CHAPTER V
HYDROGEN PRODUCTION FROM ALCOHOL WASTEWATER WITH ADDED FERMENTATION RESIDUE BY AN ANAEROBIC SEQUENCING BATCH REACTOR (ASBR) UNDER THERMOPHILIC OPERATION

(accepted in International Journal of Hydrogen Energy)

5.1 Abstract

The objective of this study was to investigate the enhancement of hydrogen production from alcohol wastewater by adding fermentation residue using an anaerobic sequencing batch reactor (ASBR) under thermophillic operation (55 °C) and at a constant pH of 5.5. The digestibility of the added fermentation residue was also evaluated. For a first set of previous experiments, the ASBR system was operated to obtain an optimum COD loading rate of 50.6 kg/m^{3}d of alcohol wastewater without added fermentation residue and the produced gas contained 31 % H_2 and 69 % CO_2. In this experiment, the effect of added fermentation residue (100-1,200 mg/l) on hydrogen production performance was investigated under the optimum COD loading rate of 50.6 kg/m^{3}d of the alcohol wastewater. At a fermentation residue concentration of 1,000 mg/l, the produced gas contained 40 % H_2 and 60 % CO_2 without methane and the system gave the highest hydrogen yield and specific hydrogen production rate of 128 ml/g COD removed and 2,880 ml/l d, respectively. Under thermophilic operation with a high total COD loading rate (51.8 kg/m^{3}d) and a short HRT (21 h) at pH 5.5, the ASBR system could only break down cellulose (41.6 %) and hemicellulose (21.8 %), not decompose lignin.

Keywords: Hydrogen production performance; Fermentation residue; Alcohol wastewater; Anaerobic sequencing batch reactor (ASBR); Thermophilic operation

5.2 Introduction

The main energy source presently used worldwide is derived from fossil fuels, oil, coal, natural gas, and shale oil. The consumption of fossil fuels has

increased steadily. In addition, when these fossil fuels are used to generate energy, large quantities of greenhouse gases, including carbon dioxide (CO_2), nitrogen oxides (NO_x) and sulfur dioxide (SO_2) are released into atmosphere, consequently resulting in global warming. Hydrogen, as an energy source, has been increasingly investigated because it does not produce carbon dioxide after combustion. Hydrogen can be produced from different raw materials with the most attractive raw materials being renewable resources, particularly concentrated organic wastewaters via dark fermentation [1]. The process can be operated under atmospheric pressure and ambient temperature. Alcohol wastewater is one of the promising sources for hydrogen production because it has a high organic concentration [2-3]. The use of alcohol wastewater not only provides hydrogen as a clean and renewable fuel but also enhances the sub-sequential step of methanogenesis to produce methane [4].

In the ethanol fermentation industry, one of the serious problems is the large quantity of fermentation residue that has to be removed from the wastewater because it upsets the process performance of the anaerobic units, especially in the upflow anaerobic sludge blanket (UASB) bioreactors. Hence, it has to be separated from the wastewater, leading to a large quantity of the fermentation residue. As a result, it causes environmental problems, bad smell and water pollution. Because of the low degradation rates of cellulosic materials under anaerobic conditions [5], the improvement of digestibility is of great interest. To enhance the digestibility of fermentation residue, several pretreatment methods are available—physical pretreatment (mechanical disturbance including milling, crushing, or grinding), chemical pretreatment (acid/base hydrolysis or solvent extraction), biological pretreatment (using enzymes or fungi) [6], and metal nanoparticle pretreatment (adding silver or nickel nanoparticles) [7-8]. All of these pretreatment methods have the unique purpose of to breaking down the resistance layer of lignin. Lignin is a complex compound with a polymeric structure, resulting in high resistance to biological degradation. Large amounts of lignin have been found to reduce the efficiency of cellulose degradation because lignin serves as a protective barrier to both cellulose and hemicelluloses [10]. One of the most innovative methods used to enhance the digestibility of cellulosic residues is to operate anaerobic bioreactors for

