CHAPTER IV
# RESULTS AND DISCUSSION

4.1 Hydrogen Production Performance

For the hydrogen production process, the cassava wastewater with added cassava residue was directly fed to the hydrogen UASB unit at the initial feed COD of about 10,000 mg/l or slightly higher and COD loading rate of 72 kg/m^{3}d based on feed COD and hydrogen UASB unit without added cassava residue. The hydrogen bioreactor was operated under thermophilic temperature (55 °C) and a constant pH 5.5. The system was operated at different cassava residue concentration (300, 600, 900, 1,200 and 1,500 mg/l).

4.1.1 COD Removal and Gas Production Rate

COD removal and gas production rate as a function of cassava residue concentration are shown in Figure 4.1. The COD removal increased with increasing cassava residue concentration and reached a maximum value of 48 % at cassava residue concentration of 1,200 mg/l and then decreased with further increasing cassava residue concentration up to 1,500 mg/l. The increase in cassava residue concentration resulted in an increase in organic compounds available for microorganisms to utilize. In contrast, beyond a cassava residue concentration of 1,200 mg/l, the hydrogen production performance was decreased because of a high VFA concentration in the system which will be discussed later. For the gas production rate, it had a similar trend to the COD removal.

Total COD loading rate (kg/m^3 d)
(based on feed COD and hydrogen UASB volume)
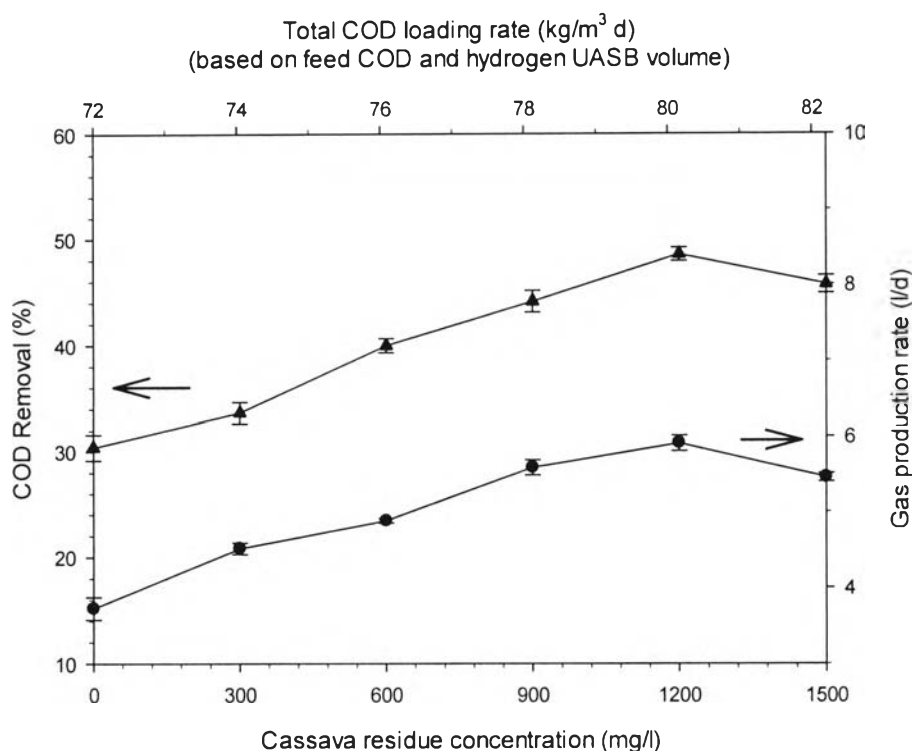
Figure 4.1 COD removal and gas production rate as a function of cassava residue concentration at 55 °C and pH 5.5.

4.1.2 Hydrogen Production Performance

Gas composition and hydrogen production rate at different cassava residue concentrations are shown in Figure 4.2. The produced gas in hydrogen UASB unit contained mainly hydrogen and carbon dioxide with a small amount of methane. Both hydrogen content and hydrogen production rate increased with increasing cassava residue concentration from 300 to 1,200 mg/l and then decreased to 38.2 % and 2.1 l/d for hydrogen content and hydrogen production rate, respectively when further increase cassava residue concentration from 1,200 to 1,500 mg/l due to the toxicity from VFA accumulation. The maximum hydrogen content (43 %) and the maximum hydrogen production rate (2.5 l/d) were found at a cassava residue concentration of 1,200 mg/l. This phenomenon can be explained with the same reason used for COD removal (Intanoo et al., 2012). For carbon dioxide, it had an opposite trend to the hydrogen content. The results suggested that an increase in

cassava residue concentration resulted in more organic compounds available for the microorganisms to convert to hydrogen.
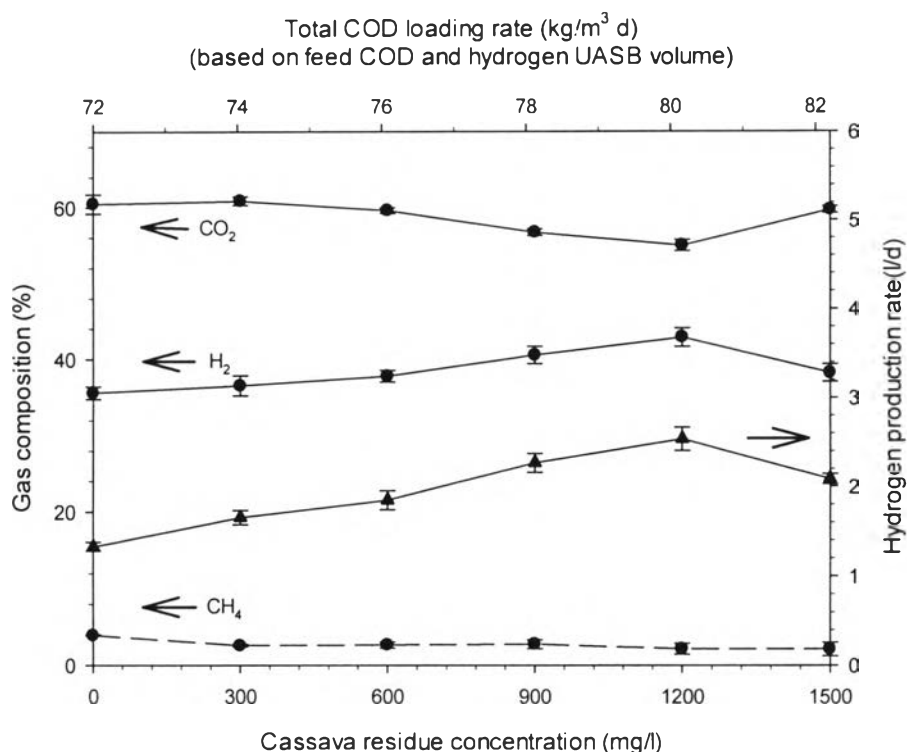

Figure 4.2 Gas composition and hydrogen production rate as a function of cassava residue concentration at 55 °C and pH 5.5.

