CHAPTER IV
RESULTS AND DISCUSSION

4.1 Hydrogen Production Performance

In this study, the biodiesel wastewater at an initial feed COD of 45,000 mg/l was directly fed to hydrogen ASBR unit. The effect of COD loading rate ranging from 33.75 to 67.50 kg/m^{3}d based on feed COD and hydrogen ASBR volume on hydrogen production was investigated. The solution pH was controlled at 5.5 and a constant temperature 37 ºC.

4.1.1 COD Removal and Gas Production Rate

The effect of COD loading rate on COD removal and gas production rate is shown in Figure 4.1. The COD removal increased with increasing COD loading rate from 33.75 to 67.50 kg/m^3 d and then decreased with further increasing COD loading rate from 67.50 to 84.38 kg/m^3 d. The highest COD removal (36.8%) and the highest gas production rate (4.2 l/d) were found at a COD loading rate of 67.50 kg/m^3 d. These results can be explained that when COD loading rate increased, more organic compounds in the system was also increased available for microbes to degrade. However, at a very high COD loading rate of 84.38 kg/m^3 d, the COD removal and the gas production rate in the system decreased because there were high amount of volatile fatty acid (VFA) in the system.

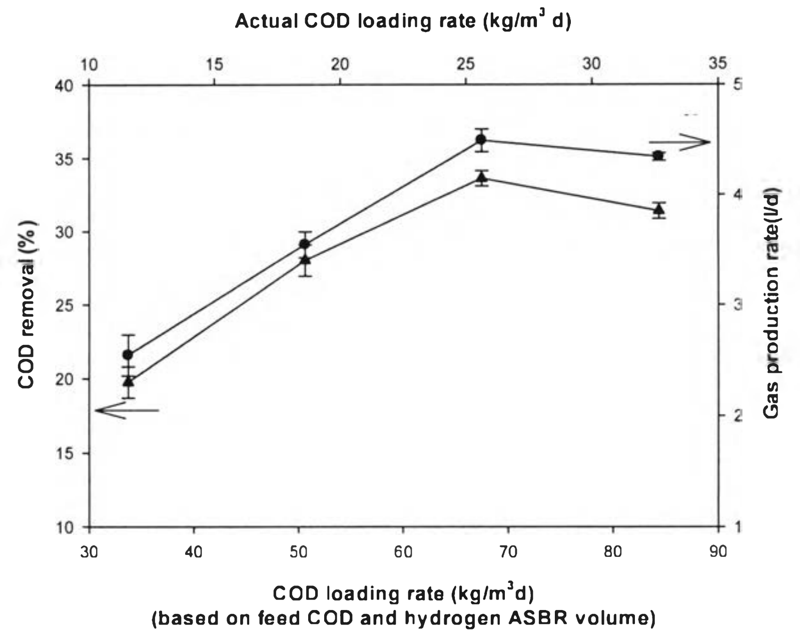

Figure 4.1 Effects of COD loading rate on COD removal and gas production rate of hydrogen ASBR unit at pH 5.5 and 37 °C.

4.1.2 Hydrogen Production Performance

The main produced gases in hydrogen ASBR unit were mainly hydrogen and carbon dioxide with small amount of methane (Figure 4.2). The hydrogen percentage and hydrogen production rate increased with increasing COD loading rate and then decreased with further increasing COD loading rate from 67.50 to 84.38 kg/m^3 d. The highest hydrogen percentage (31.7 %) and the highest hydrogen production rate (1.4 l/d) were found at the same COD loading rate of 67.50 kg/m^3 d. From the result, it can be suggest that there were high organic compounds available for producing hydrogen composition. The decrease in hydrogen production can be explained with the same explanation of COD removal.

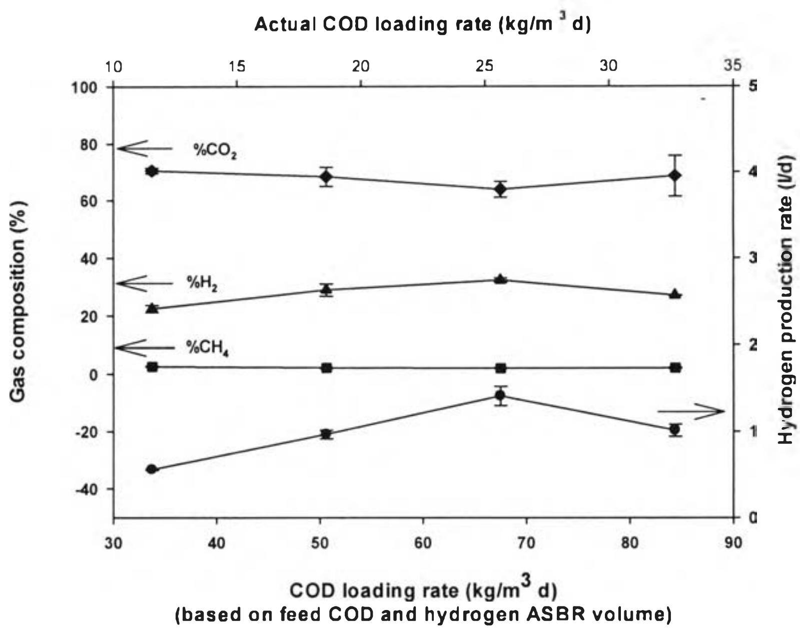

Figure 4.2 Effects of COD loading rate on gas composition and hydrogen production rate at pH 5.5 and 37 °C.

