CHAPTER IV
HYDROGEN PRODUCTION FROM ALCOHOL WASTEWATER BY AN ANAEROBIC SEQUENCING BATCH REACTOR UNDER THERMOPHILIC OPERATION: NITROGEN AND PHOSPHOROUS UPTAKES AND TRANSFORMATION

4.1 Abstract

The objective of this study was to investigate hydrogen production from alcohol wastewater using an anaerobic sequencing batch reactor (ASBR) under thermophilic operation and at a constant pH of 5.5. Under the optimum COD loading rate of 68 kg/m^3d, the produced gas contained 43 % H_2 and the system provided a hydrogen yield and specific hydrogen production rate of 130 ml H_2/g COD removed and 2,100 ml H_2/l d, respectively, which were much higher than those obtained under the mesophilic operation. Under thermophilic operation, both nitrogen and phosphate uptakes were minimal at the optimum COD loading rate for hydrogen production and most nitrogen uptake was derived from organic nitrogen. Under the thermophilic operation for hydrogen production, the nutrient requirement in terms of COD:N:P was found to be 100:6:0.5, which was much higher than that for the methenogenic step for methane production.

Keywords: Hydrogen production; Alcohol wastewater; Anaerobic sequencing batch reactor (ASBR); Thermophilic operation; Optimum condition

4.2 Introduction

A shortage of energy seems to be coming in the near future due to continued over consumption of fossil fuels. Therefore, renewable energy sources are being considered as alternatives to replace the dwindling fossil fuels supplies. Among alternative fuels, hydrogen produced from wastewater via dark fermentation is the most interesting alternative fuel because it is a clean fuel (only water is produced in

the combustion process without carbon dioxide effluent) and it also gives a high-energy yield. Hence, hydrogen has been suggested as a future fuel [1]. Additionally, hydrogen can be used to generate electricity through fuel cells [2].

Hydrogen can be produced in several ways: steam reforming of natural gas, thermal cracking of natural gas, pyrolysis or gasification of biomass, and electrolysis of water. All of these processes require high temperatures and/or high pressure for operation, leading to a high production cost. Moreover, they are not environmentally friendly [3-6] and there is a risky of explosion during operation. A better way to -produce hydrogen is by a biological process because it can be operated under ambient conditions [1]. The biological hydrogen production process can be classified into 2 types: dark and photo fermentation. The dark fermentation process is more favorable due to its constant production of hydrogen without the need of light. Various raw materials, especially wastewater, have been widely used as a substrate in hydrogen production. Cassava wastewater [7], food waste [8], starch wastewater [9], wheat powder solution [10], and industrial wastes [11] are source of the materials currently used. Sreethawong et al., [7] studied hydrogen production from cassava wastewater and achieved a specific hydrogen production rate (SHPR) of 388 ml H_2/g VSS d and a hydrogen yield of 158 l/COD removed. Furthermore, there are some parameters, such as temperature, controlled pH, organic loading rate, cycle duration, hydraulic retention time (HRT), and toxic compound present in an incoming wastewater that have been considered to affect hydrogen production performance. Shin et al., [8] found that hydrogen production from food waste under thermophilic conditions was higher than that under mesophilic conditions. Another study showed the pH-dependency for hydrogen production from cassava starch, with suitable pH level (5.5 and 6.0) that displayed better hydrogen production performances and produced a better environment for the cells to utilize starch for growth [12].

In this present work, alcohol wastewater was used to produce hydrogen using an anaerobic sequencing batch reactor (ASBR) under thermophilic operation. The uptakes and transformation of nitrogen compounds and phosphate were also investigated at different COD loading rates.

4.3 Materials and Methods

4.3.1 Seed Sludge Preparation

Seed sludge collected from the biogas plant at Sapthip Lopburi Co., Ltd., Thailand, was first concentrated by sedimentation, and the concentrated sludge was then ground and screened by sieving to remove large sand particles. In order to eliminate methane-producing bacteria or hydrogen consumers, the seed sludge was boiled for 15 min [10,13-17]. The heat-treated sludge was then added to an anaerobic sequencing batch reactor (ASBR). The microbial concentration in terms of MLVSS (mixed liquid volatile suspended solids) for a start-up in this study was about 20,000 mg/l.