Figures 4.3-4.4 show the specific hydrogen production rate (SHPR) and hydrogen yield, respectively. Both SHPR and hydrogen yield increased when cassava residue concentration increased from 300 to 1,200 mg/l and then decreased with further increasing cassava residue concentration from 1,200 to 1,500 mg/l. The maximum SHPR (633 ml H_2/l d or 130 ml H_2/g MLVSS d) and the maximum hydrogen yield (8 ml H_2/g COD applied or 15 ml H_2/g COD removed) were also found at the same cassava residue concentration of 1,200 mg/l. It can be suggested that at cassava residue concentration of 1,200 mg/l is the optimum for hydrogen production under the two stage UASB process at a temperature of 55 °C and constant pH 5.5.
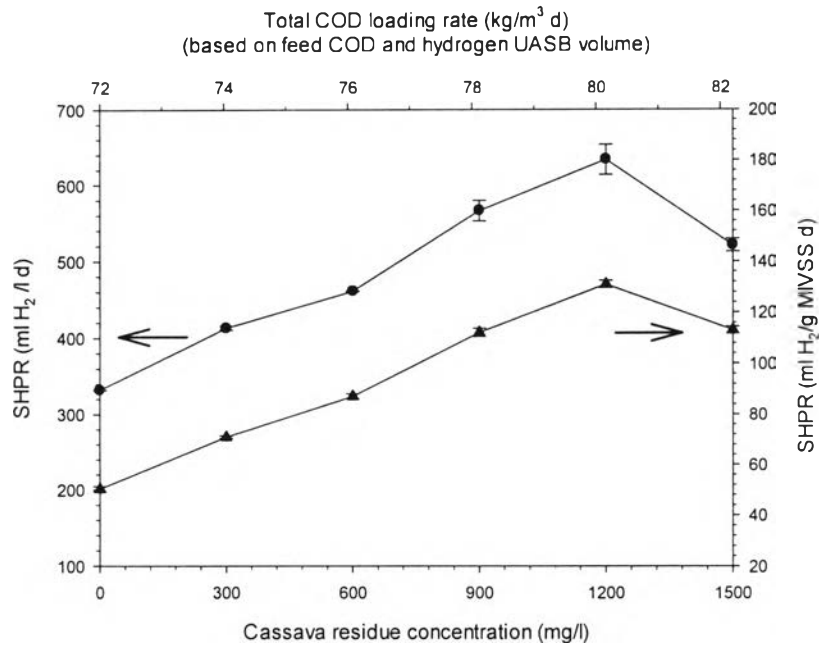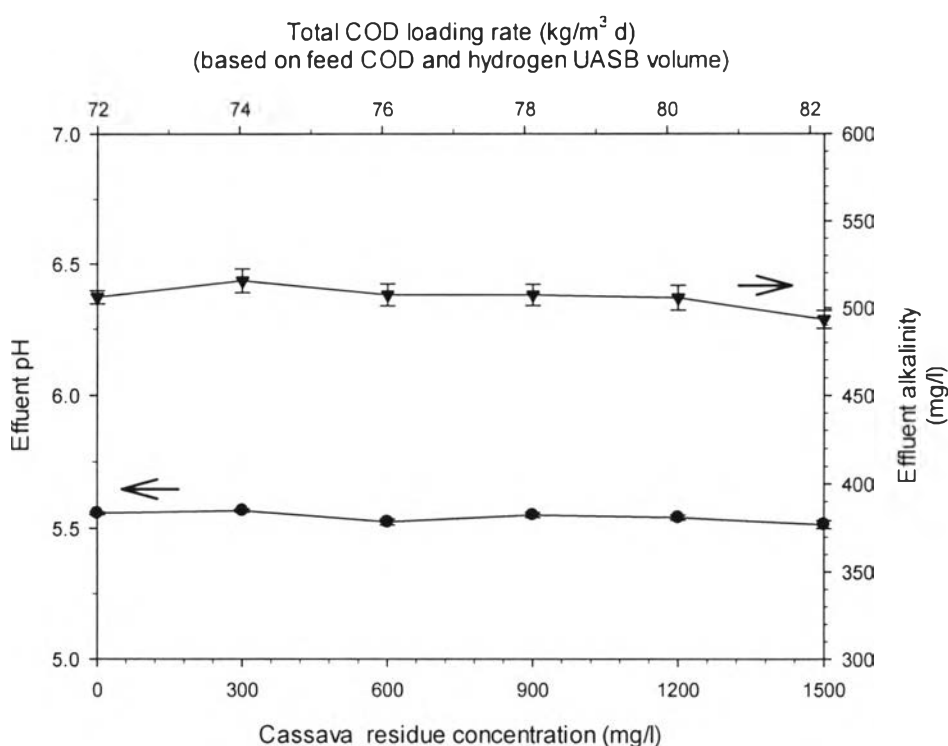
Figure 4.3 Specific hydrogen production rate (SPHR) as a function of cassava residue concentration at 55 °C and pH 5.5.
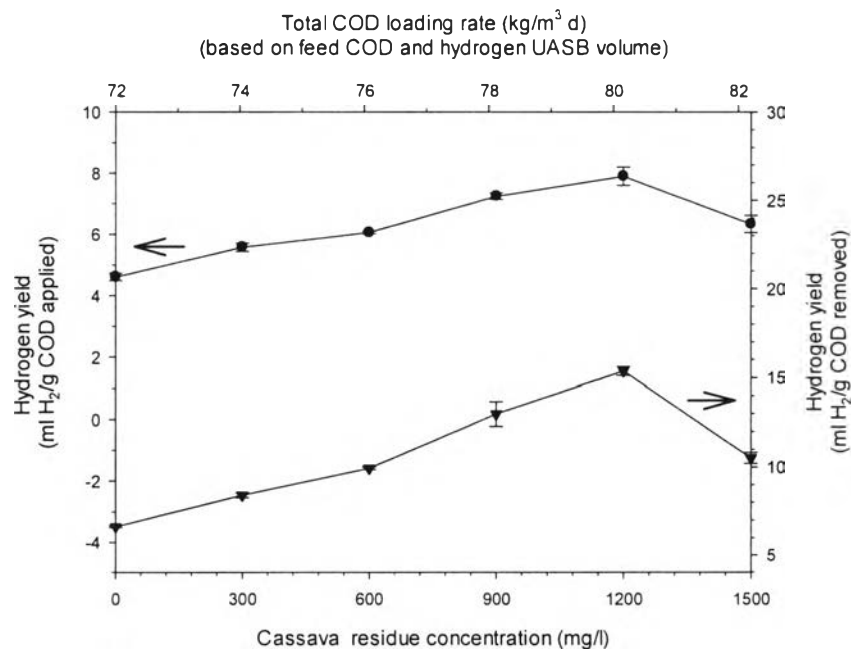
Figure 4.4 Hydrogen yield as a function of cassava residue concentration at 55 °C and pH 5.5.

Figure 4.5 shows the effluent pH and alkalinity as a function of cassava residue concentration in the hydrogen UASB unit. The effluent pH almost unchanged at any given cassava residue concentration because the pH was controlled at 5.5 by using a pH controller with a 1 M NaOH solution. The effluent alkalinity almost unchanged with increasing cassava residue concentration in the range of 300-1,200 mg/l and then slightly decreased when further increase cassava residue concentration from 1,200 to 1,500 mg/l due to the VFA accumulation in the system.

Figure 4.5 Effluent pH and alkalinity as a function of cassava residue concentration at 55 °C and pH 5.5.
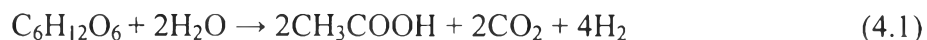
4.1.3 Volatile Fatty Acid (VFA) and VFA Composition

The total VFA concentration and its composition in the hydrogen UASB unit are shown in Figure 4.6. The total VFA concentration increased with increasing cassava residue concentration and reached a maximum value of 14,600 mg/l as acetic acid at the highest cassava residue concentration of 1,500 mg/l. When cassava residue concentration further increased from 1,200 to 1,500 mg/l, it

significantly affected to the decrease in hydrogen production performance because of the toxicity level of VFA accumulation resulting in the decrease in microbial activity (Fan *et al.,* 2006).