Specific hydrogen production rate (SHPR) and hydrogen yield (Figure 4.3-4.4) had a similar trend to COD removal result. They increased with increasing COD loading rate and then decreased with further increasing COD loading rate from 67.50 to 84.38 kg/m^3 d. The maximum specific hydrogen production rate, SHPR (88.9 ml H_2/g MLVSS d or 332.5 ml H_2/ l d) and the maximum hydrogen yield (7.8 ml H_2/ g COD removed or 4.9 ml H_2/g COD applied) were found at a COD loading rate of 67.50 kg/m^3 d corresponding to the highest COD removal and hydrogen production rate. When the system was operated at a very high COD loading rate of 84.38 kg/m^3 d, the hydrogen production decreased due to the toxicity from VFA accumulation, which will be discuss later. Therefore, the optimum COD loading rate for hydrogen production is 67.50 kg/m^3 d.

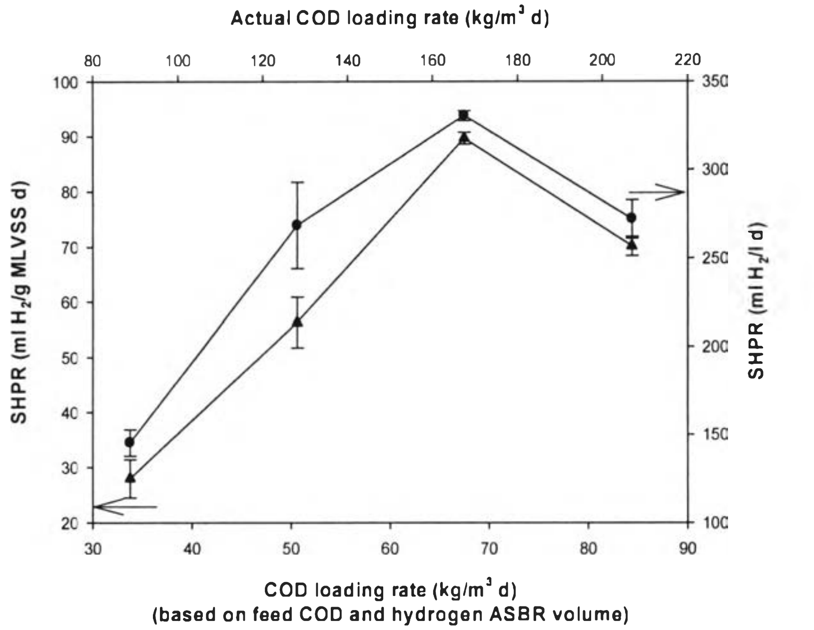

Figure 4.3 Effect of COD loading rate on specific hydrogen production rate at pH 5.5 and 37 °C.

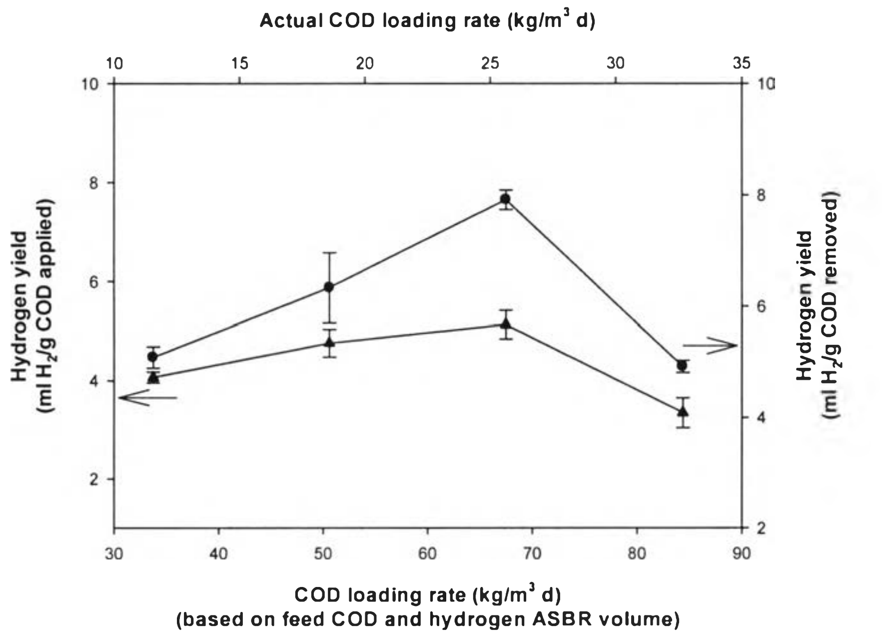

Figure 4.4 Effect of COD loading rate on hydrogen yield at pH 5.5 and 37 °C.