4.3.2 Alcohol Wastewater

Alcohol wastewater was also obtained from Sapthip Lopburi Co., Ltd., Thailand, where cassava is used as a raw material for alcohol fermentation. It was filtered through a 0.2 mm sieve to remove any large, solid particles before use. The alcohol wastewater had a chemical oxygen demand (COD) value of 60,000 mg/l and a COD nitrogen and phosphorous ratio of 100:7:1.3, indicating that the wastewater contained sufficient amounts of both nutrients (N and P) for anaerobic degradation (the theoretical ratio of COD:N:P = 100:1:0.4 for anaerobic decomposition for biogas production) (Table 4.1). Therefore, an addition of nutrients was not required in this study. Interestingly, most of the nitrogen in the wastewater was in the form of organic nitrogen with a significant amount of nitrate nitrogen. The low pH (3.4) of the alcohol wastewater resulted from a high volatile fatty acid (VFA) concentration (5,080 mg/l as acetic acid).

4.3.3 ASBR Operation

Two identical units of anaerobic sequencing batch reactors (ASBR) were used independently to perform hydrogen production at different COD loading rates. The possibilities of photosynthesis bacteria activity was inhibited by using PVC material in the construction of the bioreactors. Each of reactors had an inner diameter of 13 cm and a height of 30 cm. The bioreactors were operated with a working volume of 4 L. The experimental set-up of the studied ASBR was given

elsewhere [7]. The ASBR operation consisted of 4 steps: feeding, reacting, settling, and decanting. Each step was controlled by timers. First, for the feed step, the alcohol wastewater was introduced into the top of the reactor. A feed pump was controlled by a level probe inside the reactor to achieve a constant feed volume for each cycle. Next, the reaction step was proceeded by using a magnetic stirrer at 400 rpm to achieve homogenously mixing. A heater (equipped with thermocouple) and a pH-controller (with a dosing pump for a 1 M NaOH solution) were used to maintain a constant temperature and solution pH in the ASBR, respectively. In this work, the ASBR reactors were operated at a temperature of 55 °C and solution pH of 5.5 [12,18]. An initial quantity of 1000 ml of heat-treated sludge was added to each of the ASBR reactors. The ASBR operation times of four sequential steps at 6 cycles per day were 15, 90, 120, and 15 min for feeding, reacting, settling. and decanting, respectively. The 6 cycles per day were used to operate the ASBR because it had been proven previously to provide the highest hydrogen production performance [7]. Table 4.2 shows the flow rates of either feed or decant at different COD loading rates. The ASBR reactor was operated around two weeks to reach a steady state before taking effluent and produced gas samples for analysis and measurement. Steady state conditions were attained when both effluent COD and gas production rates did not change with time.

Table 4.1 Characteristics of the alcohol wastewater samples

Parameter	Unit	Value
pH	-	3.4
COD	mg/l	60,000
Total VFA	mg/l	5,080
Ethanol concentration	mg/l	3,120
Total solids (TS)	mg/l	10,000
Total phosphorous	mg/l	800
Total nitrogen	mg/l	4,400
Ammonium (NH_4^+-N)	mg/l	70
Nitrate (NO_3^--N)	mg/l	400
Nitrite (NO_2^--N)	mg/l	2
COD:N:P	-	100:7:1.3

4.3.4 Measurements and Analytical Methods

The gas production rate was measured by using a wet gas meter (Ritter, TGO5/5). The gas composition was analyzed by a gas chromatograph (GC, Perkin-Elmer, AutoSystem) equipped with a thermal conductivity detector (TCD) and a packed column (stainless-steel 10'x 1/8' x .085" HayeSep D 100/120 mesh, Altech). Injector, column, and detector temperatures were kept at 60, 35, and 150 °C, respectively. Argon was used as the carrier gas. The total amount of volatile fatty acids (VFA) in the effluent samples was determined by the distillation and titration method [19]. The VFA composition in the effluent samples was analyzed by another gas chromatograph (Perichrom, PR2100) equipped with a flame ionization detector and a DB-WAXetr capillary column (J & W Scientific) in the splitless mode with helium as a carrier gas, hydrogen as a combustion gas, and air zero as a combustion-