As shown in Figure 4.6, the main components of produced organic acid were butyric acid (HBu), valeric acid (HVa), acetic acid (HAc), and propionic acid (HPr). The organic acids which were important to the production of hydrogen are HBu and HPr. In this present work, HBu concentration was the highest while HPr concentration was the lowest consistent with a higher hydrogen production performance. It can be explained that the formation of butyric acid is the metabolic path way for the hydrogen production whereas the formation of propionic acid is the metabolic path way for hydrogen consumption, according to Equation 4.2-4.3. (Hawkes *et al.*, 2002 and Zhang *et al.*, 2006). In comparisons of process performance in terms of COD removal (Figure 4.1) and hydrogen production efficiency (Figures 4.2-4.4) and total VFA concentration profile (Figure 4.6), the toxicity level to hydrogen-producing bacteria was around 13,000 mg/l.
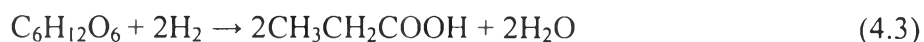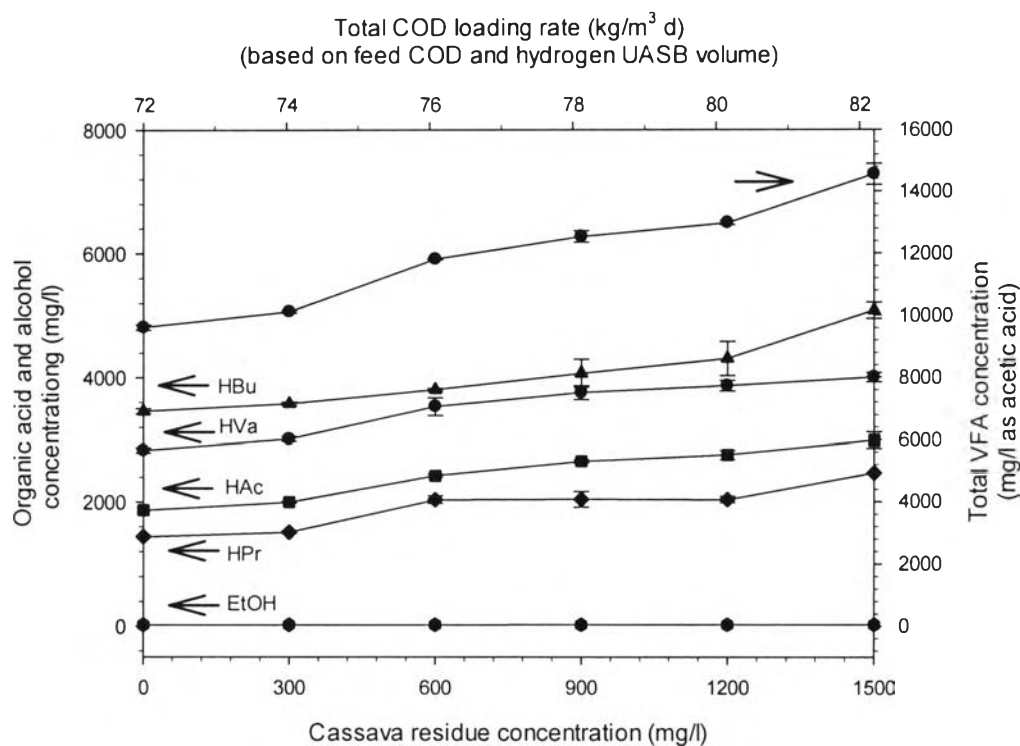
Acetic acid Production

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

Butyric acid production

$$C_6H_{12}O_6 \rightarrow CH_3(CH_2)_2COOH + 2CO_2 + 2H_2 \tag{4.2}$$

Propionic acid production

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

Total COD loading rate (kg/m³ d)
(based on feed COD and hydrogen UASB volume)

Figure 4.6 Total VFA, organic acid and alcohol concentration as a function of cassava residue concentration at 55 °C and pH 5.5.

4.2 Methane Production Performance

For the methane production process, the liquid effluent from the hydrogen UASB unit was directly fed to the methane UASB unit for further produced methane. Methane UASB unit was operated under thermophilic temperature (55 °C) without pH control.

4.2.1 COD Removal and Gas Production Rate

The COD removal as a function of cassava residue concentration is shown in Figure 3a. The COD removal increased with increasing cassava residue concentration, reached a maximum value of 76 % at a cassava residue concentration of 1,200 mg/l. After that, it decreased with further increasing cassava residue from 1,200 to 1,500 mg/l. The gas production rate also had a similar trend to the COD removal.
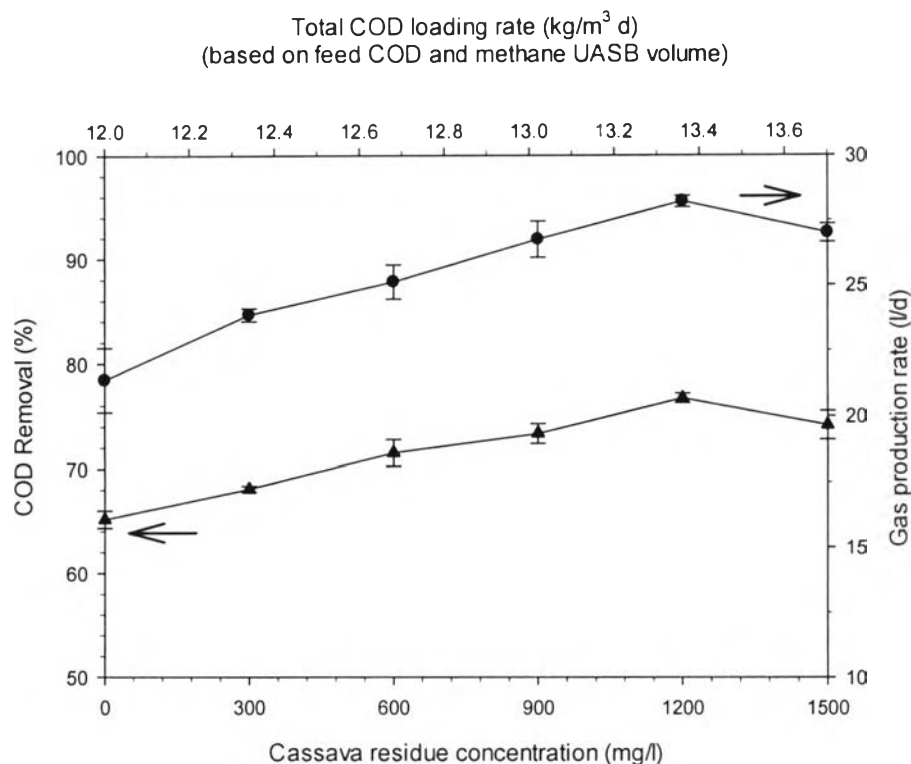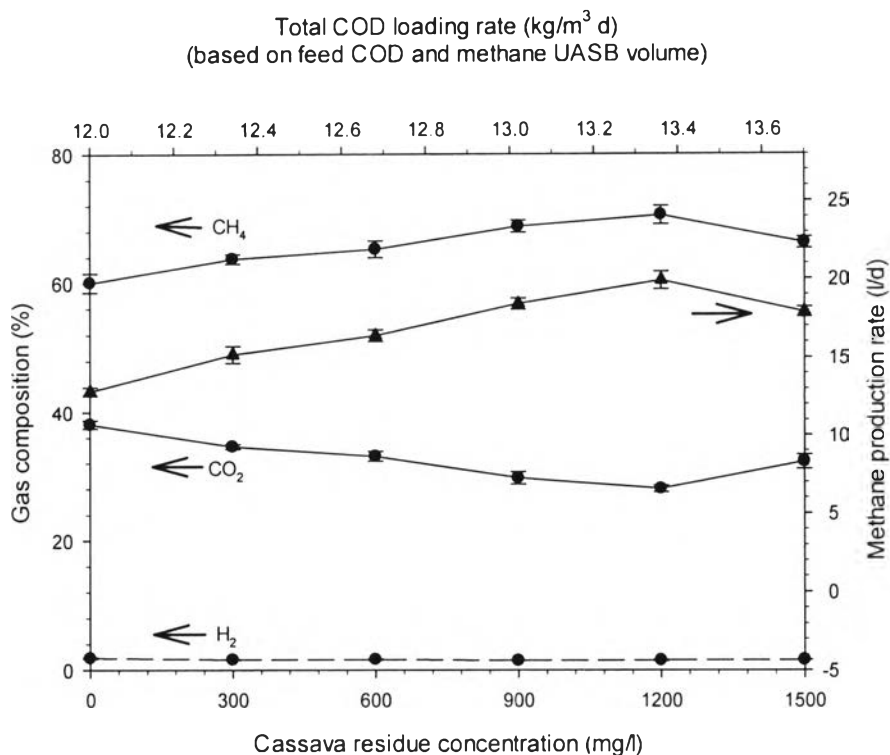
Figure 4.7 COD removal and gas production rate as a function of cassava residue concentration at 55 °C without pH control.

