initial feed COD value of 60,000 mg/l, the MLVSS rapidly dropped from 12,080 to 11,040 mg/l with increasing COD loading rate from 45 to 67.5 kg/m^{3}d. After that, it increased with further increasing COD loading rate, and reached the maximum amount of 12,740 mg/l at the COD loading rate of 112.5 kg/m^{3}d. From the comparative MLVSS results, the comparatively high MLVSS obtained at the initial feed COD value of 40,000 mg/l and a COD loading rate of 60 kg/m^{3}d implied that these conditions were the most appropriate for the growth of hydrogen-producing bacteria in this work, resulting in the highest gas production rate, as previously described. The comparatively low microbial washout from the bioreactor (effluent VSS) was also observed under these conditions (Figure 4.3b).



(a) (b)

Figure 4.3 MLVSS (a) and effluent VSS (b).

An anaerobic system not only involves the degradation of the organic materials in the wastewater to hydrogen, carbon dioxide, and methane, but also accompanies the production of volatile fatty acids (VFA). The total VFA concentrations at various initial feed COD values and different COD loading rates are shown in Figure 4.4a. It was clearly observed that the total VFA concentration increased with increasing COD loading rate for all initial feed COD values (20,000, 40,000, and 60,000 mg/l). The highest total VFA concentrations were 16,350, 23,190, and 22,320 mg/l, which were obtained at COD loading rates of 37.5, 75, and 112.5 kg/m^{3}d at the initial feed COD values of 20,000, 40,000, and 60,000 mg/l, respectively. At the initial feed COD value of 40,000 mg/l and the COD loading rate of 60 kg/m^{3}d, the maximum gas production rate (Figure 4.1c) and maximum hydrogen content (Figure 4.2b) in the produced gas were obtained with a total VFA concentration of 18,390 mg/l; however, a further increase in the COD loading rate at this initial feed COD value negatively affected the gas production rate and the hydrogen content possibly due to the production of too high total VFA concentration of 23,190 mg/l, which was the highest total VFA among the investigated three initial feed COD values. By comparing the three initial feed COD values at any given COD loading rate, the total VFA concentration was in the following order: 20,000 < 40,000 < 60,000 mg/l. As mentioned above, the gas production rate (Figure 4.1c) and the hydrogen percentage (Figure 4.2b), as well as the hydrogen production rate (Figure 4.5b), reached maximum values at the initial feed COD value of 40,000 mg/l (with a COD loading rate of 60 kg/m^{3}d and a HRT of 16 h). This implies that higher total VFA concentrations were produced at higher initial feed COD values above 40,000 mg/l, since the bacteria would shift its metabolism towards the VFA accumulation rather than the hydrogen production. Therefore, it could be concluded that too much total VFA produced at high initial feed COD values with corresponding high COD loading rate may have inhibited the growth of hydrogen-producing bacteria and also inhibited the hydrogen production (Fan *et al.,* 2006).

The concentrations of VFA and ethanol in the effluent were also analyzed. Figures 4.4b–4.4d depict the VFA and ethanol concentrations at different COD loading rates for the initial feed COD values of 20,000, 40,000 and 60,000 mg/l, respectively. It can be observed that the main VFA components were acetic acid, propionic acid, butyric acid, and valeric acid with zero concentration of ethanol. For all of the experiments (at initial feed COD values of 20,000, 40,000, and 60,000 mg/l), the amounts of acetic acid, butyric acid, and valeric acid increased with increasing COD loading rate; however, the propionic acid relatively decreased. 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, whereas the propionic acid fermentation has been found to be the metabolic pathway for the consumption of produced hydrogen (Hawkes *et al.,* 2002). The concentrations of butyric and acetic acids were quite high at the COD loading rates of 37.5, 60, and 112.5 kg/m^{3}d for the initial feed COD values of 20,000, 40,000, and 60,000 mg/l, respectively, leading to the observed maximum hydrogen content in the produced gas (Figures 4.2a–4.2c), as well as the maximum hydrogen production rate (as shown next). At the initial feed COD values of 20,000, 40,000, and 60,000 mg/l, the concentration of propionic acid decreased when the higher concentration of butyric acid was obtained. These results indicate that the simultaneously obtained high butyric acid concentration and low propionic acid concentration might be the evidence of bacterial metabolic shift towards higher hydrogen production. These results agree well with the work of Cohen et al. (1985), which reported a linear inverse relationship between butyric acid and propionic acid concentrations and also a linear positive correlation between butyric acid concentration and hydrogen production. The valeric acid concentration also had the same trend as the butyric and acetic acid concentrations; its concentration increased with increasing COD loading rate. The overloading of organic substrate in the bioreactor led to increasing the accumulation of reduced product like valeric acid (Han *et al.*, 2005). This, therefore, resulted in a higher concentration of valeric acid produced at higher COD loading rates and initial feed COD values.

