CHAPTER IV
RESULTS AND DISCUSSION

The results can be presented into two parts. Firstly, the investigation showed the optimum COD loading rate on the separate hydrogen and methane production. The second part was to study the use of limited amount of oxygen in term of oxygen supply load at optimum COD loading rate in a two-stage UASB reactor consisted of 4 and 24 l hydrogen and methane UASB unit, respectively. This research was studied under the mesophillic temperature (37 °C), with pH 5.5 in hydrogen UASB unit and without pH control in methane UASB unit at a recycle ratio (methane effluent flow rate: feed flow rate) equal to 1:1.

4.1 Performance of Separate Hydrogen and Methane Production from Ethanol Wastewater: Hydrogen Production Step

4.1.1 Gas Production Rate and COD Removal

Gas production rate and COD removal is presented in Figure 4.1. Both gas production rate and COD removal increased with increasing COD loading rate from 12.0 to 36.0 kg/m^3 d based on hydrogen UASB unit, then decreased with further increasing COD loading rate to 60.0 kg/m^3 d based on hydrogen UASB unit. The maximum gas production rate (4.5 l/d) and COD removal (36 %) was at 36 kg/m^3 d based on hydrogen UASB unit.

4.1.2 Hydrogen Production Rate and Gas Composition

The hydrogen production rate is calculated from the gas production rate multiplied by hydrogen content. From Figure 4.2, the hydrogen production rate increased with increasing COD loading rate to 36.0 kg/m^3 d based on hydrogen UASB unit, then decreased with further increasing COD loading rate. From the results, the maximum hydrogen production rate of 1.8 l/d was observed at COD loading rate of 36.0 kg/m^3 d based on hydrogen UASB unit.

The gas composition mainly consisted of hydrogen and carbon dioxide. The hydrogen content increased with increasing COD loading rate from 12 to 36.0

kg/m^3 d based on hydrogen UASB unit and reached the maximum value at a COD loading of 36.0 kg/m^3 d based on hydrogen UASB unit. After that, the hydrogen content deceased with further increasing COD loading rate to 60.0 kg/m^3 d based on hydrogen UASB unit. However, carbon dioxide content showed an opposite trend to hydrogen content.

The decrease in hydrogen production performance can be explained by the toxicity from an organic acid accumulation. The higher COD loading rate, the greater organic compounds which available to convert to gaseous product and VFA (Yang *et al.*, 2006). The greater concentration of accumulated VFA in system could diffuse into inside microbial cell and ionize to decrease inside pH which destroyed the bacteria cell (Wielen *et al.*, 2000), leading to decrease in hydrogen production performance.

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 per day (ml H_2/g MLVSS d) or per volume of reactor per day (ml H_2/L_R d). From Figure 4.3, the SHPR increased with increasing COD loading rate to 36 kg/m^3 d based on hydrogen UASB unit and declined with further increasing COD loading rate to 60.0 kg/m^3 d based on hydrogen UASB unit. At COD loading rate of 36.0 kg/m^3 d based on hydrogen UASB unit, the SHPR was maximized to 460.8 ml H_2/L_R d (or 79.3 ml H_2/g MLVSS d).

4.1.4 Hydrogen Yield

Hydrogen yield is defined as the ratio of amount of produced hydrogen to the amount of consumed organic substrate in the unit of ml H_2/g COD removed (or the ratio of amount of produced hydrogen to the amount of applied organic substrate in the unit of ml H_2/g COD applied). From Figure 4.4, the hydrogen yield increased with increasing COD loading rate from 12.0 to 36.0 kg/m^3 d based on hydrogen UASB unit and reached the maximum value at a COD loading of 36.0 kg/m^3 d based on hydrogen UASB unit, which had similar trend to SHPR. However, at higher COD loading rate (>36.0 kg/m^3 d), the hydrogen yield decreased.

The performance of a two-stage UASB system can be exhibited in term of SHPR and hydrogen yield. From the result, the optimum COD loading defined as the supreme organic loading to maximize hydrogen production performance was obtained at COD loading rate of 36 kg/m^3 d based on hydrogen UASB unit.

4.1.5 The Total Volatile Fatty Acid (VFA), Its Composition and Ethanol Concentration

Beside biogas composition, the liquid products could be used to monitor hydrogen production efficiency (Yang *et al.*, 2006). For the total VFA concentration shown in Figure 4.5, it slightly increased with increase in COD loading rate in the range of 12 to 36 kg/m^3 d based on hydrogen working volume. Then, the total VFA concentration sharply increased beyond the optimum COD loading rate. The decreased in hydrogen production performance can be explained by the toxicity from organics acid accumulation. The associated VFA in the system can diffuse and ionize to decrease inside pH of microbial cell, resulting in inhibiting the growth of cell (Wielen *et al.*, 2000). The result showed that the toxicity level of VFA in hydrogen production was 9,400 mg/l.

The composition of VFA was mainly acetic acid (HAc), butyric acid (HBu), propionic acid (HPr), valeric acid (HVa) lactic acid (HLa) and ethanol. Ethanol had the highest concentration because of the yeast presented in the system.

Acetic acid, butyric acid and ethanol generation favoured the hydrogen production, while propionic acid, valeric acid and lactic acid production deteriorated hydrogen production. The metabolic path way of VFA generation showed in Equations (4.1-4.7) (Cata Saady *et al.*, 2013).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (4.1)$$

Glucose Acetic acid

$$C_6H_{12}O_6 \rightarrow 2H_2 + CH_3CH_2CH_2COOH + 2CO_2 \quad (4.2)$$

Glucose Butyric acid

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \tag{4.3}$$

Glucose Propionic acid

$$CH_3CH_2COOH + CH_3COOH + 2H_2 \rightarrow CH_3CH_2CH_2CH_2COOH + 2H_2O \tag{4.4}$$

Propionic acid Valeric acid

$$CH_3CH_2COOH + 2CO_2 + 6H_2 \rightarrow CH_3CH_2CH_2CH_2COOH + 4H_2O \tag{4.5}$$

Propionic acid Valeric acid

$$3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + H_2CO_3 \tag{4.6}$$

Lactic acid Propionic acid

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \tag{4.7}$$

Glucose Ethanol

4.1.6 The Microbial Concentration and Microbial Washout

The microbial concentration in term of mixed liquor volatile suspended solid (MLVSS) and microbial washout in term of effluent volatile suspended solid (effluent VSS) from bioreactor are shown in Figure 4.6. The MLVSS decreased from 19,100 to 14,200 mg/l with increasing COD loading rate to 36.0 kg/m^3 d based on hydrogen UASB unit, while effluent VSS showing an opposite trend to MLVSS which increased from 3,300 to 4,600 mg/l. With further increasing COD loading rate, the MLVSS and effluent VSS remained constant, because of the toxicity level of organic. The toxicity level of organic acid referred to acid accumulation in bioreactor inhibited a growth of hydrogen-producing bacteria (Lee *et al.*, 2004), leading to wash out of an inactive microbial cells from the system (Yang *et al.*, 2006).