4.2.2 Methane Production Performance

Figure 4.8 shows the gas composition and methane production rate in methane UASB unit. The main produced gas were methane and carbon dioxide with a small amount of hydrogen (1.6 %). Both methane content and methane production rate showed a similar trend to the COD removal while that of carbon dioxide had an opposite trend. The highest methane content (70 %) and the highest methane production rate (20 l/d) were found at a cassava residue concentration of 1,200 mg/l.

Total COD loading rate (kg/m^3 d)
(based on feed COD and methane UASB volume)

Figure 4.8 Gas composition and hydrogen production rate as a function of cassava residue concentration at 55 °C without pH control.

The specific methane production rates (SMPR) and methane yields are shown in Figures 4.9-4.10, respectively. They increased with increasing the cassava residue concentration from 300 to 1,200 mg/l, and then decreased with further increasing cassava residue concentration from 1,200 to 1,500 mg/l because of the increasing toxicity of VFA accumulation. The maximum SMPR (829 ml CH_4/l d or 311 ml CH_4/g MLVSS d) and methane yield (259 ml CH_4/g COD removed or 60 ml CH_4/g COD applied) found at an optimum cassava residue concentration of 1,200 mg/l.
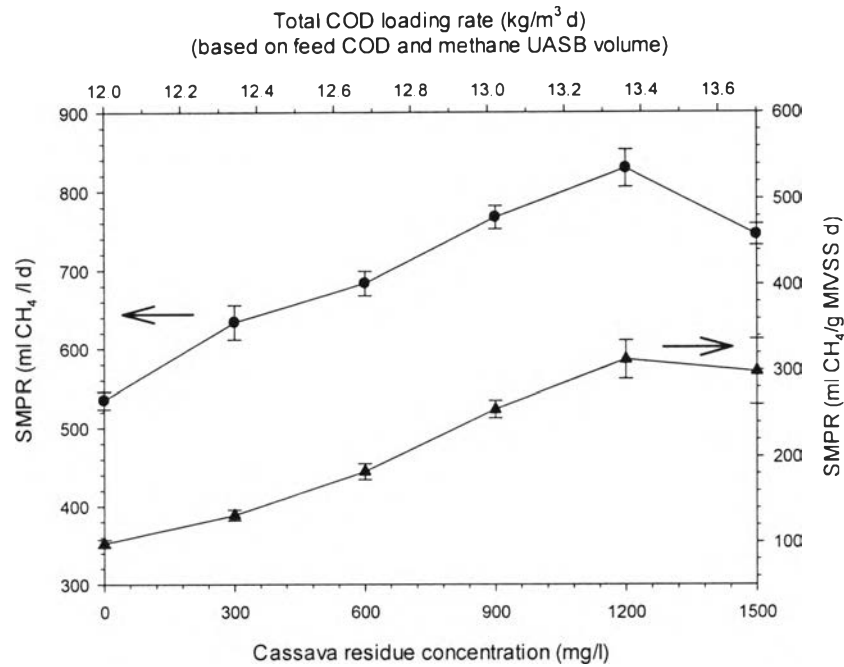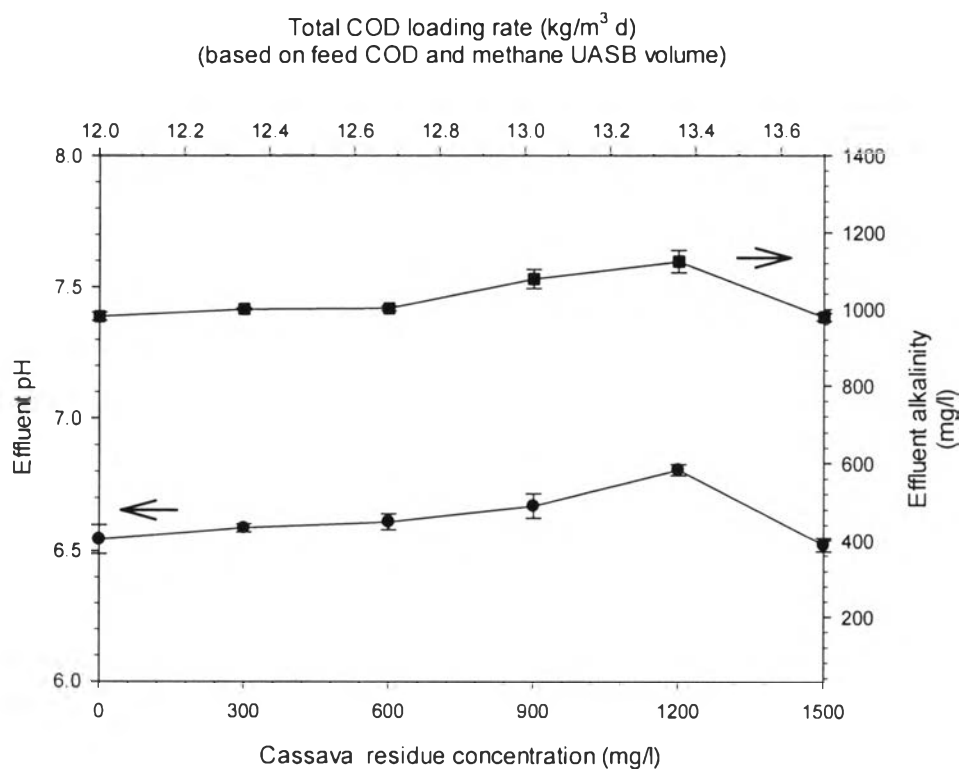
Figure 4.9 Specific methane production rate (SPMR) as a function of cassava residue concentration at 55 °C without pH control.
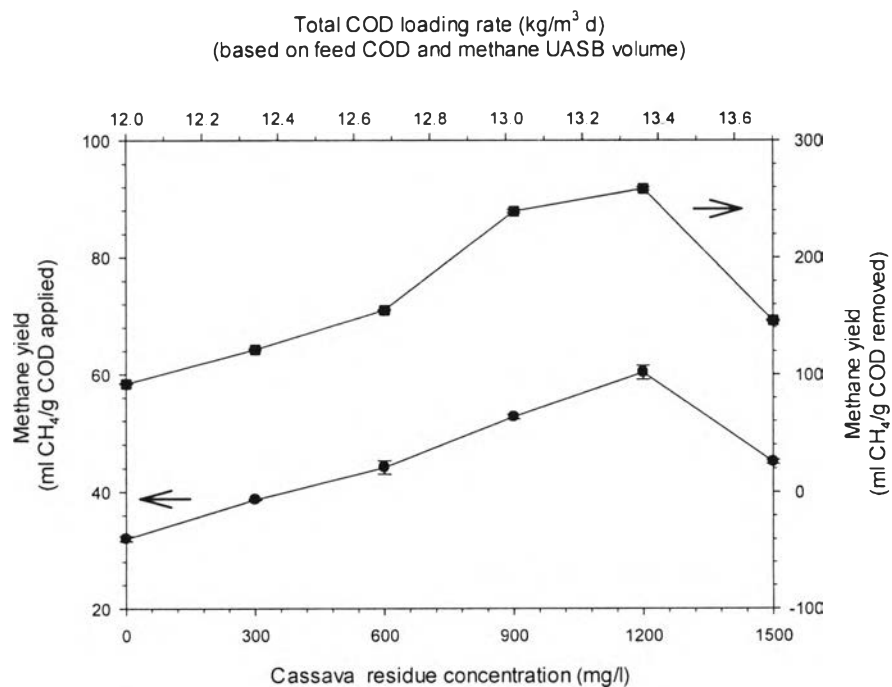
Figure 4.10 Methane yield as a function of cassava residue concentration at 55 °C without pH control.