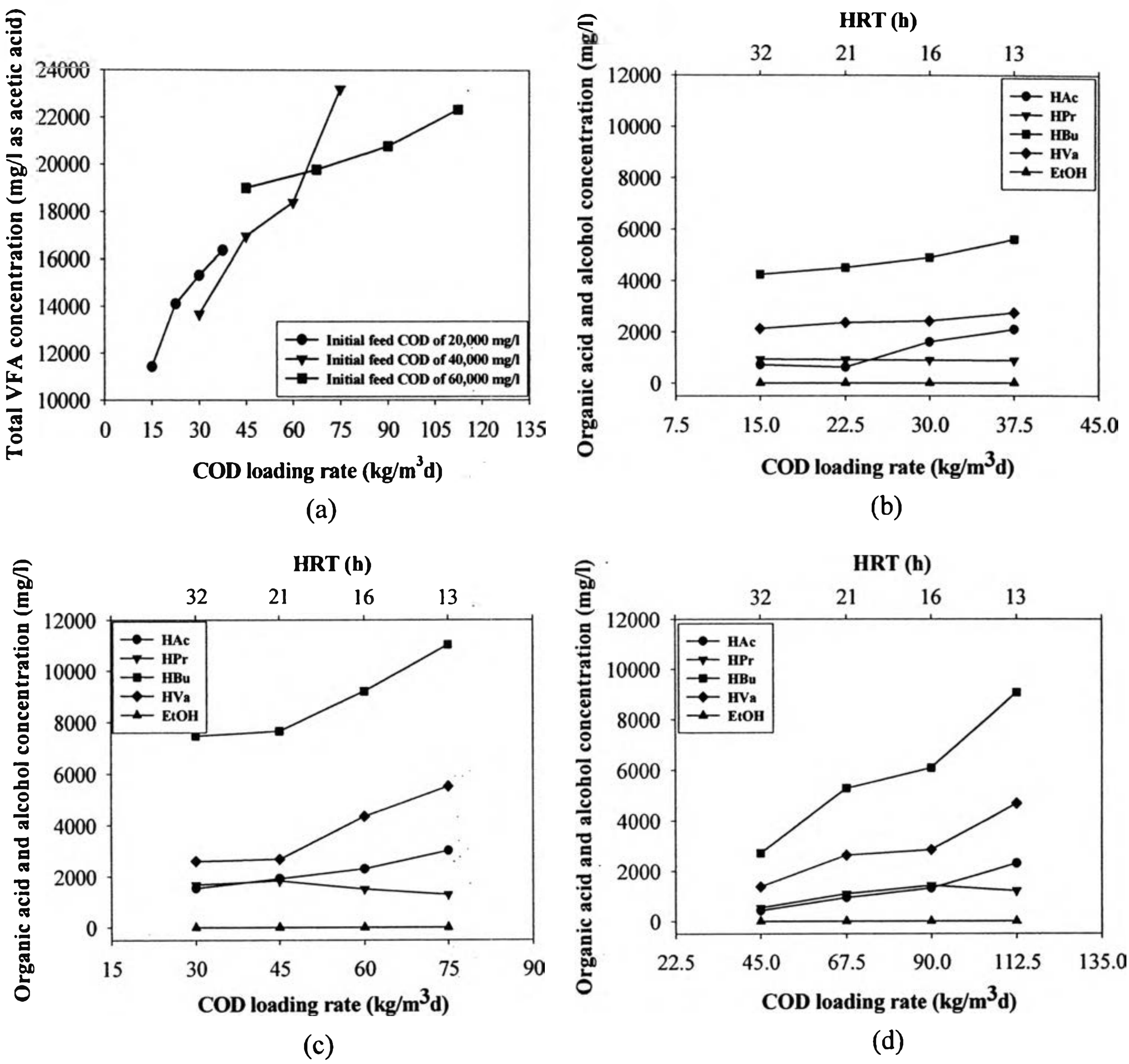


Figure 4.4 Total VFA (a), and VFA and ethanol concentrations at initial feed COD values of 20,000 mg/l (b), 40,000 mg/l (c), and 60,000 mg/l (d).

The hydrogen production rate (Figure 4.5) depends on the gas production rate and the gas composition. For the initial feed COD value of 20,000 mg/l (Figure 4.5a), the hydrogen production rate increased with increasing COD loading rate; however, it was quite low at this feed COD value, especially the COD loading rates in the range of 15–22.5 kg/m^{3}d with only the hydrogen production rate less than 0.02 l/h. This is possible due to low hydrogen percentages in the produced gas and low gas production rates. For the initial feed COD value of 40,000 mg/l (Figure 4.5b), the hydrogen production rate increased with increasing COD loading rate and reached the maximum of 0.55 l/h at a COD loading rate of 60 kg/m^{3}d because of the highest hydrogen percentage and gas production rate. After that, it rapidly decreased with further increasing COD loading rate to 75 kg/m^{3}d. Since total VFA concentration was found to be quite high at this COD loading rate (Figure 4.4a), this high total VFA concentration produced at the high COD loading rate resulted in the low hydrogen production rate due to the toxicity of VFA formation (Argun *et al.,* 2008), as mentioned in the part of total VFA concentration. For the initial feed COD value of 60,000 mg/l (Figure 4.5c), the hydrogen production rate was relatively unchanged in the range of 0.17–0.20 l/h between COD loading rates of 45–90 kg/m^{3}d, and afterwards, it increased to reach the maximum of 0.50 l/h at a COD loading rate of 112.5 kg/m^{3}d. The comparative results at different initial feed COD values revealed that the hydrogen production rate for initial feed COD value of 40,000 mg/l—with a COD loading rate of 60 kg/m^{3}d and a HRT of 16 h—was much higher than those maximum values for initial feed COD values of 20,000 and 60,000 mg/l. Therefore, the change in the initial feed COD value remarkably affected the hydrogen production rate. Although the increase in the initial feed COD value with the corresponding increased COD loading rates could stimulate the hydrogen production, they resulted in the accumulation of total VFA, which was found to inhibit the hydrogen production.