From the overall experimental results on hydrogen production step, the optimum COD loading rate was suggested at 36.0 kg/m^3 d based on hydrogen UASB unit due to the highest hydrogen production performance in term of the highest hydrogen content (41 %), hydrogen yield (33.5 ml H_2/g COD removed and 12.0 ml

H_2/g COD applied), hydrogen production rate (1.8 l/d), specific hydrogen production rate (79.3 ml H_2/g MLVSS d and 460.8 ml H_2/L_R d) and COD removal (36 %).

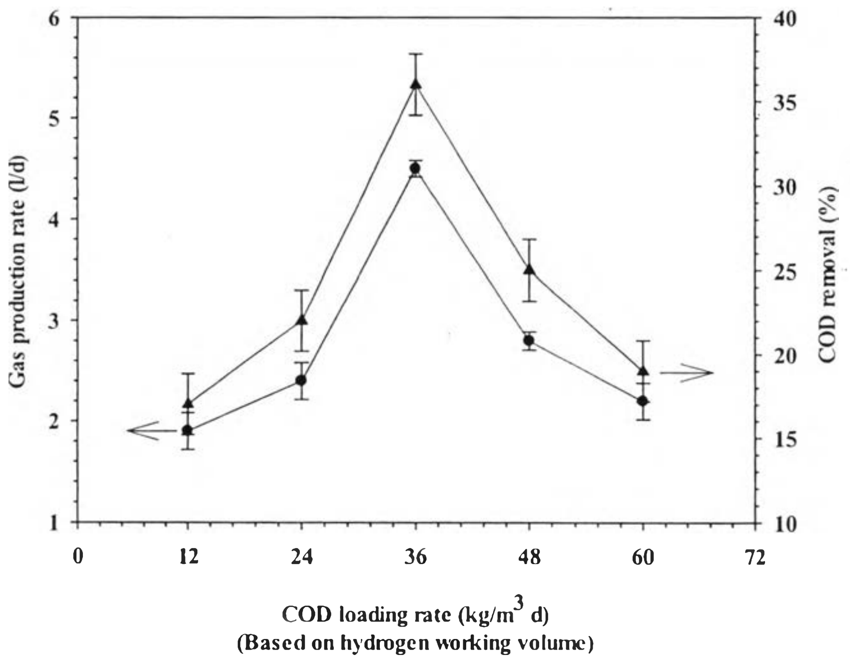

Figure 4.1 Gas production rate and COD removal as a function of COD loading rate.

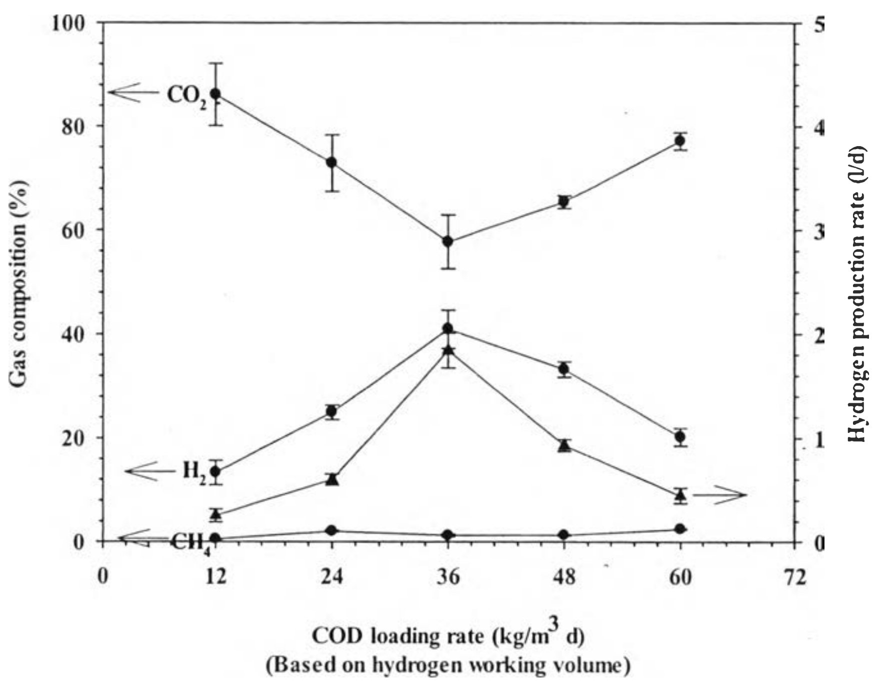

Figure 4.2 Gas composition and hydrogen production rate as a function of COD loading rate.