For the methane UASB unit, the effluent pH and alkalinity increased with increasing cassava residue concentration and reached a maximum value at a cassava residue concentration of 1,200 mg/l. After that, they sharply decreased with further increasing cassava residue concentration from 1,200 to 1,500 mg/l. (Figure 4.11) It can be confirm that the increase in VFA in the system was observed, causing toxic to the methanogen activities (Chandra *et al.*, 2006).

Figure 4.11 Effluent pH and alkalinity as a function of cassava residue concentration at 55 °C without pH control.

### 4.2.3 Volatile Fatty Acid (VFA) and VFA Composition

The total VFA concentration and its composition in methane UASB unit are shown in Figure 4.12. The total VFA concentration in the methane UASB unit increased at any given cassava residue concentration and reached the maximum value of 757 mg/l as acetic acid at the highest cassava residue concentration of 1,500 mg/l. The components of produced organic acid were acetic acid, propionic acid, butyric acid, valeric acid. In this work, acetic acid concentration was the highest because propionic acid and butyric acid from hydrogen UASB unit converted to acetic acid by acetogenic microorganism (Mohan *et al.*, 2008). The methane can be produced by acetotrophic pathway, as shown in Equation 4.4. Moreover, methane can be generated via hydrogenotrophic pathway which converts hydrogen and carbon dioxdide to methane, as shown in Equation 4.5 (Abbasi *et al.*, 2012). The higher acetic acid concentration, the higher methane production performance in the methane UASB unit. However, at a very high total VFA concentration (812 mg/l as acetic acid), the system showed the lower methane production performance due to higher toxicity from VFA accumulation to microorganism and the toxic level to methane-producing bacteria is around 700 mg/l.

Acetotrophic pathway

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{4.4}$$

Hydrogenotrophic pathway

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{4.5}$$
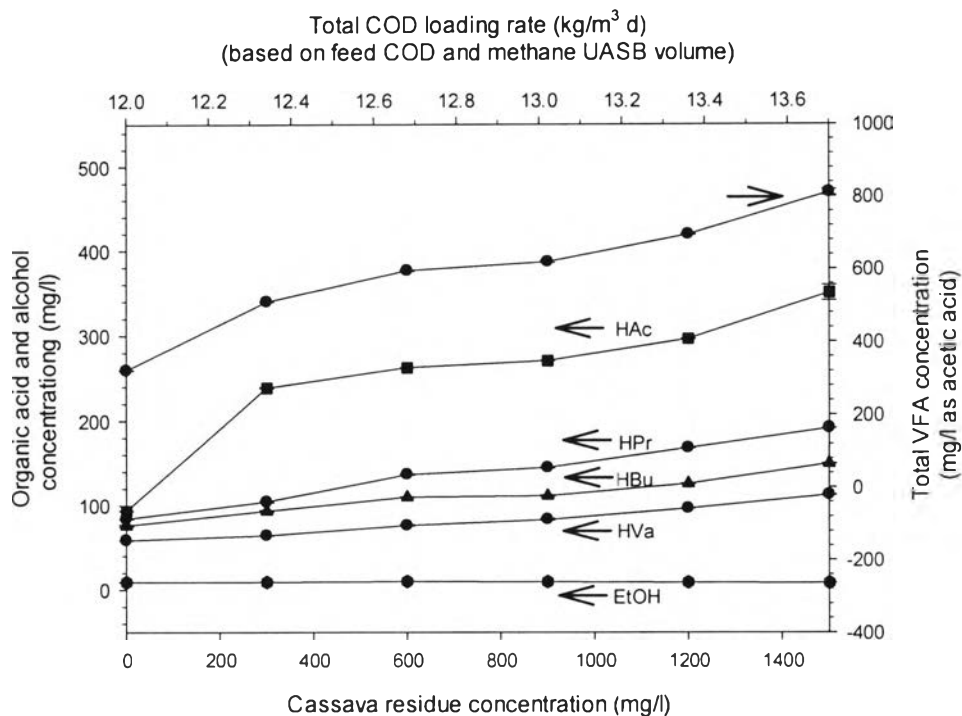
Figure 4.12 Total VFA , organic acid and alcohol concentration as a function of cassava residue concentration at 55 °C without pH control.

4.3 Microbial Concentration and Microbial Washout Results

Figure 4.13 show the microbial concentrations, along with the accumulated cassava residue in the bioreactor (MLVSS) and the microbial, together with cassava residue washout (effluent VSS) from each UASB unit. The MLVSS decreased with increasing cassava residue concentration while the effluent VSS had an opposite trend. As a result, when increase in cassava residue concentration, the microbial wash out from the system increased due to the increasing in VFA which is toxic to the microorganisms while the production performance of hydrogen (Figure 4.1-4.4) and methane (Figure 4.7-4.10) increased. It can be suggested that the microbial concentration contained mainly hydrogen-producing bacteria in the hydrogen UASB unit and methane-producing bacteria in methane UASB unit. In contrast, beyond a cassava residue concentration of 1,200 mg/l, the hydrogen and methane production