supporting gas. The column temperature program was started at 60 °C, heated to 125 °C at a ramping rate of 10 °C/min, held for 2 min, then heated to 180 °C at a ramping rate of 15 °C/min, and held for 15 min. The temperatures of both injector and conductor were kept constant 250 °C. The mixed liquor volatile suspended solids (MLVSS) in the reactor was taken during the reacting step to represent the microbial concentration and the volatile suspended solids (VSS) in effluent samples taken during the decanting step to represent the microbial washout from the system were measured according to standard methods [19]. The COD values in the feed and effluent samples were determined by the dichromate method using a COD analyzer (HACH, DR 2700). Nitrogen analyses (in terms of organic nitrogen measured by the diazotization, cadmium reduction method, and inorganic nitrogen measured by the salicylate method) in the feed and effluent samples were carried out with TNT persulfate digestion. The total phosphorous in the feed and effluent samples was determined by the molybdovanadate method with acid persulfate digestion (Hach Company). The average values of the analysis (with less than 5 % standard deviation) were used to access the process performance of the ASBR system.

Table 4.2 Operating conditions for the ASBR process at different COD loading rates

Feed and Decant (l/d)	HRT (h)	Feed or Decant (l/cycle)	COD loading rate (kg/m^{3}d)
3	32	0.5	45
3.75	25.6	0.625	56
4.5	21.3	0.75	68
5.25	18.3	0.875	79

4.3.5 Calculations

The hydrogen production rate. which is calculated from the gas production rate and gas composition as a function of COD loading rate, is shown below:

$$\text{hydrogen poduction rate}, l/h = \text{gas production rate}, l/h \times \left[\frac{\% H_2}{100}\right] \quad (1)$$

The hydrogen yield, which is calculated from a hydrogen production rate per g of COD applied or removed, is shown below:

$$\text{hydrogen yield}, ml\ H_2\ /\ g\ COD\ removed = \frac{\text{hydrogen production rate}, ml/d \times 1000}{\text{feed flow rate}, l/d \times COD\ removed, mg/l} \quad (2)$$

$$\text{hydrogen yield}, ml\ H_2\ /\ g\ COD\ applied = \frac{\text{hydrogen production rate}, ml/d \times 1000}{\text{feed flow rate}, l/d \times COD\ applied, mg/l} \quad (3)$$

4.4 Results and Discussion

4.4.1 Organic Removal and Gas Production Results

The COD removal efficiency increased with increasing COD loading rates from 45 to 68 kg/m^{3}d and then decreased with further increasing COD loading rates from 68 to 79 kg/m^{3}d (Figure 4.1a). The maximum COD removal was 32 % at a COD loading rate of 68 kg/m^{3}d. The increase in the COD loading rate resulted in an increase in organic compounds available for microbial degradation, leading to increased COD removal. However, at a very high COD loading rate—greater than 68 kg/m^{3}d—the system started having high VFA, causing increasing toxicity to the microbes, thereby lowering the COD removal and the microbial concentration in the bioreactor, as shown later. The gas production rate had a similar trend to the COD removal. The optimum COD loading rate of 68 kg/m^{3}d provided both maximum COD removal and gas production rate.

4.4.2 Hydrogen Production Rresults

Under the studied conditions, the produced gas contained mainly hydrogen and carbon dioxide without methane, suggesting the methanogenic step

was completely suppressed at high COD loading rates which is in a good agreement with previous results [20,21] (Figure 4.1b). The hydrogen percentage increased with increasing COD loading rate and reached a maximum value of 43% at a COD loading rate of 68 kg/m^{3}d. It decreased with further increasing COD loading rate from 68 to 79 kg/m^{3}d. The same explanation used for the effect of COD loading rate on the COD removal can be applied for that of the gas production rate, and hydrogen percentage in the produced gas [7,10,20,22]. Carbon dioxide percentage had an opposite trend to the hydrogen percentage.

The hydrogen production rate increased with increasing COD loading rate and reached a maximum at a COD loading rate of 68 kg/m^{3}d (Figure 4.1b). It decreased with further increasing COD loading rate beyond 68 kg/m^{3}d. There was an increase in the hydrogen production rate with increasing COD loading rate because of the increase in organic compounds in the system available to convert to hydrogen gas. However, with a very high COD loading rate of 79 kg/m^{3}d, the hydrogen production rate decreased because of the toxicity from VFA accumulation, as mentioned later.