Another factor that may inhibit the hydrogen production is the toxicity of potassium. The alcohol distillery wastewater used in this work contains high concentration of potassium (about 9,000 mg/l in the as-received wastewater containing a COD value of 120,000 mg/l), and the potassium concentrations contained in alcohol distillery wastewater at each initial feed COD value are shown in Table 4.1. 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 4.2), which can be toxic to acedogenic bacteria in an anaerobic digestion. This may be the reason why the increase in the initial feed COD value to 60,000 mg/l (with a potassium concentration of 4,500 mg/l) or higher (the results are shown in Appendix E4) resulted in the low hydrogen production. However, the present work only focused on the feasibility study of hydrogen production from the alcohol distillery wastewater without any pretreatment step, and a high potential for hydrogen production was observed. Therefore, an interesting aspect for the future work is to reduce the toxicity of potassium, aiming to enhance the hydrogen production efficiency.

Table 4.1 Potassium concentrations contained in alcohol distillery wastewater feeds at various initial feed COD values

Initial feed COD value (mg/l)	Potassium concentration (mg/l)
20,000	1500
40,000	3,000
60,000	4,500

Table 4.2 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

The specific hydrogen production rate (SHPR) is also used to identify the ability of bacteria to produce hydrogen in the bioreactor, and the results are shown in Figure 4.6. The maximum SHPR of 270 ml H_2/g MLVSS d (or 3,308 ml H_2/l d) was obtained at a COD loading rate of 60 kg/m^{3}d for the initial feed COD of 40,000 mg/l since the maximum gas production rate and maximum hydrogen percentage in the produced gas were achieved at these operating conditions (Figure 4.1c and 4.2b).