performance decreased because the large amount of the toxic level of VFA accumulation become toxic to the microorganisms.
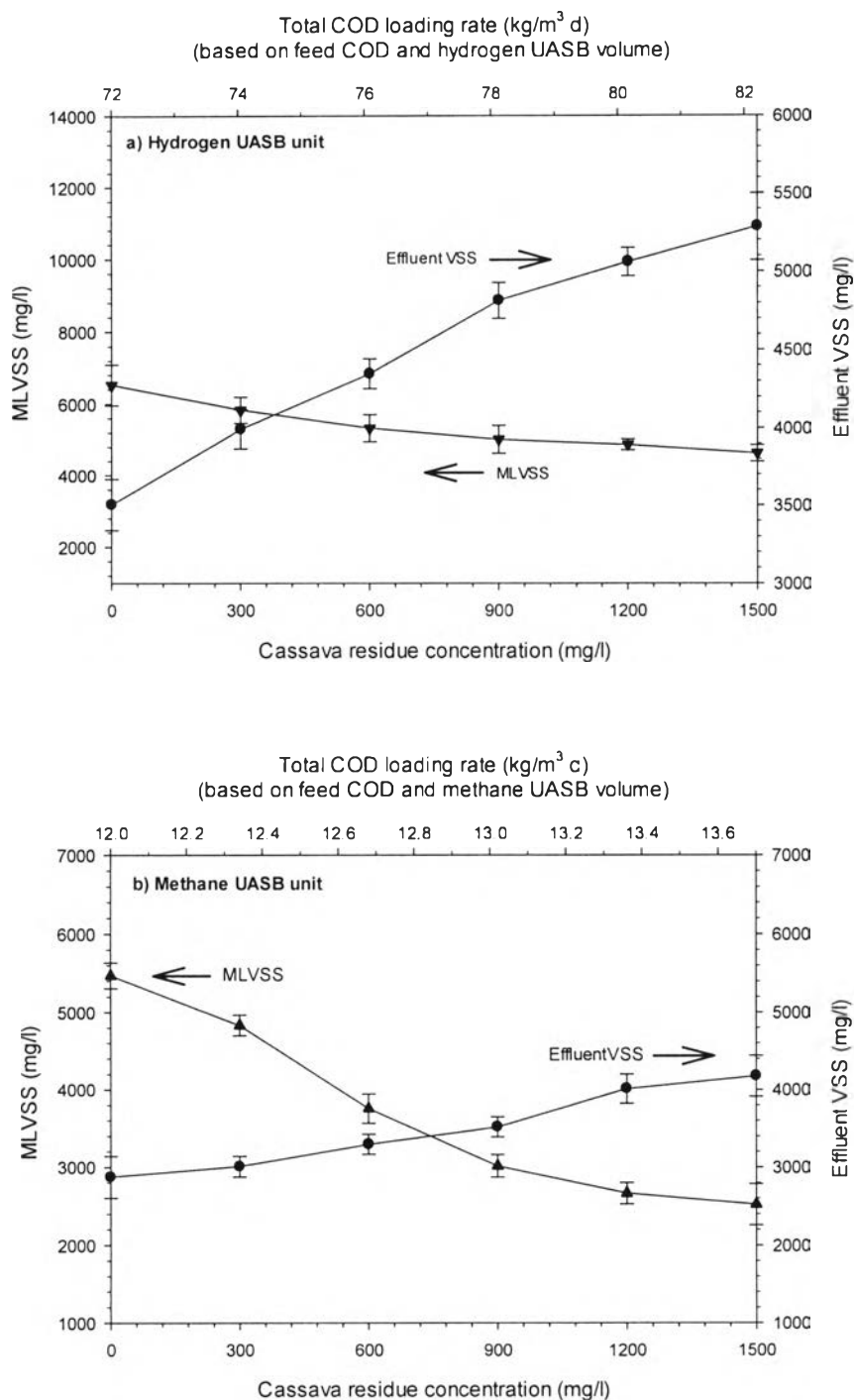
Figure 4.13 MLVSS and Effluent VSS as a function of cassava residue concentration on (a) hydrogen UASB unit, and (b) methane UASB unit.
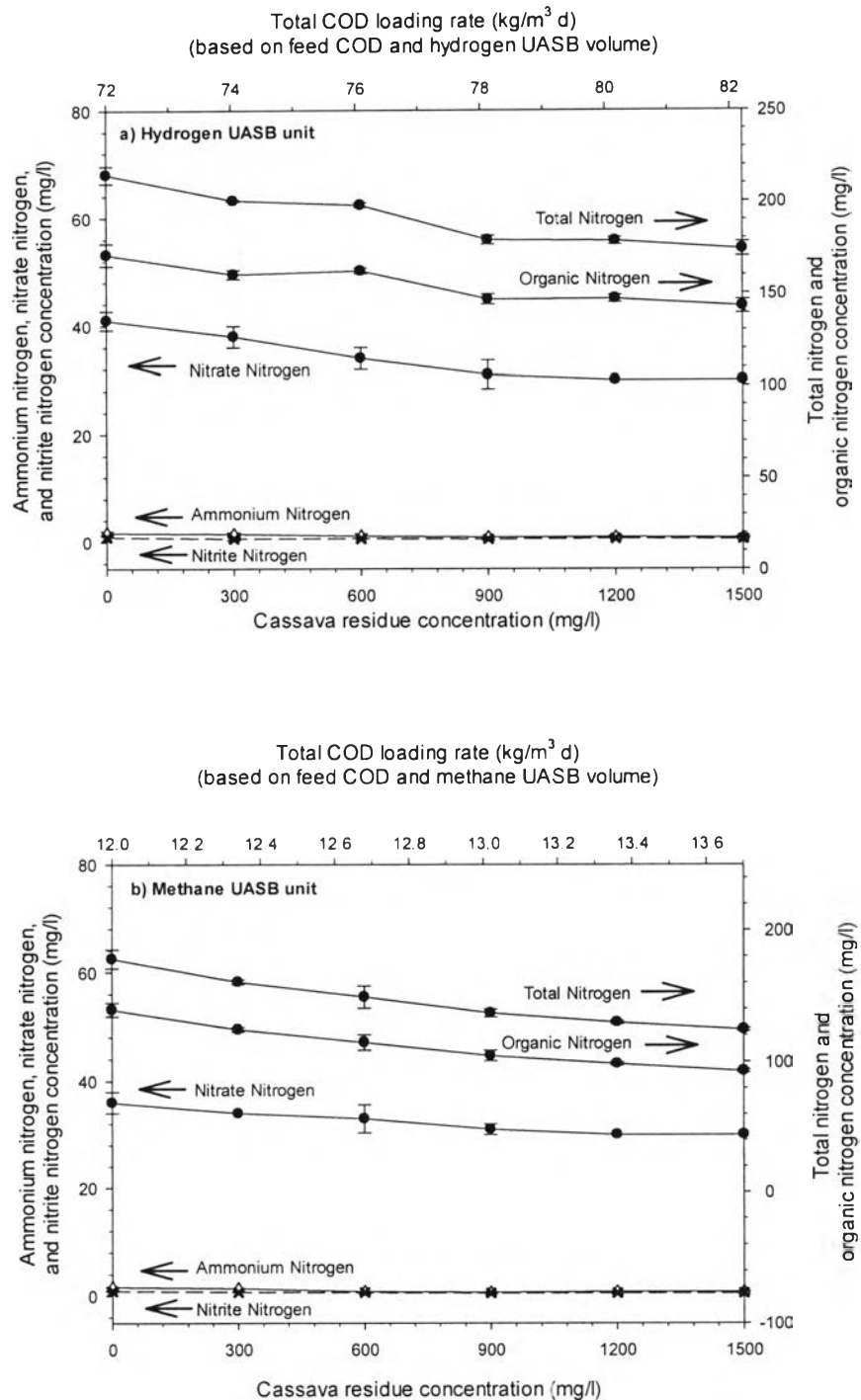
4.4 Nitrogen and Phosphorous Results