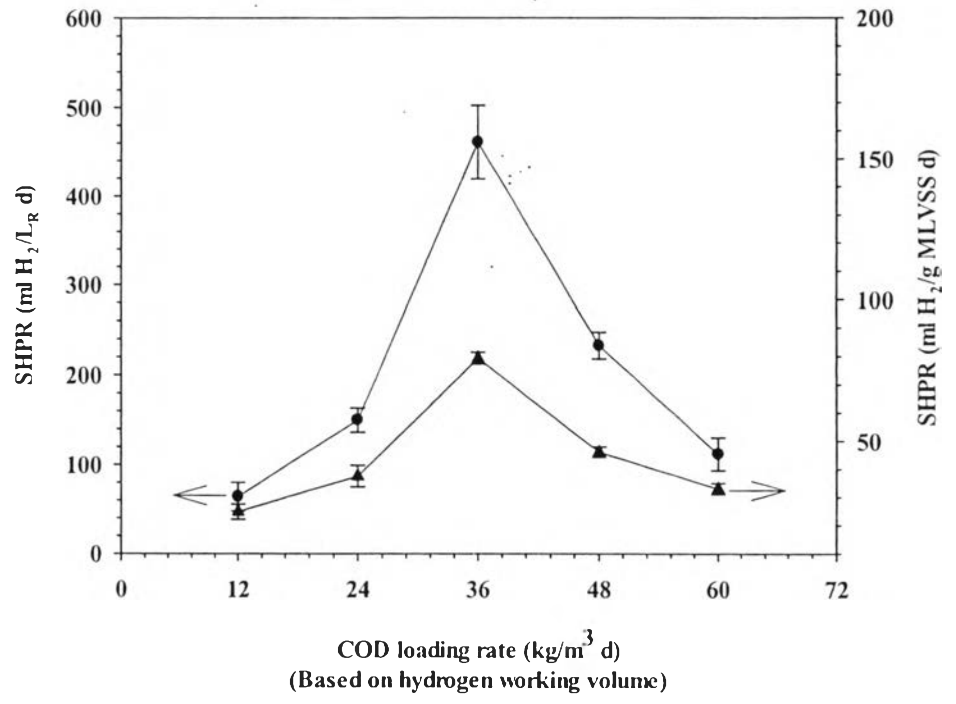

Figure 4.3 Specific hydrogen production rate as a function of COD loading rate.

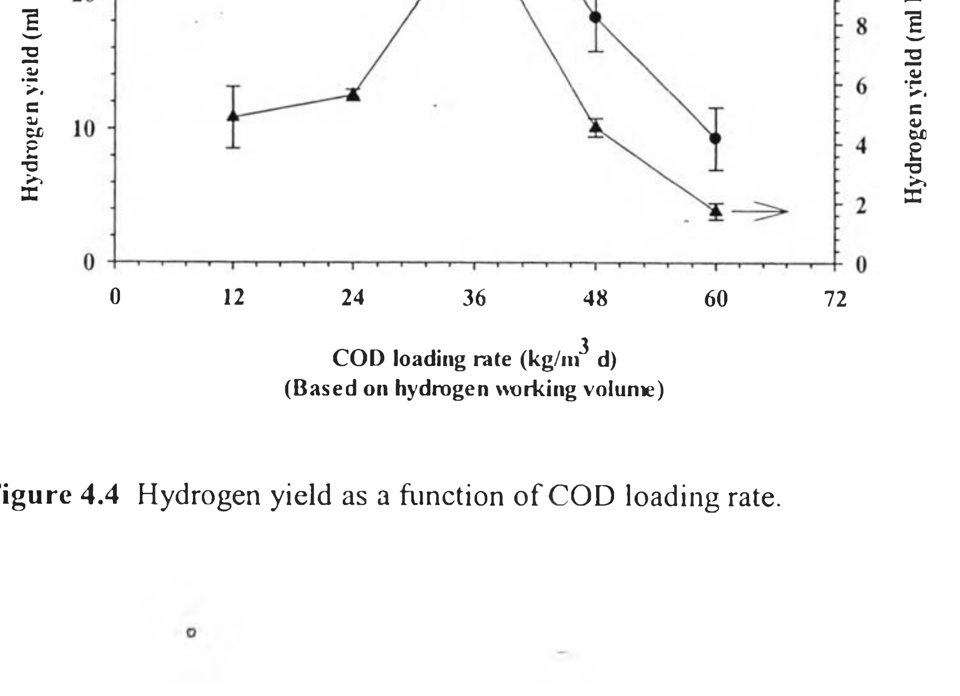

Figure 4.4 Hydrogen yield as a function of COD loading rate.

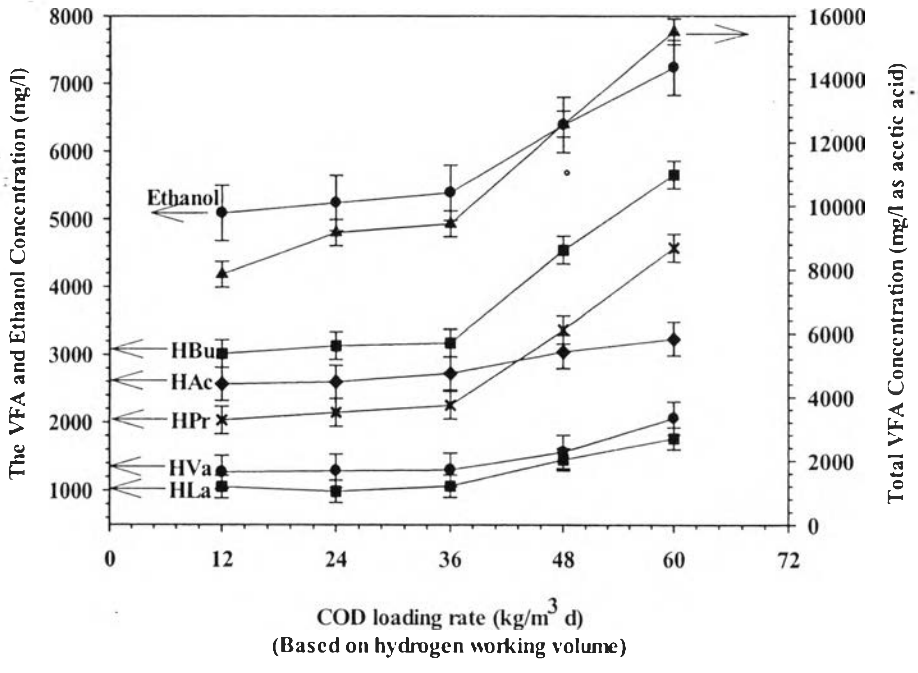

Figure 4.5 The volatile fatty acid, its composition and ethanol concentration as a function of COD loading rate.