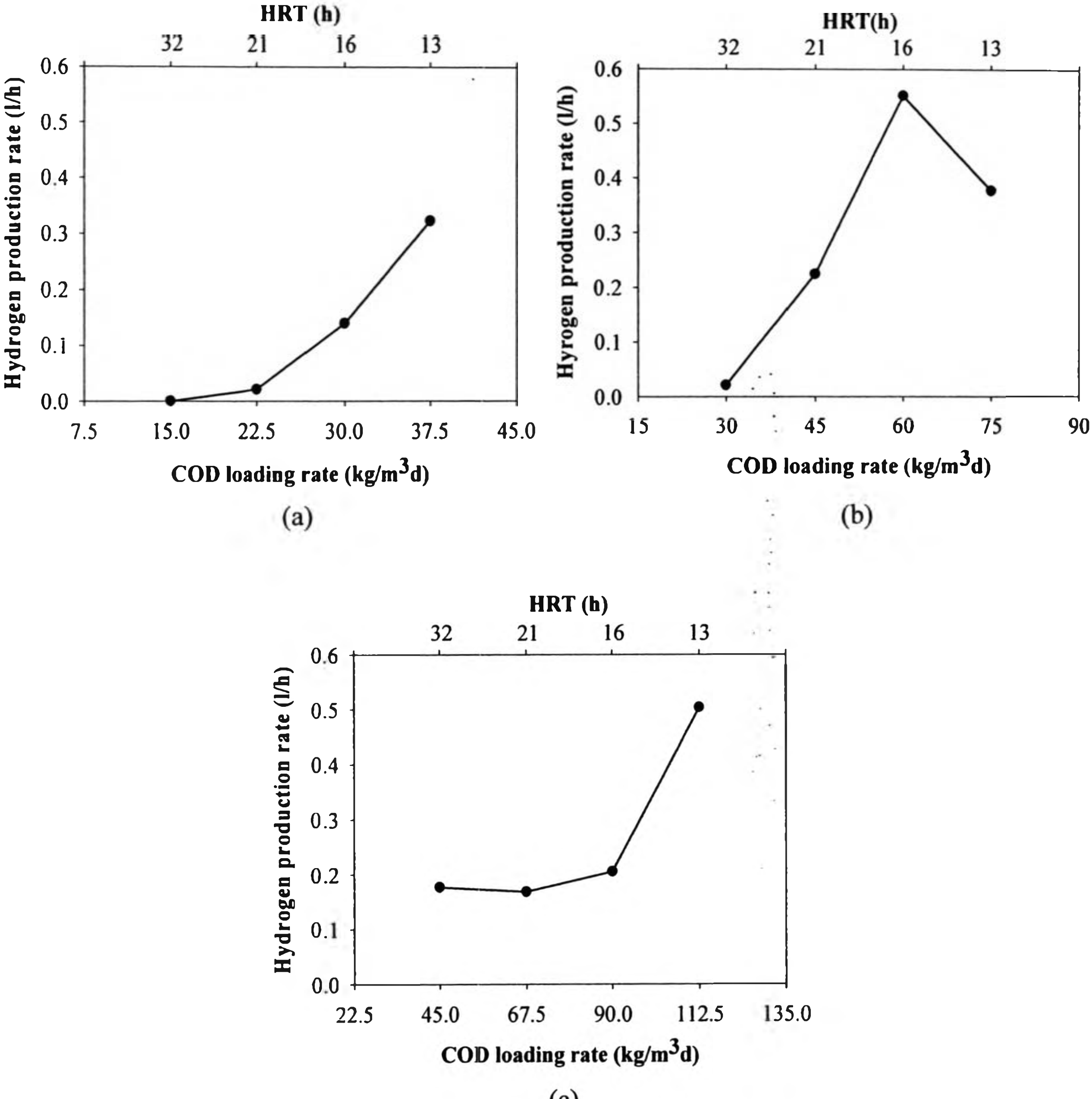
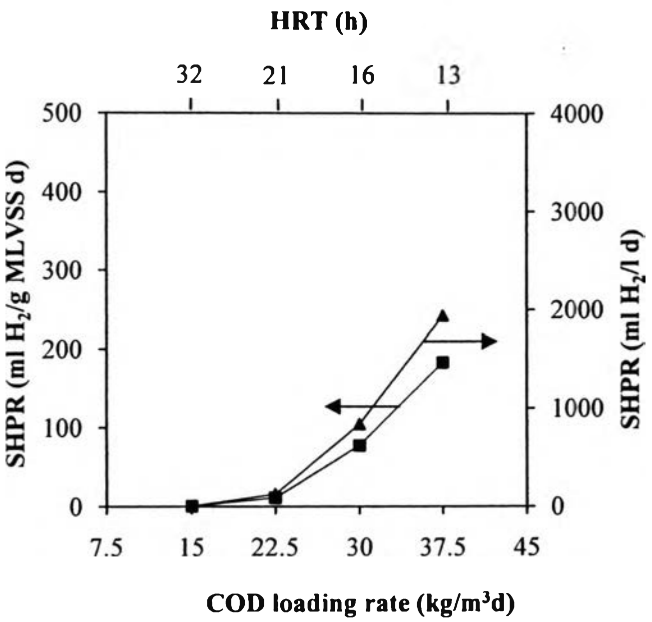
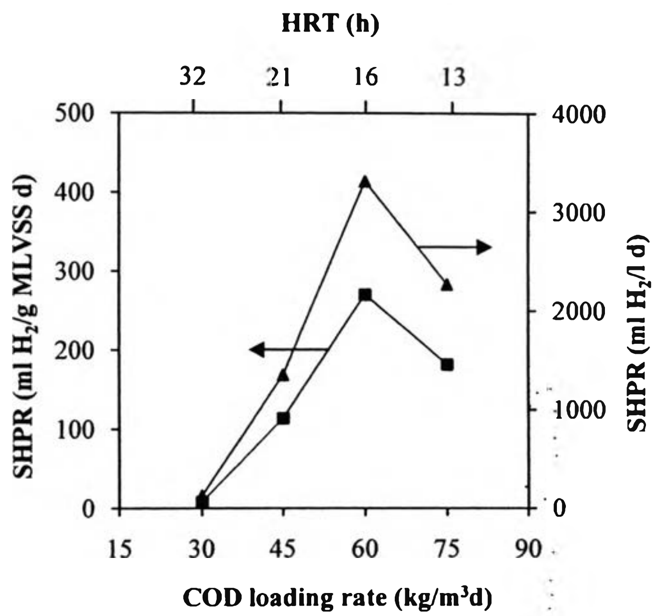