Figure 4.14 shows the nitrogen and phosphorous uptake in both hydrogen and methane reactors at different cassava residue concentrations. For any given cassava residue concentration, both nitrogen and phosphorous uptake increased. The results can be implied that hydrogen and methane-producing bacteria used nitrogen and phosphorous as nutrients for their growth. Most of the nitrogen-uptake used for growing microorganisms are organic nitrogen (Intanoo *et al.*, 2012). The concentrations of ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, organic-nitrogen, and total-nitrogen as function of cassava residue concentration are shown in Figure 4.15. In both hydrogen and methane bioreactors, the organic-nitrogen and nitrate-nitrogen concentrations decreased with increasing cassava residue concentration while ammonium-nitrogen and nitrite-nitrogen remained almost unchanged.
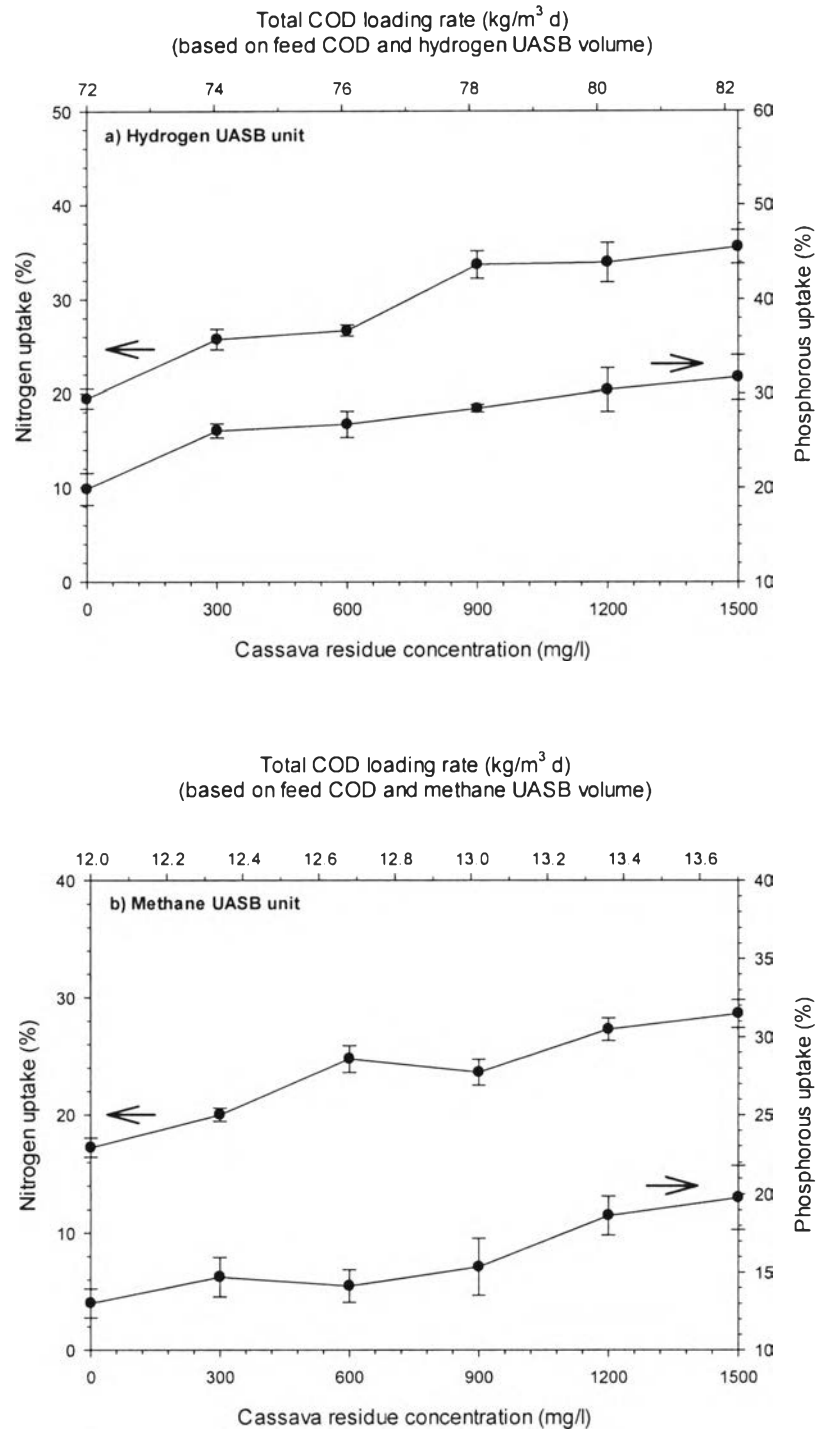
Figure 4.14 Nitrogen and phosphorous uptake as a function of cassava residue concentration on (a) hydrogen UASB unit, and (b) methane UASB unit.

Total COD loading rate (kg/m³ d)
(based on feed COD and hydrogen UASB volume)

Total COD loading rate (kg/m³ d)
(based on feed COD and methane UASB volume)

Figure 4.15 Concentrations of ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, organic-nitrogen, and total-nitrogen and as function of cassava residue concentration as a function of cassava residue concentration on (a) hydrogen UASB unit, and (b) methane UASB unit.

4.5 Digestibility Results

In both hydrogen and methane UASB unit, the digestibility of cassava residue and the microbial concentration are shown in Figure 4.16. The digestibility of cellulose, starch, and hemicellulose gradually increased from 300-1,200 mg/l. After that, it remained almost unchanged with further increasing cassava residue concentration from 1,200 to 1,500 mg/l. Surprisingly, the digestibility of starch was found to be lower than that of cellulose because anaerobic microorganisms had to hydrolyze the layer of cellulose and hemicellulose previous to the hydrolysis the layer of embedded starch by external enzymes released by microorganisms. Therefore, the digestibility of cellulose was higher than starch. For the lignin and extractive fractions, they remained almost constant because lignin and extractive compose of group of aromatic polymers, making them rigid (Vanholme *et al.*, 2010 and Teghammar, 2013). So their structures are hard to hydrolyze by anaerobic microorganisms. The results indicate that microorganisms have ability to degrade lignocellulosic materials (Nathao *et al.*, 2013). Only cellulose and hemicellulose fractions could be hydrolyzed by microorganisms under thermophilic operation. These results agree well with Magnusson et al. (2008). They found the using of mixed culture microorganism in dark fermentation process at thermophilic condition, the anaerobic microorganisms have capable to degrade organic compounds (such as cellulose and hemicellulose) to organic acids and alcohol. Finally, those organic acids and alcohol were convert to hydrogen and carbon dioxide.
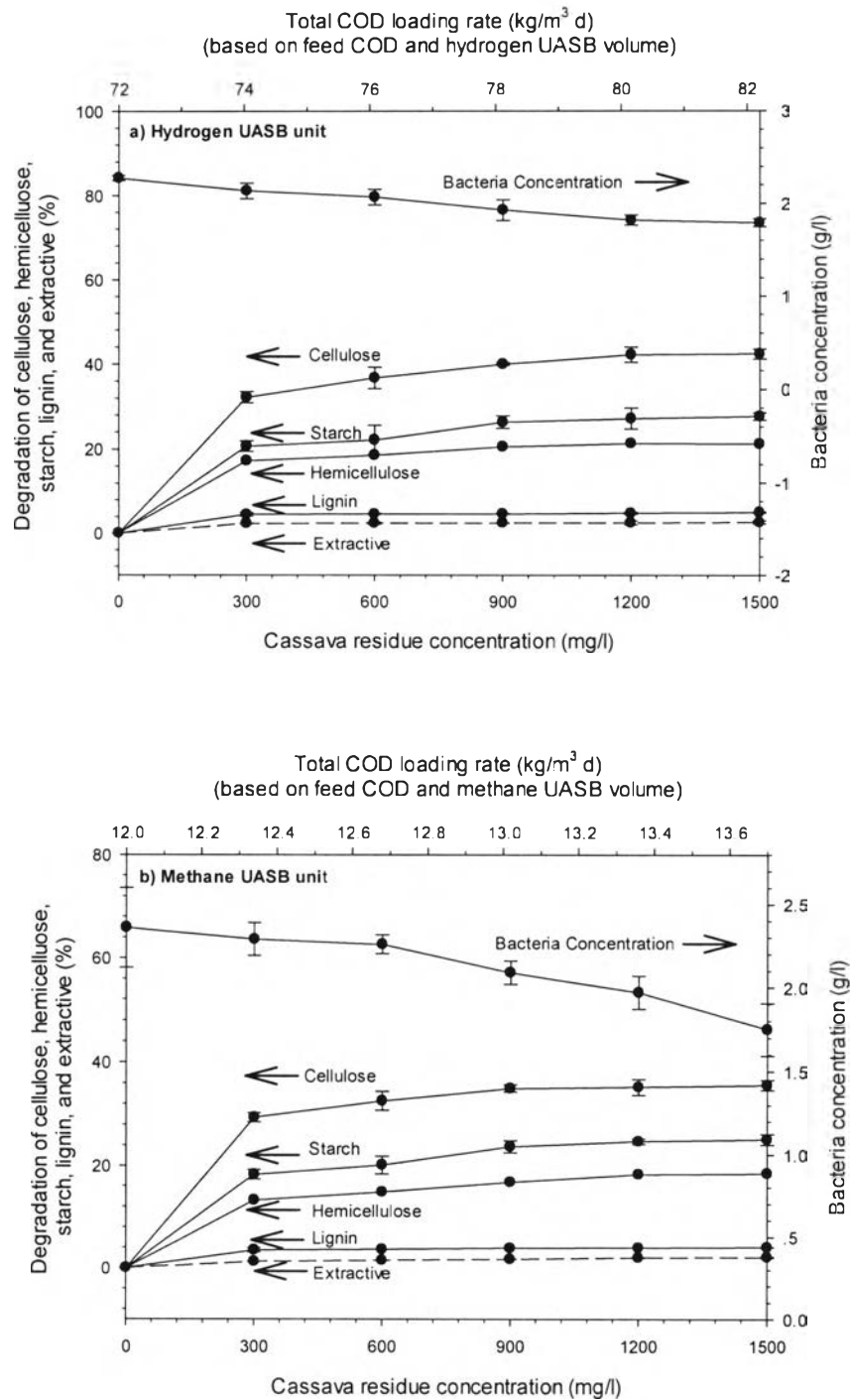
Figure 4.16 Degradation of lignocellulosic materials and bacteria concentration as a function of cassava residue concentration on (a) hydrogen UASB unit, and (b) methane UASB unit.