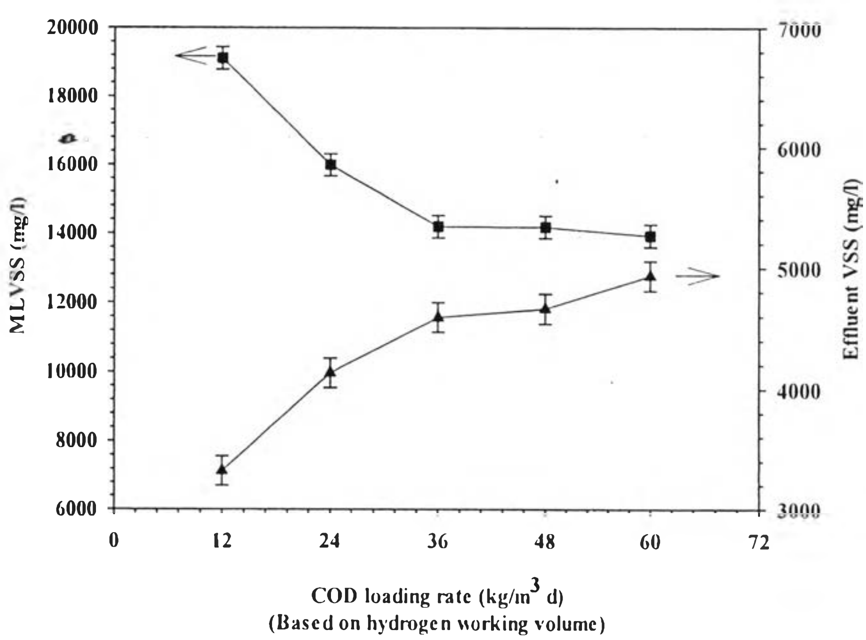

Figure 4.6 The microbial concentration and microbial washout as a function of COD loading rate.

4.2 Performance of Separate Hydrogen and Methane Production from Ethanol Wastewater: Methane Production Step

4.2.1 Gas Production Rate and COD Removal

The gas production rate and the COD removalof methane production in methane UASB unit at different COD loading rates is presented in Figure 4.7. Both the gas production rate and COD removal increased with increasing COD loading rate from 2 to 6 kg/m^3 d based on methane UASB unit to 27.5 l/d and 55 %, respectively, then decreased with further increasing COD loading rate to 10 kg/m^3 d. So, the maximum gas production rate and COD removal of 27.5 l/d and 55 %, respectively were at a COD loading rate of 6.0 kg/m^3 d based on methane UASB unit.

4.2.2 Methane Production Rate and Gas Composition

The methane production rate is calculated from the gas production rate multiplied by percentage of hydrogen. From Figure 4.8, the methane production rate increased from 1.2 to 19 l/d with an increase in COD loading rate from 2 to 6 kg/m^3 d based on methane UASB unit, then decreased with further increasing COD loading rate to 10.0 kg/m^3 d based on methane UASB unit. The maximum methane production rate was at a COD loading rate of 6.0 kg/m^3 d based on methane UASB unit. The composition of produced gas consisted mainly of methane and carbon dioxide. The methane percentage had a similar trend to COD removal. A maximum methane percentage of 69.6 % was at a COD loading of 6.0 kg/m^3 d based on methane UASB unit. Meanwhile, Carbon dioxide composition showed an opposite trend to methane content.

At COD loading rate of 6.0 kg/m^3 d based on methane UASB unit, the methane production rate (1.9 l/d) and methane content (69.6 %) were maximized. The result can be described that the hydrogen and VFA which are the main source of methane production process might cause the higher methane production. Beyond 6 kg/m^3 d based on methane UASB unit, the pH in system is too low, resulting in negative effects to methane production performance. The most suitable pH for the growth of methane-producing bacteria was reported in the range of 6.5 - 7.2 (Speece, 1983).

4.2.3 Specific Methane Production Rate

Specific methane production rate (SMPR) is defined as the methane production rate per unit weight of the microbial cells in the bioreactor (ml CH_4/g MLVSS d) or methane production rate per reactor volume (ml CH_4/L_R d). From Figure 4.9,The SMPR increased with an increase in COD loading rate and reached a maximum value of 98.0 ml CH_4/g MLVSS d (or 797.5 ml CH_4/L_R d) at a COD loading rate of 6 kg/m^3 d based on methane UASB unit. At a higher COD loading rate than 6 kg/m^3 d based on methane UASB unit, the specific methane production ratedecreased to 31.9ml CH_4/g MLVSS d (or 271.7ml CH_4/L_R d). The specific methane production exhibited a similar trend to methane composition and methane production rate.

4.2.4 Methane Yield

Methane yield is defined as the ratio of the amount of produced methane to the amount of consumed organic substrate in the unit of ml CH_4/g COD removed or the ratio of the amount of produced methane to the amount of applied organic substrate in the unit of ml CH_4/g COD applied. From Figure 4.10, methane yield increased from77.4 ml CH_4/g COD removed (or 12.5 ml CH_4/g COD applied) with an increase in COD loading rate and reached the maximum value of 163.5 ml CH_4/g COD removed (or 62.5 ml/g COD applied) at a COD loading rate of 6.0 kg/m^3 d based on methane UASB unit. Then, methane yield decreased to 67.3 ml CH_4/g COD removed (or 12.8 ml/g COD applied) with further increasing COD loading rate to 10 kg/m^3 d based on methane UASB unit.

From the results of SMPR and methane yield, the optimum COD loading rate for methane production was 6 kg/m^3 d based on methane UASB unit which maximized methane yield and SMPR, as well as methane composition.

4.2.5 The Total Volatile Fatty Acid (VFA), Its Composition and Ethanol Concentration

The effect of COD loading rate on total VFA concentration showed in Figure 4.11. The total VFA concentration slightly increased with an increase in COD loading rate to 6 kg/m^3 d based on methane UASB unit. Beyond the optimum COD loading rate, the total VFA concentration sharply increase with further increasing COD loading rate. The metabolic path way of methane generation showed in Equations (4.8-4.10) (Venkata Mohan *et al.*, 2008).

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (4.8)$$

Acetic acid

$$2CH_3CH_2OH + CO_2 \rightarrow CH_4 + 2CH_3COOH \quad (4.9)$$

Ethanol Acetic acid

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (4.10)$$

The main VFA composition in methane production was acetic and propionic acid. The VFA and ethanol produced from hydrogen production can be recovered to methane. Both VFA and ethanol concentration slightly increased with COD loading rate and reached the maximum value at an optimum COD loading rate with the pH in the range of and. After that, the VFA concentration sharply increased with a COD loading rate to 10 kg/m^3 d based on methane UASB unit which lowered the pH of system to. The suitable pH for methane production was in the range of 6.5 to 7.2 (Speece, 1983)

At an optimum COD loading rate of 6.0 kg/m^3 d, the total VFA and ethanol concentration were 226.9 mg/l as acetic acid and 79.8 mg/l, respectively.