methane production under severe conditions (50-60 °C and pH 4-5) [9]. The use of thermophilic operation at a low pH (4-5) was also found to enhance the digestibility of cornstack for hydrogen production [6]. Lynd *et al.*, [10] reported that the anaerobic degradation of cellulose at 60 °C was much higher than that at a low temperature (30-40 °C). Under high temperatures, microorganisms can produce cellulolytic enzymes to hydrolyze cellulose [11]. Pavlostathis *et al.*, [12] studied cellulose destruction at a low temperature (37 °C) and found that the cellulose destruction could be increased by increasing HRT from 0.25 to 2 d, leading to a larger bioreactor volume being required.

Several attempts to utilize biomass residues to produce hydrogen (agricultural residues including rice straw [13], wheat straw [14], cassava starch [15], and grasses [16]) for the co-digestion of wastewaters using pretreated seed sludges have been reported [17]. Lo *et al.*, [13] reported that, in a batch test, hydrogen yields increased from 0.70 mol H_2/mol xylose to 0.76 mol H_2/mol xylose when rice straw was pretreated with NaOH. Fan *et al.*, [14] reported that, in batch fermentation tests, hydrogen yield at a HRT of 126.5 h increased from 13.8 ml H_2/g TVS to 68.1 ml H_2/g TVS when increasing the concentration of the 2.0 % HCl solution pretreated wheat straw from 5 to 25 g/l. The wheat straw was pretreated by a 2.0 % HCl solution with microwave heating for 8 min. The highest hydrogen yield of 68.1 ml H_2/g TVS from the microwave treated wheat straw was about 136 times more as compared with that from the un-treated wheat straw [14]. Sigurbjornsdottir *et al.*, [16] found the hydrogen concentration increased from 28.0±0.2 mmol/l to 40.5±2.1 mmol/l when the grass was pretreated with a 0.75 %NaOH solution.

Alcohol wastewater has been investigated for hydrogen production without adding cellulosic residue, as reported in our previous works [18-19]. Poontaweegeratigarn *et al.*, [18] studied hydrogen production from alcohol wastewater by using UASB under mesophilic temperature (37 °C) and at pH 5.5. The maximum hydrogen production rate of 18 L/d and the highest H_2 yield of 125.1 ml H_2/g COD removed were found at a COD loading rate of 46 kg/m^{3}d. Intanoo *et al.*, [19] further studied hydrogen production from the same wastewater under thermophilic temperature (55 °C) and pH 5.5 using an ASBR system and the highest hydrogen yield of 130 ml H_2/g COD removed was found at a COD loading rate of 68

kg/m^3d. They concluded that hydrogen production performance, in terms of hydrogen yield and SHPR at thermophilic temperature, were much higher than those at the mesophilic temperature.

This present work is the second part of a series, of our research group, to investigate the enhancement of hydrogen production performance by adding fermentation residue without pretreatment to alcohol wastewater under thermophilic operation. The ability of thermophiles to degrade the un-treated fermentation residue as an additional biomass substrate under thermophilic temperature (55 °C) and a constant pH of 5.5 by using an anaerobic sequencing batch reactor (ASBR) was also investigated. The ASBR was fed by the alcohol wastewater at an optimum COD loading rate 50.6 kg/m^3d with a short HRT of 21 h [19]. The fermentation residue was added to the alcohol wastewater at different concentrations of suspended solids (SS; ranging from 100-1200 mg/l) in order to determine an optimum loading of the fermentation residue for maximization of hydrogen production performance.

5.3 Materials and Methods

5.3.1 Seed Sludge

A seed sludge sample collected from the UASB 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 particles. In order to eliminate or block the growth of methane-producing bacteria or hydrogen consumers, the seed sludge was boiled at 95 °C for 15 min [20-24]. The heat-treated sludge enriching hydrogen-producing bacteria was then added to an ASBR. The microbial concentration in terms of MLVSS (mixed liquid volatile suspended solids) for the start-up in this study was about 12,000 mg/l.