The SHPR increased with increasing COD loading rate and attained a maximum value of 560 ml H$_2$/g MLVSS d (or 2100 ml H$_2$/l d) at a COD loading rate of 68 kg/m^{3}d, which corresponded to the maximum hydrogen production, the maximum hydrogen percentage, and the maximum COD removal (Figure 4.1c). The SHPR decreased to 185 ml H$_2$/g MLVSS d (or 570 ml H$_2$/l d) with further increasing COD loading rate from 68 to 79 kg/m^{3}d, corresponding to the decreases in the hydrogen production rate, hydrogen percentage, and COD removal.

The hydrogen yield increased with increasing COD loading rate and reached a maximum value of 130 ml H$_2$/g COD removed or 30 ml H$_2$/g COD applied at a COD loading rate of 68 kg/m^{3}d which also corresponded to the maximum SHPR and the maximum COD removal (Figure 1d). It decreased markedly to 31 ml H$_2$/g COD removed or 5 ml H$_2$/g COD applied with further increasing COD loading rate from 68 to 79 kg/m^{3}d. As expected, the higher the COD removal, the higher the hydrogen production efficiency as a result of greater amounts of organic compounds being made available for the bacteria to convert to hydrogen gas.
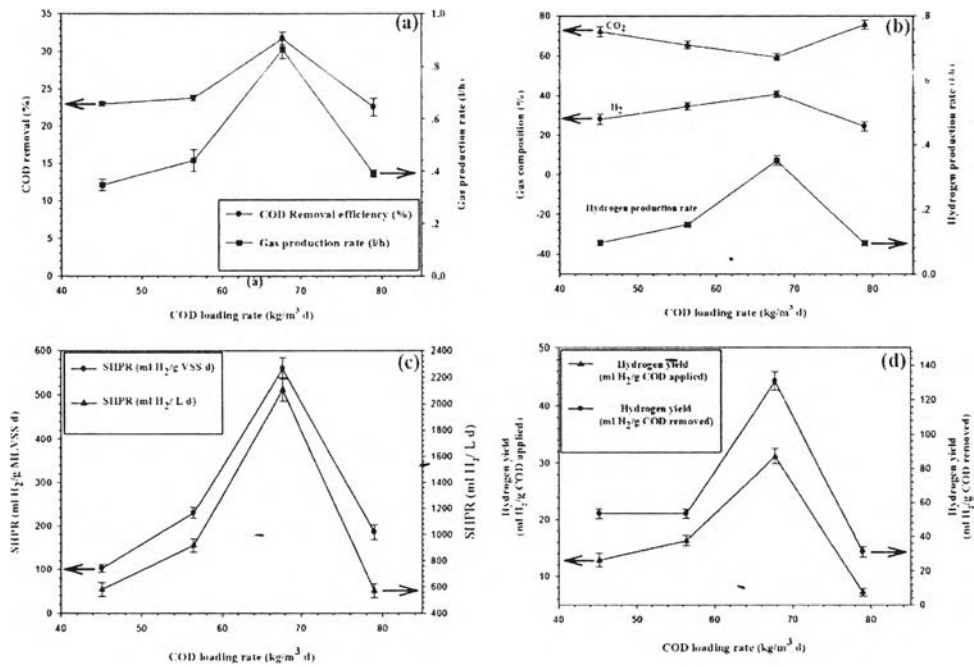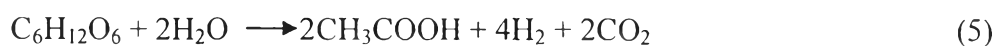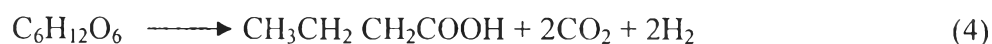
Figure 4.1 Effects of COD loading rate on (a) COD removal and gas production rate, (b) gas composition and hydrogen production rate, (c) specific hydrogen production rates and (d) hydrogen yield at pH 5.5 and 55 °C

4.4.3 Volatile Fatty Acid (VFA) Results

The total VFA increased with increasing COD loading rate. The highest total VFA concentration of 13300 mg/l was found at a COD loading rate of 79 kg/m³d which was responsible for both reductions in COD removal and hydrogen production efficiency (Figure 4.2). The increase in VFA from 8,000 mg/l to 13,300 mg/l exhibited significant toxicity to the microbial activity toward hydrogen production performance. It can be concluded that a maximum VFA for hydrogen production of this alcohol wastewater may be around 13,000 mg/l as acetic acid.