4.2.6 The Microbial Concentration and Microbial Washout

The microbial concentration in term of MLVSS and the microbial washout in term of Effluent VSS in bioreactor are shown in Figure 4.12. The MLVSS decreased with an increased in COD loading rate from 2 to 10 kg/m^3 d based on methane UASB unit which it had an opposite trend to Effluent VSS. The results suggest that the microbial concentration decreased by washing out from the system with increasing COD loading rate because the microbes cannot tolerate to acid condition and affect to methane production performance.

From the overall experimental results on methane production, the optimum COD loading rate was suggested at 6.0 kg/m^3 d due to the highest methane content (69.6 %), methane yield (163.5 ml CH_4/g COD removed and 62.5 ml CH_4/g COD applied), methane production rate (19.1 l/d), specific methane production rate (98.1 ml CH_4/g MLVSS d and 797.5 ml CH_4/L_R d) and COD removal (55.0 %).

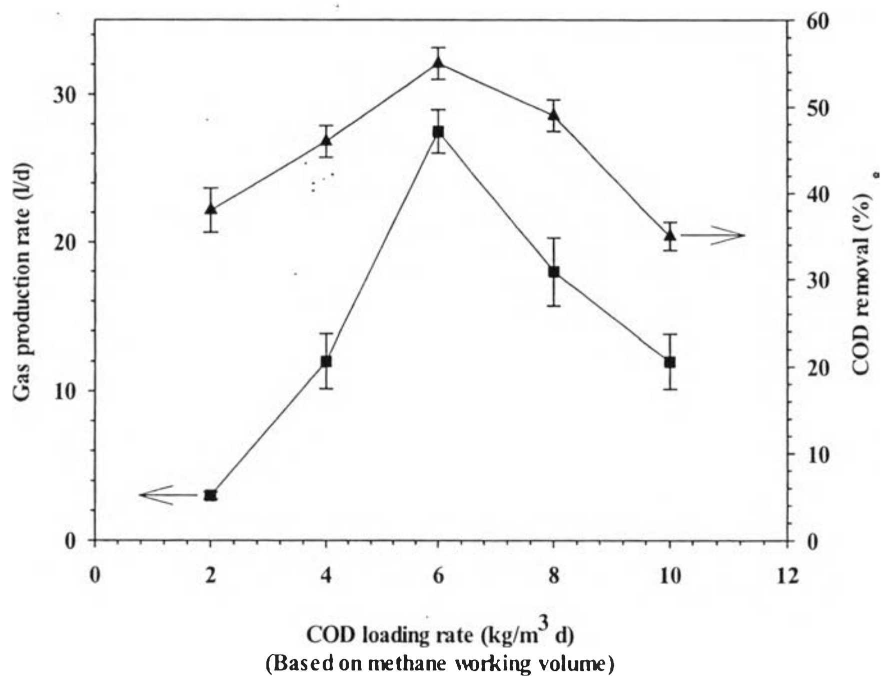

Figure 4.7 Gas production rate and COD removal as a function of COD loading rate.

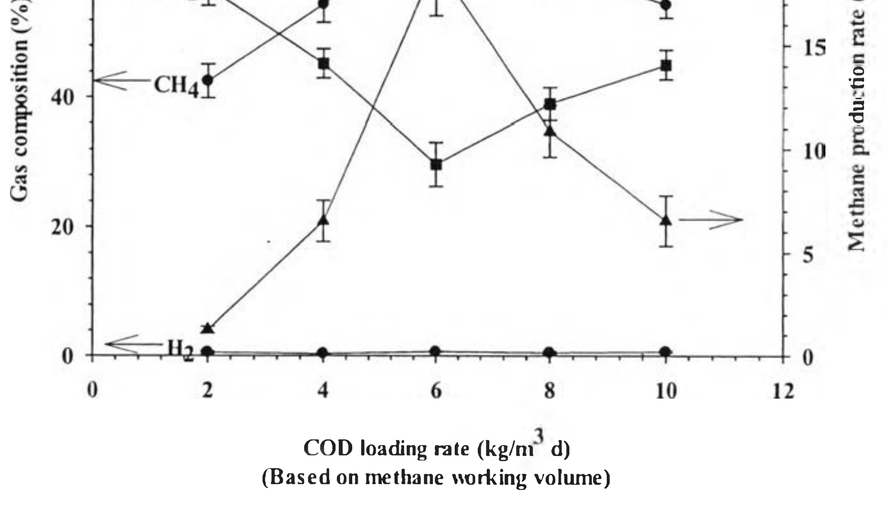

Figure 4.8 Gas composition and methane production rate as a function of COD loading rate.

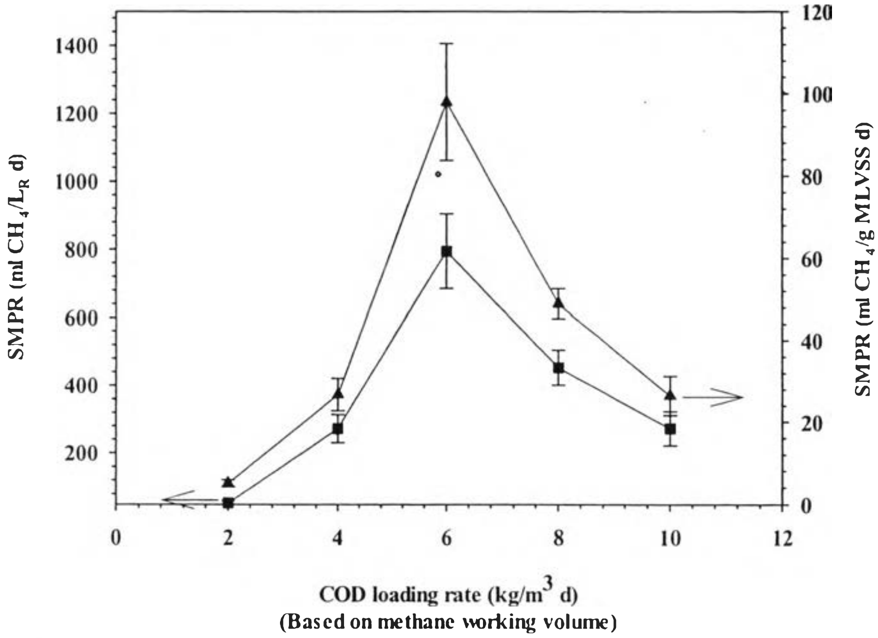

Figure 4.9 Specific methane production rate as a function of COD loading rate.