5.3.2 Alcohol Wastewater and Fermentation Residue

The alcohol wastewater used in this study was also obtained from Sapthip Lopburi Co., Ltd., Thailand with cassava chips being used as the raw material for alcohol fermentation. The discharge from the bottom of the distillation columns still contains a large quantity of unfermented cassava chips, which is

preliminarily removed by decantation before being pumped to the existing UASB biogas production plant. The collected alcohol wastewater was filtered through a 0.2 mm sieve to remove any large solid particles and kept at 4 °C before use. A sample of the fermentation residue, taken from the decanter, was dried at 105 °C, then crushed and milled to reduce particle size and finally sieved to 40-60 mesh (average diameter of the fermentation residue was about 250 μm).

5.3.3 Anaerobic Sequencing Batch Reactor (ASBR) Operation

Two identical units of anaerobic sequencing batch reactors (ASBR) were used independently to produce hydrogen from the alcohol wastewater with added fermentation residue at different fermentation residue concentrations. The possibility of photosynthetic bacteria activity was inhibited by using PVC material in the construction of the bioreactors. Each of the bioreactors had an inner diameter of 13 cm and a height of 30 cm. The bioreactors were operated with a liquid working volume of 4 L. Each ASBR unit was equipped with a magnetic stirrer (450 rpm) to suspend both microbial cells and the added fermentation residue homogeneously. The heat-treated sludge (1,000 ml) was added as a seed sludge to each of the ASBR units. Both ASBR units were operated under the optimum COD loading rate of 50.6 kg/m^3 d [19] at a temperature of 55 °C, a constant solution pH of 5.5 [25-26] and at 6 cycles per day with operating time of 15, 90, 120 and 15 min for feeding, reacting, settling and decanting, respectively [19]. To investigate the effect of the added fermentation residue, different concentrations (100-1,200 mg/l) of fermentation residue were added to the alcohol wastewater. The system was operated for approximately two weeks to reach a steady state for any fermentation residue concentration before taking the effluent and producing gas samples for analysis and measurement. Steady state conditions were attained when both effluent COD and the gas production rate did not change with time. After the ASBR systems reached steady state, the samples taken during the reacting step were filtered for the analysis of microbial and fermentation residue concentrations and chemical composition of remaining fermentation residue. The effluent samples taken during the decanting step were also filtered and analyzed for the same parameters to indicate the washout of both microbial cells and fermentation residue.

5.3.4 Measurements and Analytical Methods

The gas production rate was measured by using a wet gas meter (Ritter, TGO5/5). The gas composition of the produced gas was analyzed by a gas chromatograph (GC, Perkin-Elmer, AutoSystem) equipped with a thermal conductivity detector (TCD) and a packed column (HayeSep D 100/120 mesh, Altech) according to previous experiments [19]. The total amount of volatile fatty acids (VFA) in each effluent sample was determined by the distillation and titration method [27]. The VFA composition of each effluent sample after the distillation step was analyzed by a different 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 analysis conditions were described in our previous work [19].

The COD values in the feed and effluent samples were determined by the dichromate oxidation method with an absorbance measurement using a spectrophotometer (HACH, DR 2700). Nitrogen analyses (organic nitrogen measured by the diazotization and cadmium reduction method, and inorganic nitrogen measured by the salicylate method) in the feed and effluent samples were carried out by TNT persulfate digestion. The total phosphorous content in the feed and effluent samples were determined by the molybdovanadate method with acid persulfate digestion (Hach Company) [19].

The mixed liquor suspended solids (MLSS) and the mixed liquor volatile suspended solids (MLVSS) in the samples taken during the reacting step, were used to represent the microbial concentration with the accumulated fermentation residue in the bioreactor and the SS and the volatile suspended solids (VSS) in the effluent samples, taken during the decanting step, and used to represent the microbial washout and the fermentation residue floating out from the system were measured according to standard methods [27].