4.1.3 Volatile Fatty Acid (VFA) and VFA Composition

Total volatile fatty acid (VFA) concentration and its composition are shown in Figure 4.5. The total VFA concentration increased from 10,555 mg/l as acetic acid to 16,247 mg/l as acetic acid with increasing COD loading rate and attained a maximum value at the highest COD loading rate of 84.38 kg/m^3 d while hydrogen production performance decreased at this the highest COD loading rate. Because at a high VFA concentration inhibited microbial activity in the system due to high VFA accumulation. The main component of the VFA were butyric acid, valeric acid, acetic acid, and propionic acid with a small amount of 1,3-propanediol and ethanol. It can be concluded that the toxicity level of total VFA to the microorganism was ≈ 13,000 mg/l as acetic acid.

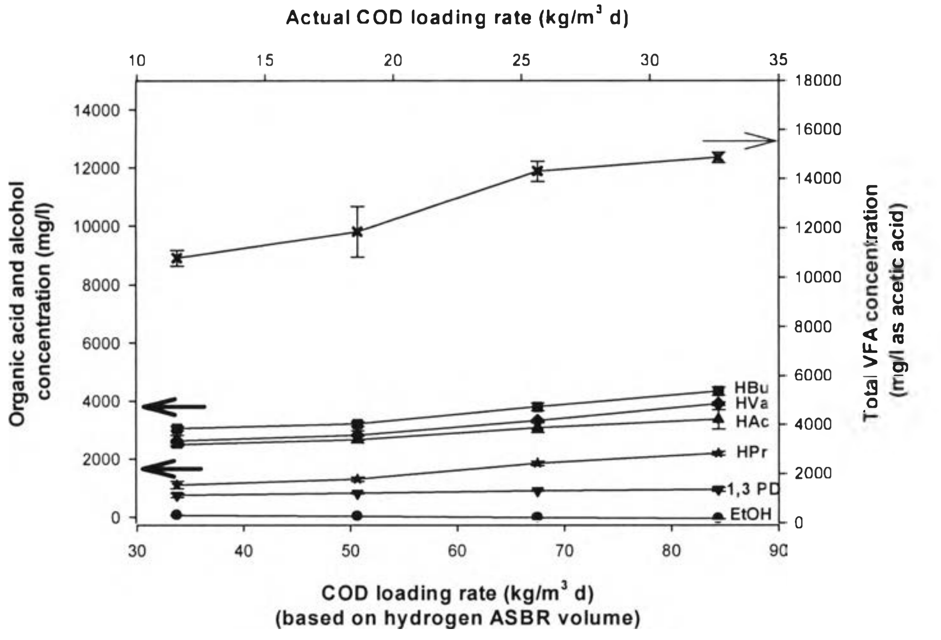

Figure 4.5 The COD loading rate versus total VFA, VFA composition, ethanol and 1,3-propanediol concentration at 37 °C and pH 5.5 in hydrogen ASBR unit.

Hydrogen production by bioconversion of glycerine can be explained by oxidative and reductive metabolisms of glycerine. The oxidative metabolism of glycerine produced pyruvate as an intermediate product (Drożdżyńska *et al.*, 2011) and it was metabolized to other difference endproducts such as ethanol, butanol, acetate, butyrate, and lactate (da Silva *et al.*, 2009) which is the same as oxidative metabolism of glucose (Sarma *et al.*, 2012). Moreover, the main fermentative products of pyruvate are acetic acid and butyric acid (Zeng, 1996). While, the reductive metabolism converted glycerine to 1,3-propanediol (Sarma *et al.*, 2012). In this studied, the component that affected to hydrogen production performance were butyric acid, 1,3-propanediol, and ethanol. The butyric acid concentration was the highest while 1,3-propanediol and ethanol concentration were the lowest which corresponding to high hydrogen production performance. It can be explained in the fact that butyric acid pathway was favorable for hydrogen production (Arooj *et al.*, 2008) as shown in equation 4.3. Whereas 1,3-propanediol and ethanol were not satisfied for hydrogen production according to equation 4.5-4.6. The production of 1,3-propanediol directly affected to hydrogen production performance because the production of one mole of 1,3-propanediol, one mole of hydrogen was consumed (Sittijunda and Reungsang , 2012).

Acetic acid Production

$$C_3H_8O_3 + H_2O \longrightarrow CH_3COOH + CO_2 + 3H_2 \qquad (4.1)$$

Propionic acid Production

$$C_3H_8O_3 \longrightarrow CH_3CH_2COOH + H_2O \qquad (4.2)$$

Butyric acid Production

$$2C_3H_8O_3 \longrightarrow CH_3CH_2CH_2COOH + 2CO_2 + 4H_2 \qquad (4.3)$$

Ethanol Production

$$CH_3COOH + H_2 \longrightarrow CH_3CH_2OH + H_2 \qquad (4.5)$$

1,3-propanediol Production

$$C_3H_8O_3 + H_2 \longrightarrow C_3H_8O_2 + H_2O \qquad (4.6)$$

4.2 Methane Production Performance

Under methane ASBR unit, the effluent from hydrogen ASBR unit was directly fed to methane ASBR unit. The effect of COD loading rate ranging from 5.63 to 14.06 kg/m^{3}d based on feed COD and methane ASBR volume on methane production was investigated under mesophilic temperature (37 °C) without control pH.