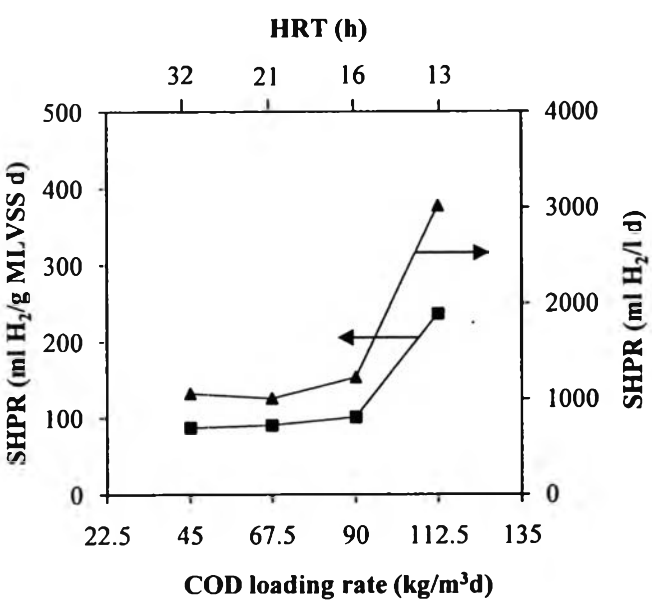
Figure 4.5 Hydrogen production rate at initial feed COD values of 20,000 mg/l (a), 40,000 mg/l (b), and 60,000 mg/l (c).