The main components of VFA were acetic acid, propionic acid, butyric acid, and valeric acid. Both acetic and propionic acids increased slightly with increasing COD loading rate throughout the range of COD loading rates (Figure 4.2). However butyric and valeric acids increased substantially where the COD loading rate increased from 68 to 79 kg/m³d. At any given COD loading rate, butyric acid was the highest, followed by valeric acid, acetic acid and propionic acid. A high concentration of butyric acid or acetic acid can indicate a higher hydrogen

production performance [23]. In contrast, greater amounts of propionic acid can result in lowered hydrogen production performance [26]. To maximize hydrogen production performance, an anaerobic system should be operated to increase butyric acid and acetic acid concentrations with a very low propionic concentration [23-25]. O-thong *et al.*, [26] also found that high amounts of butyric acid (6,200 mg/l), and acetic acid (4300 mg/l) with a low amount of propionic acid (120 mg/l) contributed to the highest hydrogen production (from palm oil wastewater). Butyric acid and acetic acid are formed via the metabolic pathway for production of hydrogen and propionic acid is formed via the metabolic pathway for consumption of hydrogen [27]. The microbial pathways of both hydrogen production and consumption are shown in the following reactions:

$$C_6H_{12}O_6 \longrightarrow CH_3CH_2 CH_2COOH + 2CO_2 + 2H_2 \qquad (4)$$

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 4H_2 + 2CO_2 \qquad (5)$$

$$C_6H_{12}O_6 + 2H_2 \longrightarrow CH_3CH_2COOH + 2H_2O \qquad (6)$$

Another factor that can affect hydrogen production performance is the formation of ethanol, as shown in Equation 7.

$$C_6H_{12}O_6 + H_2O \longrightarrow C_2H_5OH + CH_3COOH + 2H_2 + 2CO_2 \qquad (7)$$

Ethanol concentration increased with increasing COD loading rate and attained a maximum value of 5,600 mg/l at a COD loading rate of 68 kg/m^{3}d (Figure 2). The highest ethanol concentration corresponds to the highest hydrogen production performance (Figure 1c-1d) which is in good agreement with previous work [28]. Apart from the production of hydrogen via the metabolism pathway of ethanol production, the produced ethanol can also reduce the acidity in the reactor, leading to increased efficiency of hydrogen production [28].
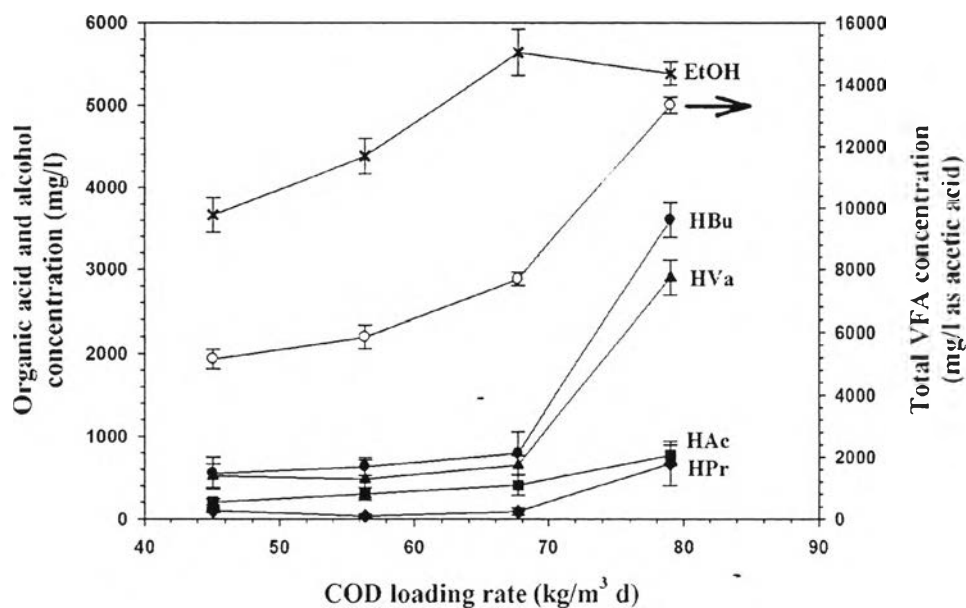
Figure 4.2 Total VFA, VFA composition and ethanol concentration versus COD loading rate at 55°C and pH 5.5