4.2.1 COD Removal and Gas Production Rate

The COD removal increased with increasing COD loading rate from 5.63 to 11.25 kg/m^3 d and decreased with further increasing COD loading rate from 11.25 to 14.06 kg/m^3 d (Figure 4.6). The COD removal and gas production rate reached a maximum value of 63.2 % and 21.6 l/d, respectively at COD loading rate of 11.25 kg/m^3 d. After that, both COD removal and gas production rate decreased with further increasing COD loading rate from 11.25 to 14.06 kg/m^{3}d. These results could be explained that most organic compound completely degraded to methane gas under anaerobic condition

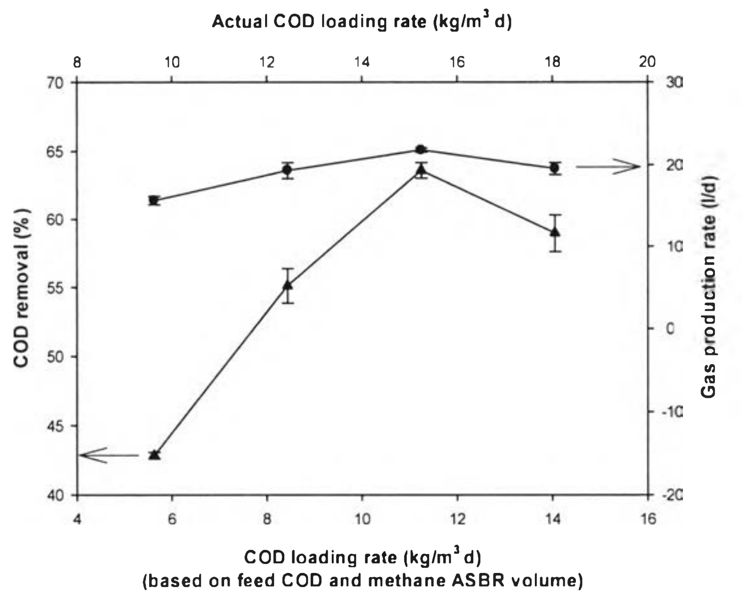

Figure 4.6 Effects of COD loading rate on COD removal and gas production rate at 37 °C without control pH in methane ASBR unit.

4.2.2 Methane Production Performance

The main produced gas in methane ASBR unit were methane and carbon dioxide with small amount of hydrogen (Figure 4.7). Both methane percentage and methane production rate increased with increasing COD loading rate from 5.63 to 11.25 kg/m^3 d and then decreased with further increasing COD loading rate up to 14.06 kg/m^3 d. Carbon dioxide had an opposite to the methane percentage. The highest methane percentage (74.8 %) and the highest methane production rate (16.15 l/d) were found at a COD loading rate of 11.25 kg/m^3 d. These results indicated that when more organic compound feeding to system, methanogenic bacteria converted those organic compound to methane gas causing the increase in methane content in ASBR unit. The decrease in the methane percentage and methane production rate due to the fact that the reduction of pH in methane bioreactor effect to methane production performance (Arooj *et al.*, 2008). Both SMPR and methane yield had the similar trend to methane percentage and methane production rate which were they increased with increasing COD loading rate from 5.63 to 11.25 kg/m^3 d and then decreased with further increasing COD loading rate up to 14.06 kg/m^3 d (Figure 4.8-4.7). The maximum specific methane production rate, SMPR (233 ml CH_4/g MLVSS d or 672.8 ml CH_4/l d) and the maximum methane yield (128.4 ml CH_4/g COD removed or 47.3 ml CH_4/g COD applied) was found at a COD loading rate of 11.25 kg/m^3 d consistent with the highest COD removal and methane production rate.

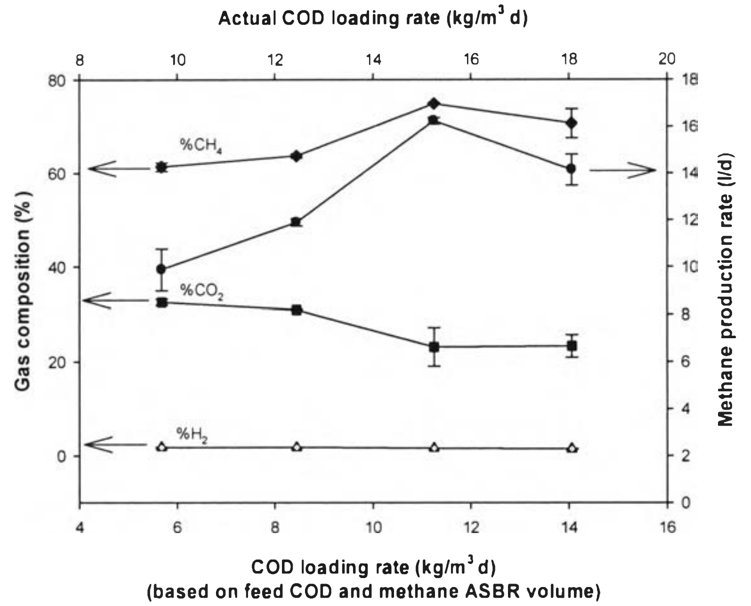

Figure 4.7 Effects of COD loading rate on gas composition and methane production rate at 37 °C without control pH in methane ASBR unit.