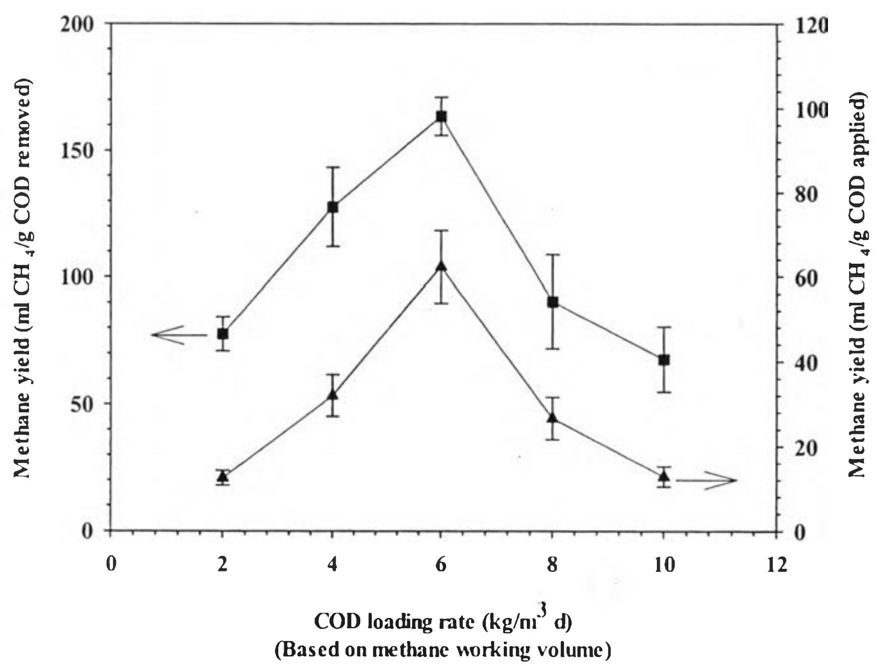

Figure 4.10 Methane yield as a function of COD loading rate.

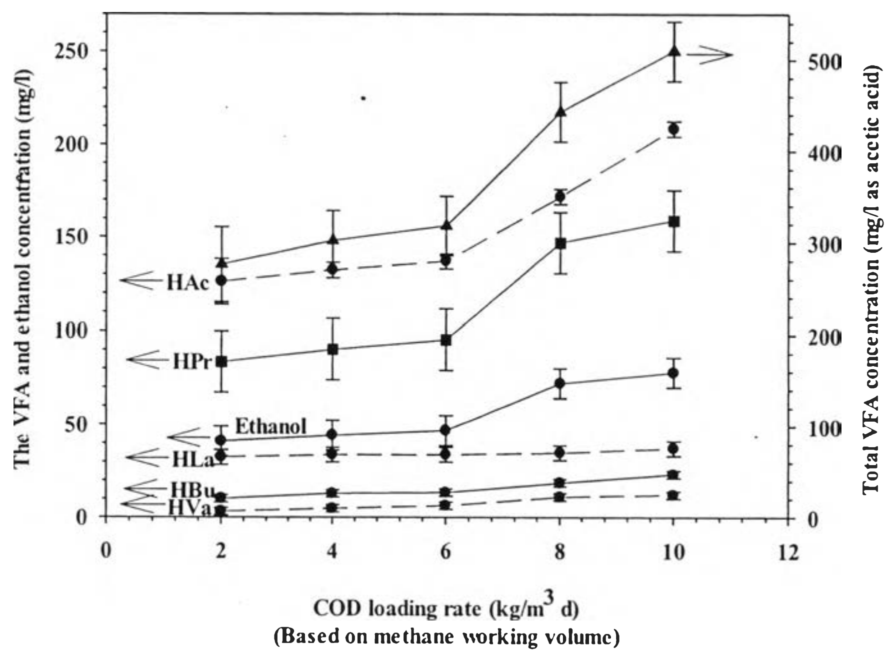

Figure 4.11 The volatile fatty acid, its composition and ethanol concentration as a function of COD loading rate.

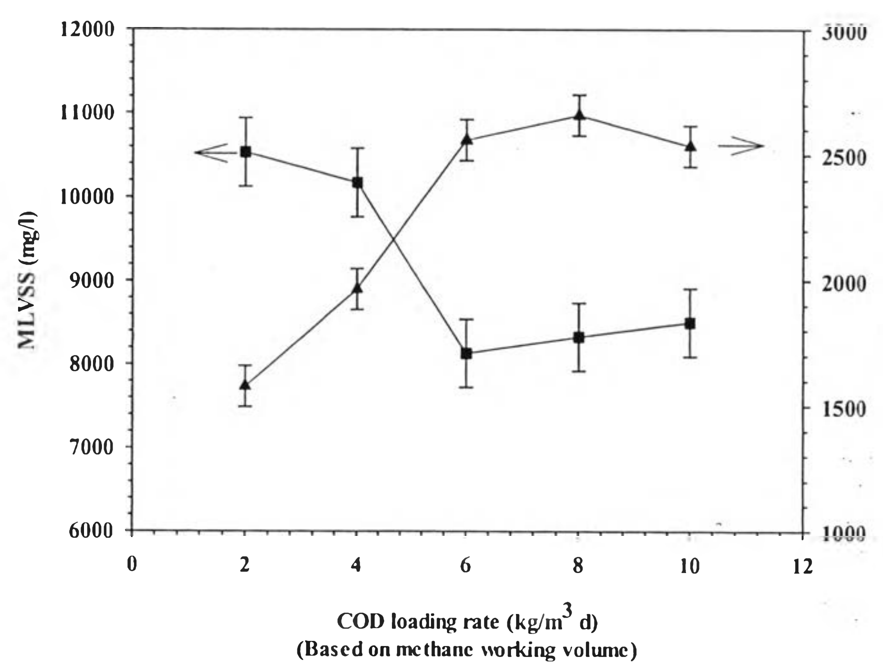

Figure 4.12 The microbial concentration and microbial washout as a function of COD loading rate.