4.6 Overall Performance

The overall performance of two stage UASB processes operated under the thermophilic temperature is shown in Figure 4.17. From the result, it can be concluded that at the optimum cassava residue concentration of 1,200 mg/l, the total COD removal efficiency was 88 % and the total gas production rate was 34 l/d. The mixed gas contents 8.7 % H_2, 58.6 % CH_4, and 32.7 % CO_2. The total methane production rate was 20 l/d, giving a total methane yield of 260 ml CH_4/g COD removed or 61 ml CH_4/g COD applied and a total specific methane production rate of 715 ml CH_4/l d at the optimum cassava residue concentration of 1,200 mg/l. For hydrogen production result, the total hydrogen production rate was 3 l/d, giving a total hydrogen yield of 18 ml H_2/g COD removed or 8 ml H_2/g COD applied and a total specific hydrogen production rate of 106 ml H_2/l d at the cassava residue concentration of 1,200 mg/l. The degradation of lignocellulosic materaials in overall two stage system was also investigated. Under study condition, the degradation performance of cellulose and hemicellulose were 62.5 % and 35 %, respectively while the degradation of starch was 45 %. The cellulose degradation in this study is slightly higher than 59 % cellulose degradation reported by Gadow et al. (2012), which is operated under CSTR reactor at 55 °C using cellulose as substrate.
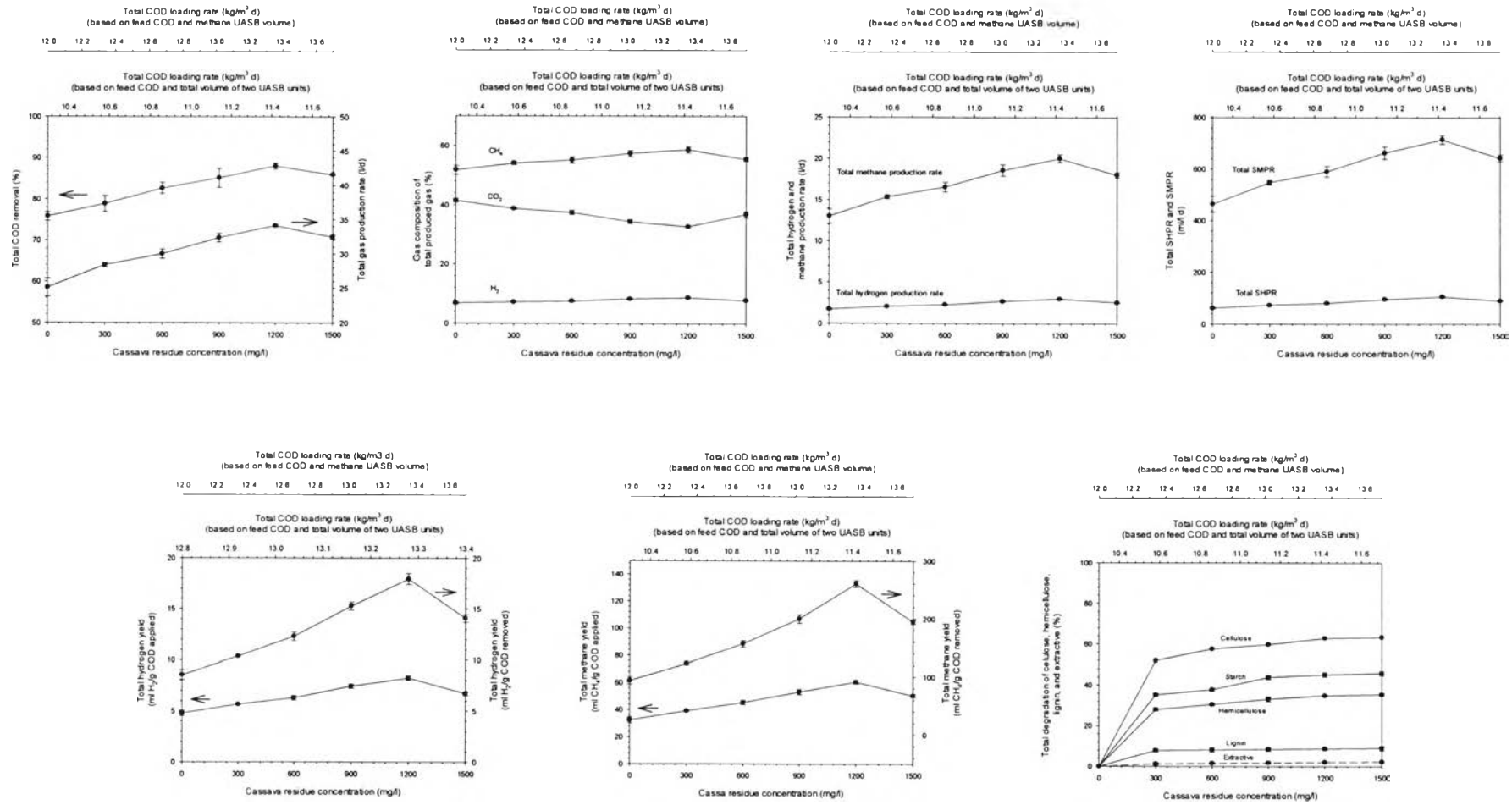
Figure 4.17 Overall performance of two stage UASB processes.