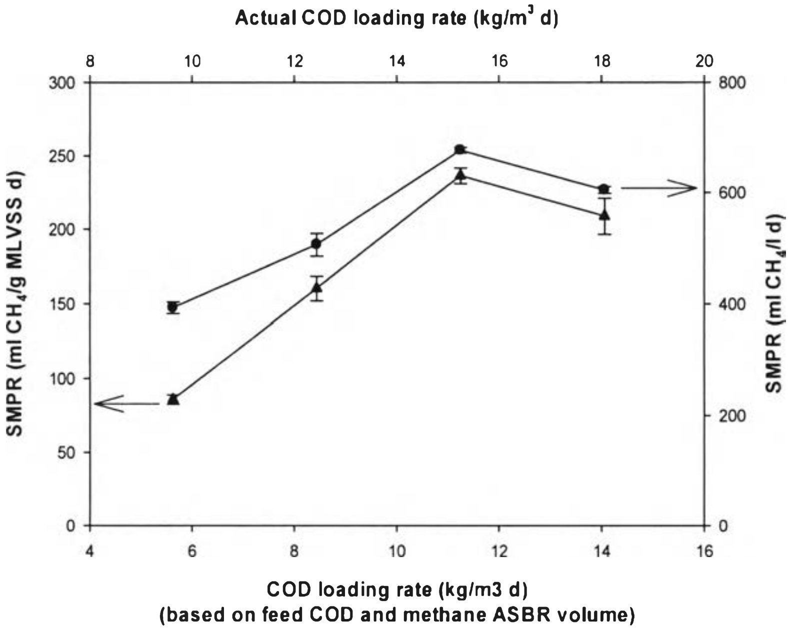

Figure 4.8 Effects of COD loading rate on specific methane production rates at 37 °C without control pH in methane ASBR unit.

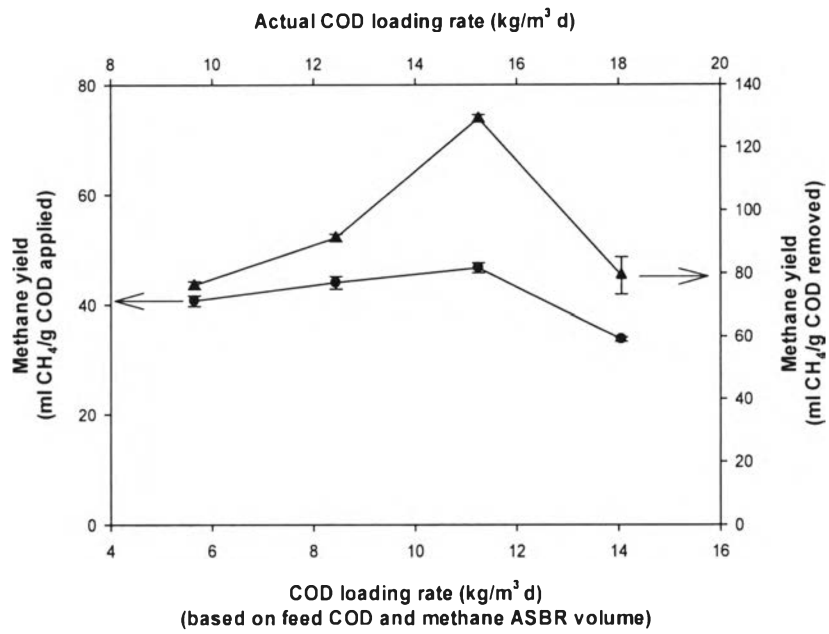

Figure 4.9 Effects of COD loading rate on methane yield at 37 °C without control pH in methane ASBR unit.

4.2.3 Volatile Fatty Acid (VFA) and VFA Composition

The total VFA concentration in methane ASBR unit increased with increasing COD loading rate and reached the maximum value (1,712 mg/l) at high COD loading of 14.06 kg/m^3 d (Figure 4.10) while methane production performance decreased because of high amount of organic acid formation. Those organic acid compounds could be accumulated in the system resulting in toxicity to microbes for methane production. The component of VFA were acetic acid, propionic acid ,butyric acid, valeric acid with a very small amount of 1,3-propanediol and ethanol. The highest concentration was acetic acid which can be converted to methane and carbon dioxide gases by acetotrophic pathway according to equation 4.7. Moreover, hydrogen and carbon dioxide gases from previous step will be further converted to methane by hydrogenotrophic pathway (Abbasi *et al*, 2012) as shown in equation 4.8.