4.3 Performance of Separate Hydrogen and Methane Production from Ethanol Wastewater: Methane Production Step under Microaeration System

4.3.1 Gas Production Rate and COD Removal

The gas production rate and COD removal as a function of COD loading rate are presented in Figure 4.13. The gas production rate increased from 27.5 to 45.54 l/d with an increase in oxygen supply load from 0 to 39 kg O_2/kg COD applied. Then, it decreased with further increasing oxygen supply load. For the COD removal, it has the similar trend to the gas production rate. The maximum COD removal of 62.4 % and the maximum gas production rate of 45.5 l/d were obtained at the oxygen supply load of 39 kg O_2/kg COD applied.

4.3.2 Methane Production Rate and Gas Composition

From Figure 4.14, the methane composition and methane production rate were increased from 69.5 to 72 % and 22.0 to 32.85 l/d, respectively, with the increase in the oxygen supply load from 0 to 4 ml O_2/LR d. However, the methane composition and methane production rate decreased with an increase in oxygen supply load from 4 to 6 ml O_2/L_R, while the large amount CO_2 (57 %) and O_2 (23 %) could be observed at the oxygen supply load of 6 ml O_2/L_R d. The result can be explained that the increase in oxygen supply load enable the facultative anaerobic bacteria activity to switch their respiration type to either aerobic or anaerobic function which improve the degradation of complex organic compound. At higher oxygen supply load (>4 6 ml O_2/L_R d) the system went to aerobic condition, resulting in higher CO_2 content.

4.3.3 Specific Methane Production Rate and Methane Yield

From Figures 4.15 and 4.16, the specific methane production rate (SMPR) and methane yield show the similar trend to methane production rate. The maximum SMPR of 1,400 ml CH_4/L_R d (or 168.5 ml CH_4/ g MLVSS d) and the maximum methane yield of 171.6 ml CH_4/g COD removed (or 107.2 ml CH4/g COD applied) was at the oxygen supply load of 4.0 ml O_2/L_R d. Therefore, the oxygen supply load of 4.0 ml O_2/L_R d is the optimum oxygen content for methane production.

In addition, 0.13 % hydrogen sulfide produced from ethanol wastewater via anaerobic digestion was eliminated to 0.0 %, using oxygen supply load of 4 ml O_2/L_R d. It can be explained that, the sulphide-oxidising bacteria converted dissolved hydrogen sulphide to elemental sulphur (S^0) (Tang *et al.*, 2009). The consumption reaction of hydrogen sulfide occurred simultaneously with anaerobic digestion reaction (Van der Zee *et al.*, 2007), was shown in Equation (4.11).

$$H_2S\text{-consuming bacteria} + H_2S + \text{Organic substrate} + O_2 \rightarrow S^0 + \text{New cells} \qquad (4.11)$$

4.3.4 The Volatile Fatty Acid (VFA), Its Composition and Ethanol Concentration

The total VFA concentration increases with the increase in the oxygen supply load and attains the maximum value of 324.6 mg/l as acetic acid at the oxygen supply load of 4.0 ml O_2/L_R d (Figures 4.16), whereas the methane production rate decreases with the increase in the oxygen supply load from 4.0 to 6.0 ml O_2/L_R d (Figures 4.14). The results indicate that, under the oxygen supply load from 0.0 to 4.0 ml O_2/L_R d, both acidogenic and acetogenic bacteria perform well. For the VFA composition, acetic acid concentration was highest, because this condition is suitable for the growth of acetogenic bacteria which was key microorganisms to convert VFA to acetic acid.

4.3.5 The Microbial Washout

The microbes washout in term of effluent volatile suspended solid (Effluent VSS) was shown in Figure 4.15. The Effluent VSS sharply increased with an increase in the oxygen supply load from 0 to 4 ml O_2/L_R d. Then, it slightly increased with further increasing oxygen supply load to 6 ml O_2/L_R d. At optimum oxygen supply load, the Effluent VSS was 2,000. The results indicate that under the microaerobic condition, facultative anaerobic bacteria could switch their respiration type to aerobic function, which promptly consumed the total supplied oxygen in the UASB unit, resulting in no effect to strict anaerobic bacteria (such as acetogenic and methanogenic bacteria). Besides, the facultative anaerobic bacteria contained a superoxide dismutase which detoxified oxygen radicals in the UASB system. Under the aerobic condition, facultative anaerobic bacteria cannot consume the total supplied oxygen in the tank due to the large amount of oxygen. Then, excess oxygen in biogas composition. (Botheju and Bakke, 2011).

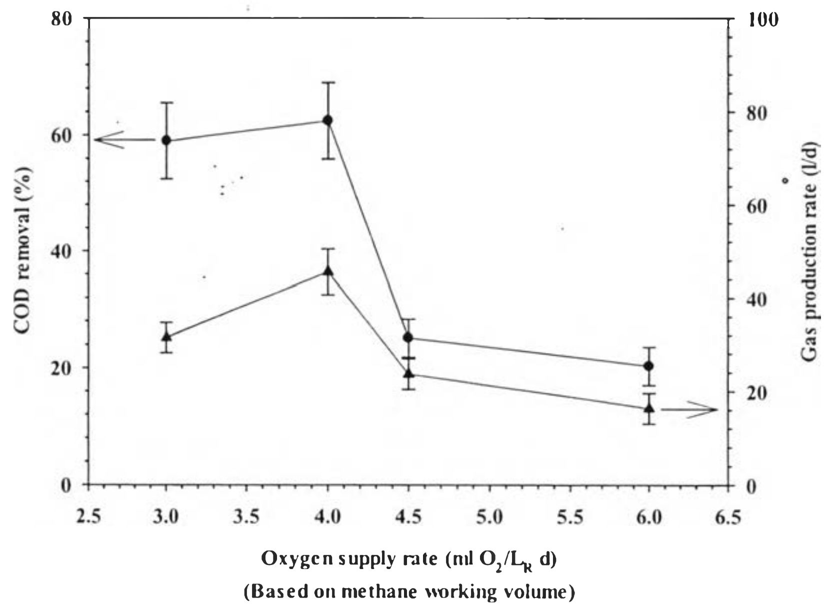

Figure 4.13 COD removal and gas production rate as a function of oxygen supply load.