4.4.4 Microbial Concentration and Microbial Washout Results

The MLVSS decreased with increasing COD loading rate whereas the microbial washout from the system in terms of VSS increased with increasing COD loading rate from 45 to 68 kg/m³d (Figure 4.3). The microbial concentration only slightly changed when the COD loading rate increased from 68 to 79 kg/m³d. The microbial washout in terms of effluent VSS had an opposite trend to the microbial concentration in the system (MLVSS). The results suggest that an increase in microbial washout from the system and a decrease in the microbial concentration in the bioreactor with increasing COD loading rate (resulted from increasing VFA in the system) are responsible for the decreasing hydrogen production performance at a very high COD loading rate. Arooj et al., [9] reported that the decreasing MLVSS with decreasing hydraulic retention times (HRTs) caused the population shift of hydrogen-producing bacteria, leading to increasing hydrogen production. However, a further increase in HRT caused the system to collapse, and lowered the hydrogen production rate. The highest hydrogen production performance was found at a COD loading rate of 68 kg/m³d (Figure 1). With the maximum hydrogen production

performance, the system had the lowest microbial concentration, indicating that at this optimum COD loading rate, the system contained mostly hydrogen-producing bacteria.
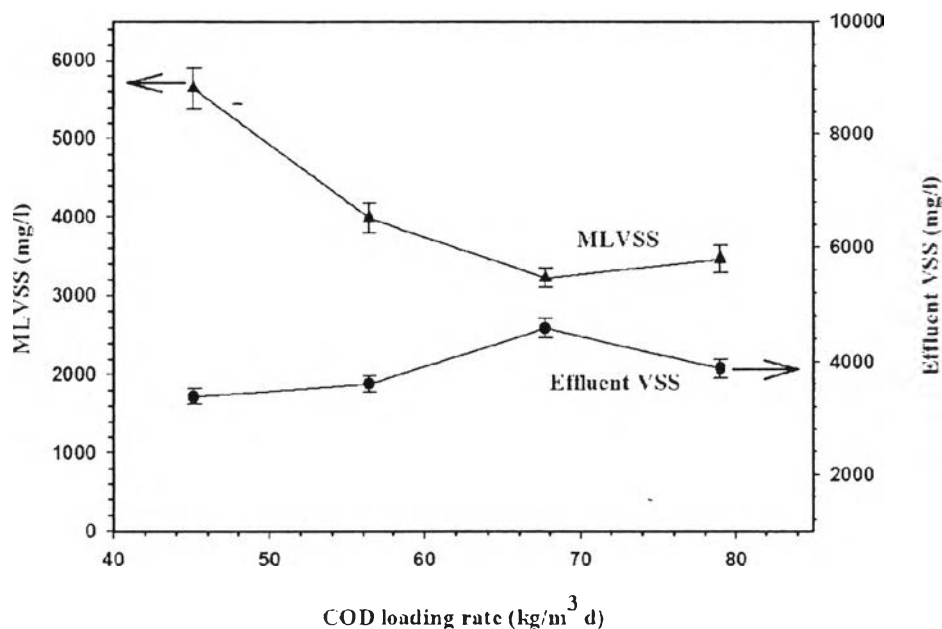
**Figure 4.3** MLVSS and effluent VSS versus COD loading rate at 55°C and pH 5.5.

4.4.5 Nitrogen and Phosphorous Results

Both nitrogen and phosphorous are known as essential nutrients for the growth of microbes, including hydrogen-producing bacteria, and the uptake of nitrogen can come from different forms of nitrogen compounds [30]. Both nutrients for the growth of hydrogen-producing bacteria -and how nitrogen transformation occurs during the hydrogen production step are great of interest. Both nitrogen and phosphorous removals mirrored the microbial concentration in the bioreactor (Figure 4.4a). They decreased with increasing COD loading rate and reached minimum values at a COD loading rate of 68 kg/m^3d. They slightly increased with further increasing COD loading rate from 68 to 79 kg/m^3d. The results confirm that the higher the microbial concentration in the system, the higher the nutrient uptakes. Under the optimum COD loading rate, the removal ratio of COD:N:P was found to be 100:6:0.5, which is much higher than the theoretical ratio for the anaerobic organic decomposition for biogas production (100:1:0.2). The very high nutrient

uptakes of the acidogenic step with thermophilic operation resulted from the system was operated under extremely high organic loading rates in which the microbes required very high nutrient uptakes for their growth.