Acetotrophic pathway

$$CH_3COOH \longrightarrow CH_4 + CO_2 \quad (4.7)$$

Hydrogenotrophic pathway

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \quad (4.8)$$

Buswell formula equation

$$C_3H_8O_3 \longrightarrow 1.75CH_4 + 1.25CO_2 + 0.5H_2O \quad (4.9)$$

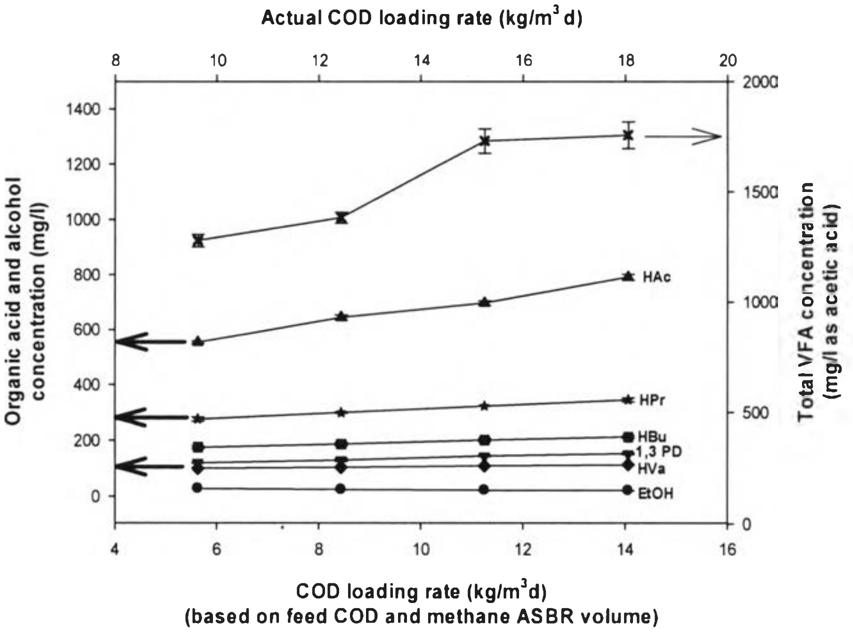

Figure 4.10 The COD loading rate versus total VFA, VFA composition, ethanol and 1,3-propanediol concentration at 37 °C without control pH in methane ASBR unit.

4.3 Microbial Concentration and Microbial Washout Results

Mixed liquor volatile suspended solid (MLVSS) represents microbes concentration in ASBR unit and effluent volatile suspended solid (VSS) represent microbial wash out from ASBR unit. In both ASBR unit, the MLVSS decreased with increasing COD loading and the effluent VSS show an opposite trend to MLVSS. When MLVSS decreased, the hydrogen and methane production performance increased because in hydrogen reactor contained mainly hydrogen-forming bacteria and hydrogen-consuming bacteria was washed out from hydrogen reactor according to low produced methane in hydrogen ASBR unit. In methane ASBR unit, the main bacteria was methane-forming bacteria in methane reactor and methane-consuming bacteria was washed out from methane reactor.

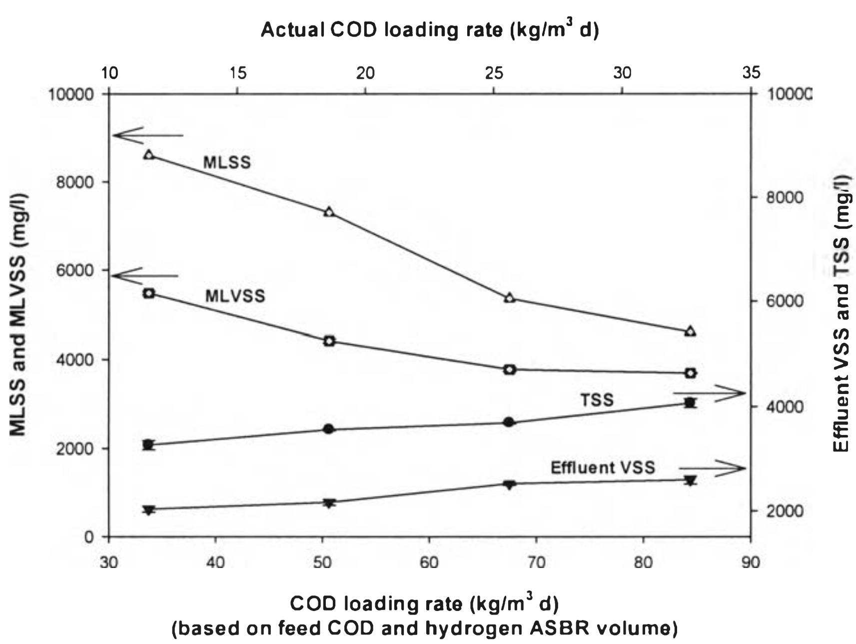

Figure 4.11 The COD loading rate versus MLSS, MLVSS, TSS, and effluent VSS in hydrogen ASBR unit.