(a)



(b)



(c)

Figure 4.6 Specific hydrogen production rate at initial feed COD values of 20,000 mg/l (a), 40,000 mg/l (b), and 60,000 mg/l (c).

When considering the variation of hydraulic retention time (HRT) with respect to the hydrogen production, it can be seen that the HRT greatly affected metabolic products and microbial population, leading to the variations in gas composition, gas production rate, hydrogen production rate, and specific hydrogen production rate. The experiments showed that for both systems with the initial feed COD values of 20,000 and 60,000 mg/l, the hydrogen production rate (Figure 4.5) and SHPR (Figure 4.6) tended to increase when the HRT was reduced from 32 to 13 h and reached the maximum values at 13 h HRT. This indicated that more carbohydrate substrates in the wastewater were converted to hydrogen gas at a shorter HRT. On the other hand, at the initial feed COD value of 40,000 mg/l, the hydrogen production rate and SHPR also increased to reach the highest values of 0.55 l/h and 270 ml H_2/g MLVSS d (or 3,308 ml H_2/l d), respectively, when the HRT was reduced from 32 to 16 h. Further reduction in the HRT to 13 h resulted in the decreases in both the hydrogen production rate and SHPR to 0.38 l/h and 182 ml H_2/g MLVSS d (or 2,265 ml H_2/l d), respectively. This could be described with the aid of VFA concentration results (Figure 4.4). The highest hydrogen production rate and specific hydrogen production rate at 16 h HRT were observed by accompanying a comparatively low propionic acid concentration as compared to 13 h HRT. As mentioned above, the propionic acid fermentation was not favorable for the hydrogen production, so the increase in the propionic acid concentration at 13 h HRT would lead to a lower hydrogen production. However, it was clearly found that the HRT in the range of 13 to 16 h was suitable for the hydrogen production, depending upon the COD loading rate used. Therefore, it can be concluded that the enhancement of hydrogen production efficiency can be obtained by properly controlling HRT, as well as COD loading rate.

The hydrogen yield was calculated from the hydrogen production rate and COD removal per day, and the results of hydrogen yield are shown in Figure 4.7. For the initial feed COD value of 20,000 mg/l (Figure 4.7a), the hydrogen yield sharply increased with increasing COD loading rate and reached the maximum of 178 ml H_2/g COD removed at a COD loading rate of 37.5 kg/m^{3}d (13 h HRT). For the initial feed COD value of 40,000 mg/l (Figure 4.7b), the hydrogen yield increased with increasing COD loading rate and reached the maximum of 172 ml H_2/ g COD removed at a COD loading rate of 60 kg/m^{3}d (16 h HRT), and then it decreased with further increasing COD loading rate higher than 60 kg/m^{3}d. For the initial feed COD value of 60,000 mg/l (Figure 4.7c), the hydrogen yield dropped when increasing COD loading rate to 67.5 kg/m^{3}d, and then became nearly constant in the COD loading rate range of 67.5 to 90 kg/m^{3}d. Afterwards, it rapidly increased with further increasing COD loading rate to 112.5 kg/m^{3}d. The maximum hydrogen yield was 83 ml H_2/ g COD removed at a COD loading rate of 112.5 kg/m^{3}d (13 h HRT). By comparing the results of the three initial feed COD values (20,000, 40,000, and 60,000 mg/l), the maximum hydrogen yield tended to decrease with increasing initial feed COD value, and a higher COD loading rate was required to obtain a maximum hydrogen yield at a corresponding higher initial feed COD value. The initial feed COD value of 20,000 mg/l and the COD loading rate of 37.5 kg/m^{3}d (13 h HRT) gave the highest hydrogen yield (178 ml H_2/g COD removed). However, the initial feed COD value of 40,000 mg/l and the COD loading rate of 60 kg/m^{3}d (16 h HRT) were considered to be the optimum conditions for the hydrogen production since the maximum hydrogen production rate and maximum SHPR were obtained, and the hydrogen yield of 171 ml H_2/g COD removed was only slightly lower than that at the initial feed COD value of 20,000 mg/l and the COD loading rate of 37.5 kg/m^{3}d.