Figure 4.4b shows the concentrations of ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, organic-nitrogen, and total nitrogen at different COD loading rates. The concentrations of all the nitrogen-containing compounds (organic, ammonium and nitrate nitrogen) increased with increasing COD loading rates except the nitrite-nitrogen concentration, which remained almost unchanged. The results suggest that most of the organic nitrogen was utilized by the hydrogen-producing bacteria for their growth. It has been reported that the presence of ammonium-nitrogen concentrations greater than 200 mg/l [29, 31-32, 35] and in the range of 800-3,900 mg/l [34] showed moderate and severe toxicity to hydrogen-producing bacteria [33], respectively, suggesting that the ASBR treated the alcohol wastewater did not have ammonium-nitrogen toxicity.

The concentration of nitrate-nitrogen decreased with increasing COD loading rate. Denitrifying bacteria are responsible for the nitrate reduction under anaerobic condition. Most of the nitrogen uptake came from the organic-nitrogen. As confirmed experimentally, the nitrogen gas produced by the denitrifying bacteria was very low and could not be detected in the produced gas. Hence, under the thermophilic temperature of the acidogenic step, denitrification cannot occur and the hydrogen-producing bacteria may be responsible for the nitrate uptake.
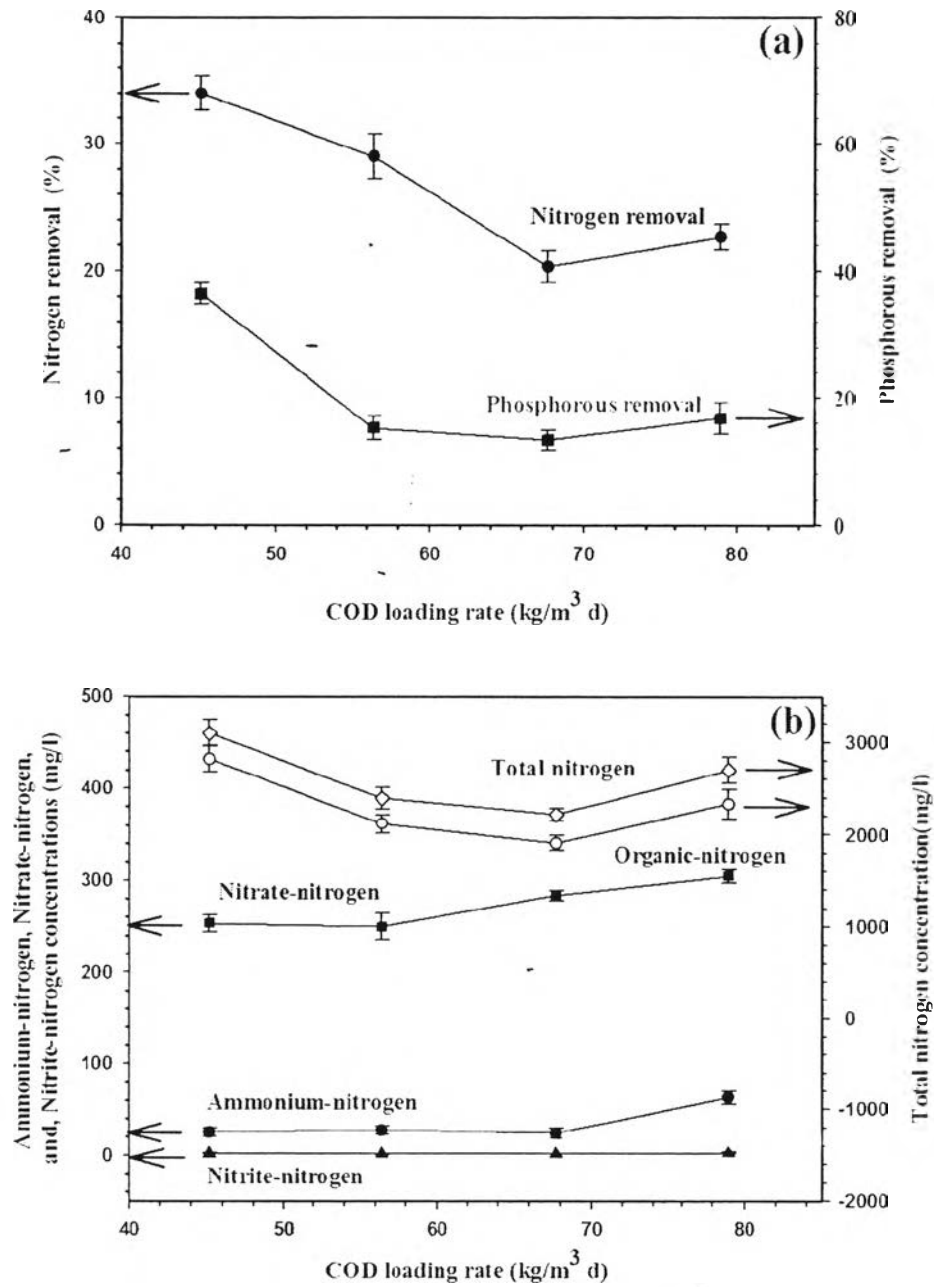
Figure 4.4 Nitrogen and phosphorous removal (a), and total nitrogen, organic nitrogen and inorganic nitrogen concentration (b) in the system as a function of COD loading rate at 55°C and pH 5.5.