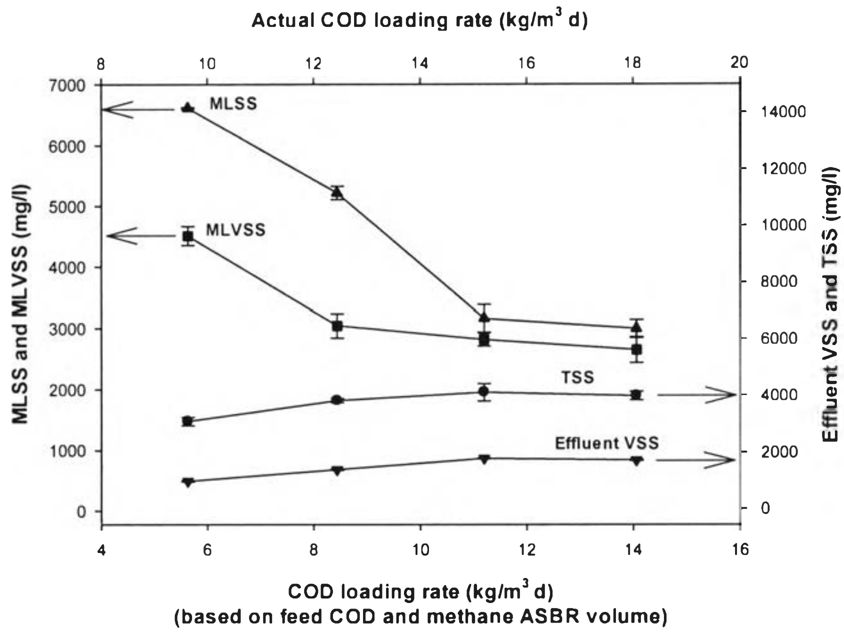

Figure 4.12 The COD loading rate versus MLSS, MLVSS, TSS, and effluent VSS in methane ASBR unit.

4.4 Nitrogen, Phosphorous, and Glycerine Results

Nitrogen, phosphorous, and glycerine uptake increased with increasing COD loading rate and then they slightly changed with further increasing COD loading rate from 67.50 to 84.38 kg/m^3 d based on feed COD and hydrogen volume and from 11.25 to 14.06 kg/m^3 d based on feed COD and methane volume (Figure 4.13-4.14). As the amount of microbial concentration in both system increased, it indicated that the utilized of nitrogen, phosphorous, and glycerine also increased. Nitrate and organic nitrogen in hydrogen and methane production process decreased with increasing COD loading rate whereas ammonium nitrogen and nitrite nitrogen in hydrogen and methane ASBR units were unchanged (Figure 4.15-4.16). Therefore, most nitrogen using by hydrogen and methane-producing bacteria were nitrate and organic nitrogen.

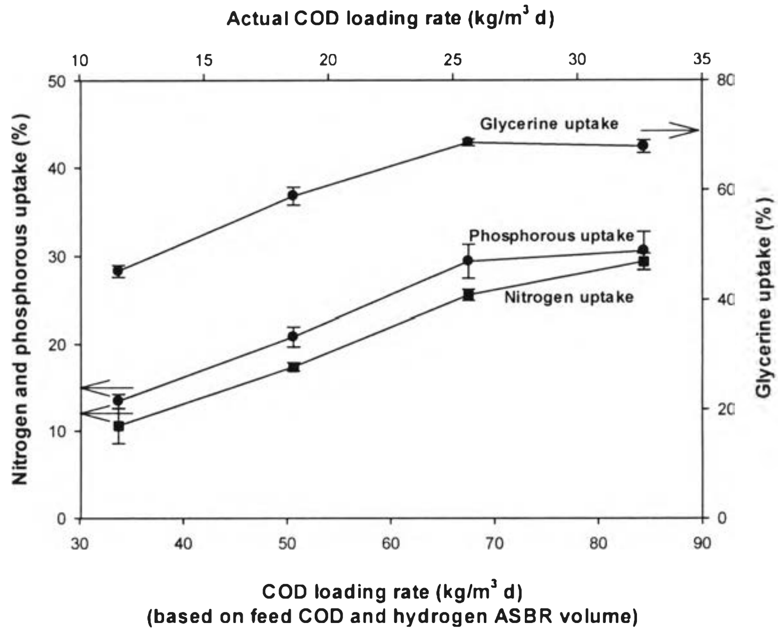

Figure 4.13 Nitrogen uptake, phosphorous uptake, and glycerine uptake in hydrogen production system as a function of COD loading rate at 37°C and pH 5.5.

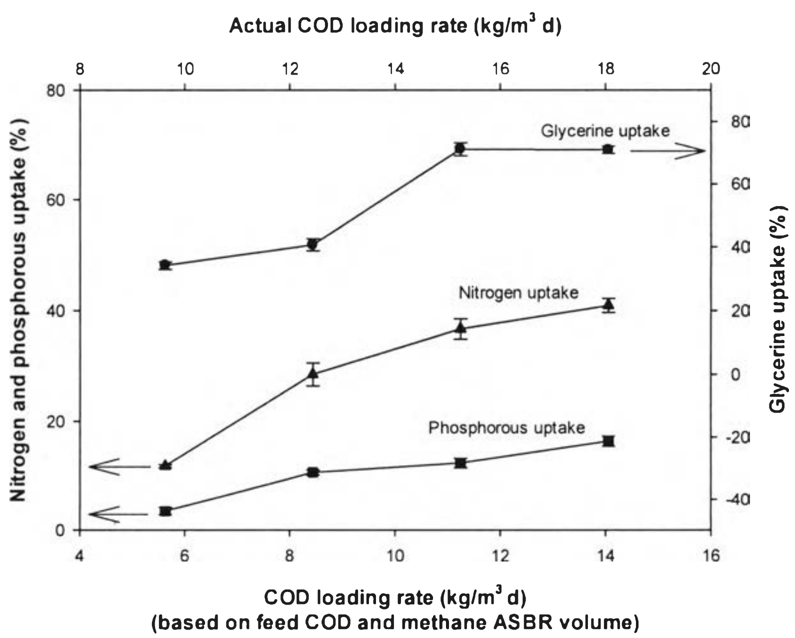

Figure 4.14 Nitrogen uptake, phosphorous uptake, and glycerine uptake in methane ASBR unit as a function of COD loading rate at 37°C without control pH.