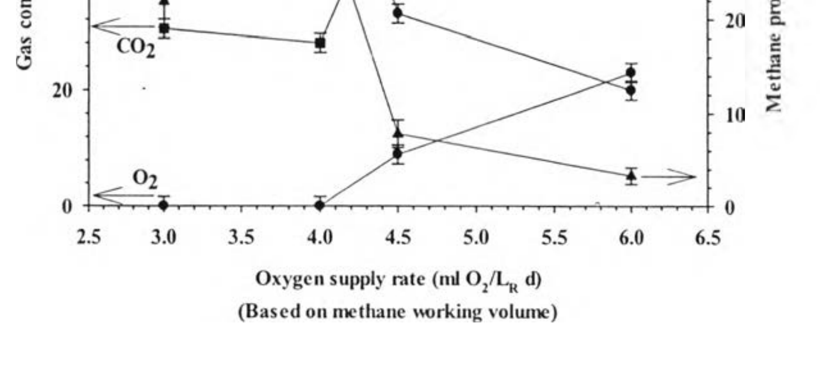

Figure 4.14 Gas composition and methane production rate as a function of oxygen supply load.

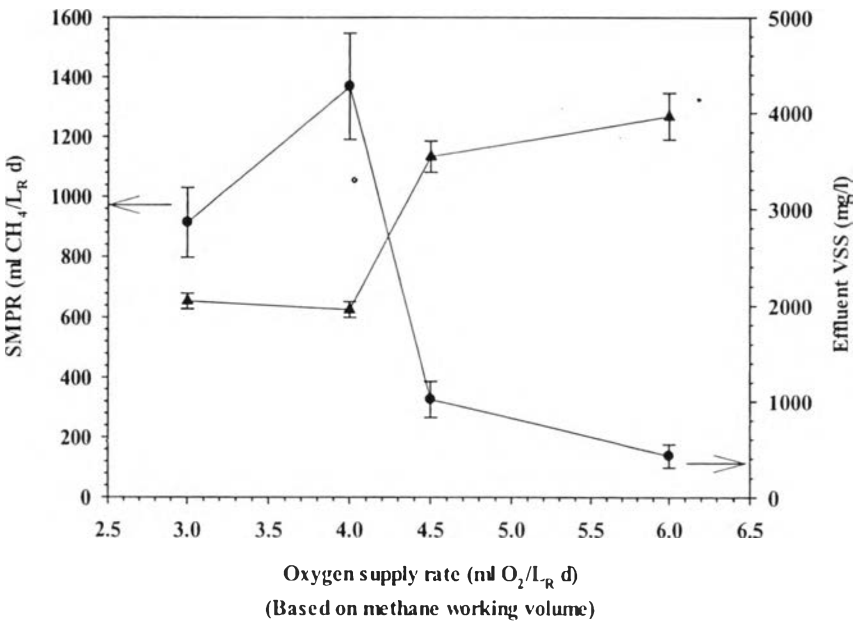

Figure 4.15 Specific methane production rate and effluent VSS as a function of oxygen supply load.

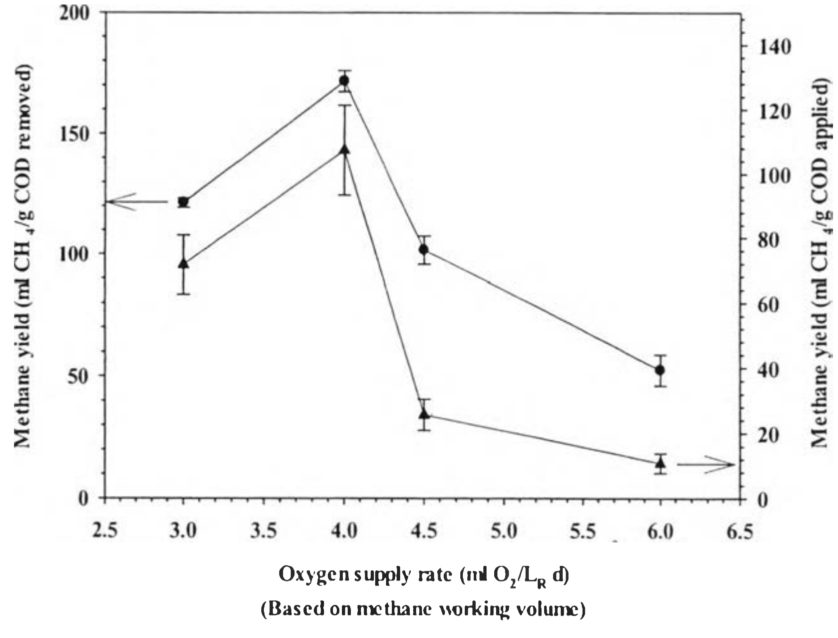

Figure 4.16 Methane yield as a function of oxygen supply load.

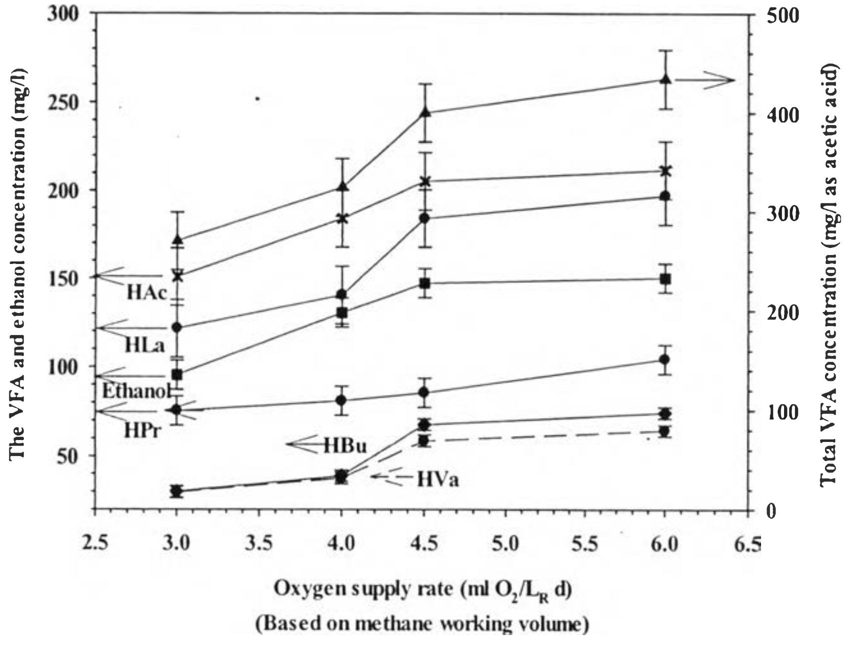

Figure 4.17 The volatile fatty acid, its composition and ethanol concentration as a function of oxygen supply load.