4.4.6 Comparisons of Thermophilic and Mesophilic for Hydrogen Production Performance

As shown in Table 4.3, the ASBR operated under the thermophilic temperature of 55 °C provides a much higher optimum COD loading rate (68 kg/m^3d) as compared to that of the system operated under the mesophilic temperature of 37 °C (30 kg/m^3d) using upflow anaerobic sludge blanket (UASB). Moreover, the thermophilic operation gave a much higher hydrogen production performance in terms of both hydrogen yield and SHPR than that operated under the mesophilic operation. Interestingly, the COD removal, the system VFA, and the microbial washout were higher. Whereas the microbial concentration was lower for the thermophilic operation than that for the mesophilic operation. The results suggest that the thermophiles are more effective than mesophilic bacteria in producing hydrogen.

Table 4.3 Comparison between thermophilic and mesophilic process for hydrogen production performance at optimum conditions

	(Thermophillic operation, present study)	(Mesophillic operation, [36])
	ASBR	UASB
Optimum COD loading rate (kg/m³d)	68	30
%Hydrogen in produced gas	43±0.32	38±0.45
Hydrogen yields		
- ml H_2/g COD applied	30±0.31	19±0.27
- ml H_2/g COD removed	130±0.73	94±0.
Specific hydrogen production rates (SHPR)		
- ml H_2/l d	2100±0.23	550±0.28
- ml H_2/g MLVSS d	560±0.31	42±0.33
Microbial concentration in bioreactor, MLVSS (mg/l)	3,200±0.22	13,000±0.21
Microbial washout concentration, VSS (mg/l)	4,500±0.42	4,000±0.35
COD removal (%)	32±0.26	20±0.31
Total VFA concentration in bioreactor (mg/l as acetic acid)	13,300±0.33	12,600±0.37
VFA and alcohol concentrations in bioreactor		
- butyric acid (mg/l)	2,600±0.22	2,500±0.24
- valeric acid (mg/l)	2,400±0.34	1,800±0.31
- acetic acid (mg/l)	2,200±0.21	3,000±0.20
- propionic acid (mg/l)	1,800±0.23	2,200±0.21
- ethanol concentration (mg/l)	5,600±0.33	5,600±0.32

4.5 Conclusions

Hydrogen production from alcohol wastewater with an anaerobic sequencing batch reactor under thermophilic operation, and a controlled pH of 5.5 was investigated. At a COD loading rate of 68 kg/m^{3}d, the system gave the best hydrogen production performance with a maximum specific hydrogen production rate of 560 ml H$_2$/g MLVSS d and maximum hydrogen yield of 130 ml H$_2$/g COD removed. Thermophilic operation was found to be superior to the mesophilic operation for hydrogen production. The nutrient requirement for hydrogen production under thermophilic operation is much higher than that for biogas production under mesophilic temperature.

4.6 Acknowledgements

4.7 References

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