4.5 Overall Performance

The overall performance of two stage ASBR process is shown in Figure 4.15. The results found that the optimum total COD loading rate of 9.64 kg/m^3 d provide the total COD removal of 77.9% and total gas production rate of 26.3 l/d. The gas compositions of total produced gas were mainly methane (63.5%), hydrogen (6.5%), and carbon dioxide (30%). Moreover, total hydrogen production performance shows in terms of a total hydrogen production rate (1.80 l/d), a total hydrogen yield (52.8 ml H_2/g COD removed or 12.3 ml H_2/g COD applied) and a total specific hydrogen production rate (60.6 ml H_2/ l). For methane production performance, a total methane production rate was 16.40 l/d which provided methane yield of 514.7 ml CH_4/g COD removed or 121.2 ml CH_4/g COD applied and a total specific methane production rate of 580.9 ml CH_4/l d.

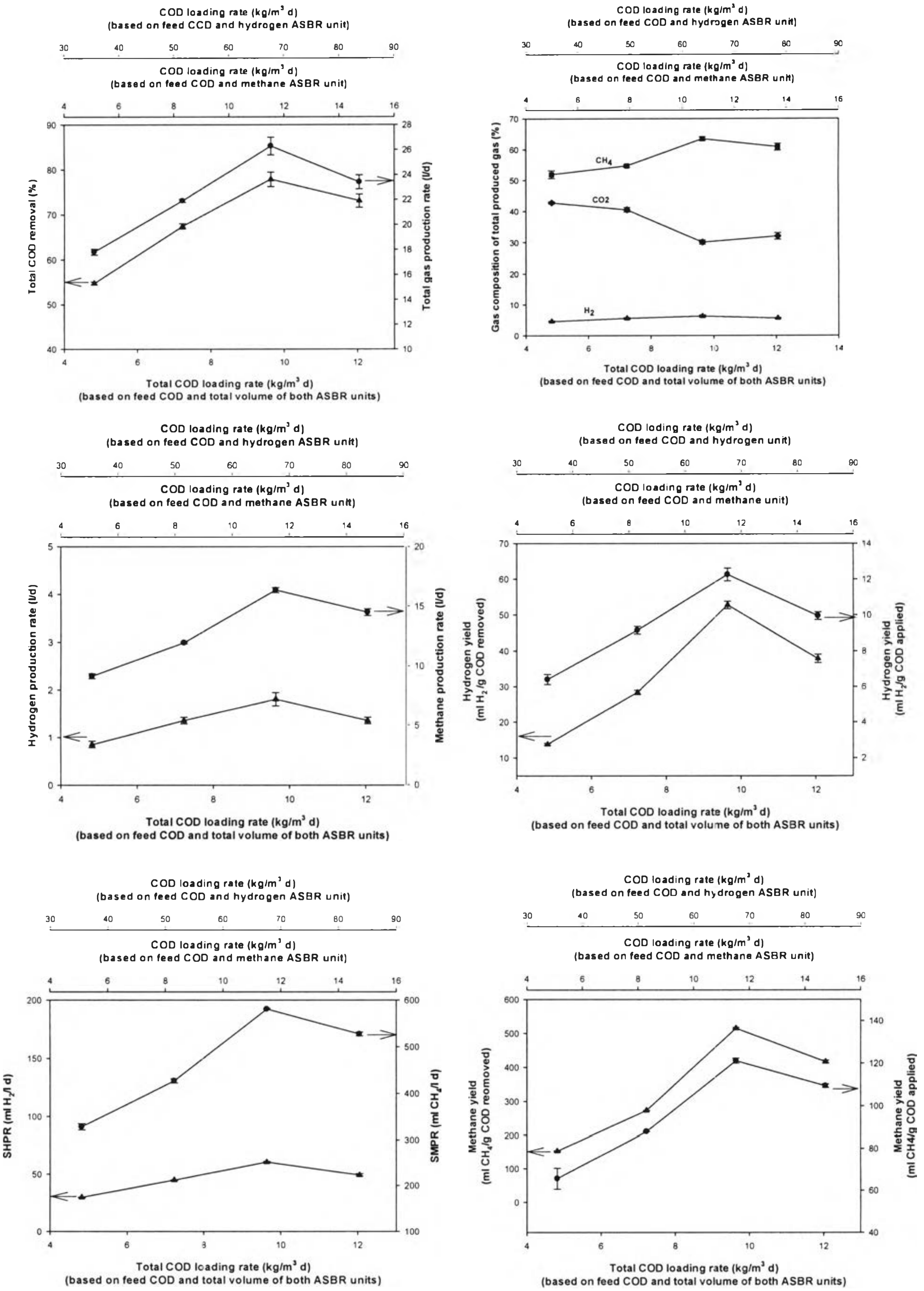

Figure 4.15 Overall performance of two stage ASBR processes.