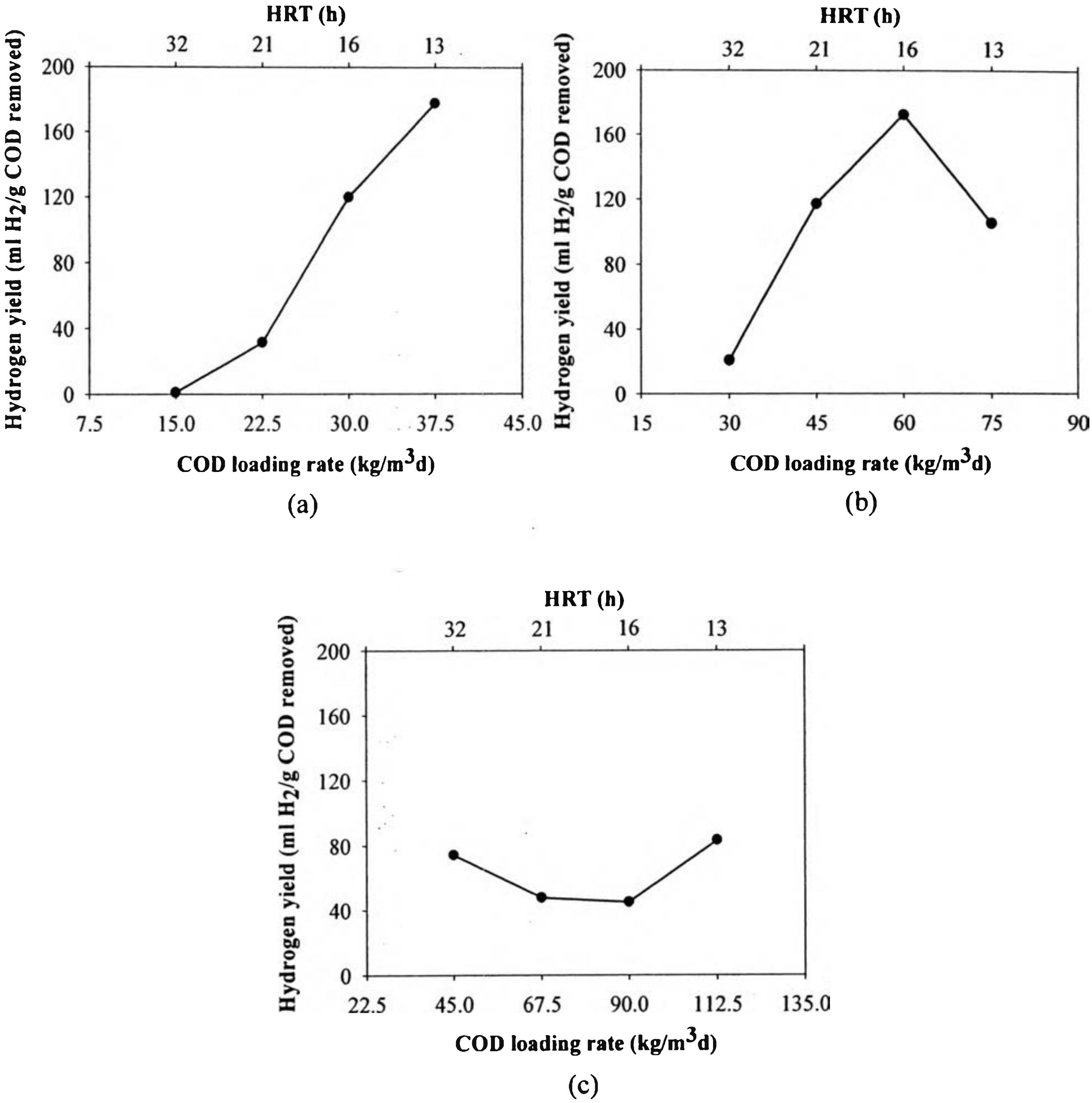


Figure 4.7 Hydrogen yield at initial feed COD values of 20,000 mg/l (a), 40,000 mg/l (b), and 60,000 mg/l (c).