The dried sample of fermentation residue was analyzed for elemental and chemical compositions. An elemental analyzer (TruSpec-CHN) was used to determine C, H, O, N and S contents in the sample. Combustion and burner

temperatures were kept at 950 °C and 850 °C, respectively, with oxygen, helium, and air used as carrier gases. The concentrations of glucose in the dried sample of fermentation residue and the effluent were determined by the enzymatic method with a glucose (HK) assay kit (Sigma-Aldrich, Inc).

For the analyses of both fractions of microbial cells and remaining fermentation residue, the dried samples of the fermentation residue, the MLSS from the ASBR units and the effluent SS were analyzed according to suggested methods of Lin [28]. Firstly, the organic nitrogen of the dried microbial sludge sample was analyzed and converted into a microbial concentration with a known nitrogen-content of 11.21 % (in the dried weight basis) in the microbial cells which was obtained from the growth of microbes with the studied wastewater without added fermentation residue. The remaining fermentation residue fraction was obtained after substracting the microbial weight fraction. For the chemical composition analysis of the fermentation residue, a dried sample was first extracted by acetone. The weight loss of the acetone extraction step represented oils and phenolic compounds in the sample. Next, the acetone-extracted sample was dissolved in a 0.5 M NaOH solution at 90 °C for 2 h. The weight loss from this step indicated the fraction of hemicellulose and starch. The starch fraction in the NaOH dissolution solution was then determined by the amylase/amyloglocosidase method using a starch assay kit (Sigma-Aldrich, Inc). Next, the remaining residue, after the NaOH dissolution step, was further treated with a 72 wt% H_2SO_4 solution at 8 °C for 24 h to dissolve the lignin fraction. The weight of the H_2SO_4 undissolved solids represented the fractions of cellulose and ash. Finally, the undissolved solids were placed in an oven at 550 °C for 1 h to burn out the cellulose fraction. The weight loss in this step represents the cellulose fraction. The average values of the analysis results (with less than 5 % standard deviation) were used to determine the process performance of the studied ASBR system.

5.3.5 Calculations and Process Performance Evaluation

Calculations for process performance evaluation were similar to our previous work [21]. The hydrogen yield was determined from a volume of hydrogen produced per g of COD applied or removed. The specific hydrogen production rates

(SHPR) were based on both the bioreactor volume and microbial dried weight, which are important parameters for both design and operation of a hydrogen production unit, and were also determined at different fermentation residue concentrations. The digestibility of the added fermentation residue was determined by performing mass balance under steady state conditions, as expressed below:

$$Digestibility\ or\ degradation = Input - Output - Accumulation \quad (1)$$

5.4 Results and Discussion

5.4.1 Chemical Composition of Alcohol Wastewater and Fermentation residue

As shown in Table 5.1, the alcohol wastewater has a high chemical oxygen demand (COD) value of 45,000 mg/l with a COD:nitrogen:phosphorous ratio of 100:1.33:1.23, indicating that the wastewater contains sufficient amounts of both nutrients (N and P) for anaerobic degradation (the theoretical ratio of COD:N:P = 100:1:0.2 for anaerobic decomposition with biogas production [19]). Therefore, an addition of nutrients was not required in this study. Most of the nitrogen in the wastewater was in the form of nitrate and organic nitrogen with a significant amount of ammonium nitrogen.

The elemental and chemical composition of the fermentation residue samples are shown in Table 5.2. The major elements of the fermentation residue are hydrogen, oxygen and carbon based on molar basis. It should be mentioned here that both protein and sugar were found to be extremely low and they were excluded from the list. Surprisingly, 39.74 % starch was found to present in the fermentation residue sample, indicating that a significant amount of starch was embedded in the rigid structure of the lignocellulosic fiber network. Apart from the starch fraction, the order of all fractions in the fermentation residue sample was cellulose > lignin ≈ hemicellulose > ash >> extractive. The high fraction of ash (12.48 %) present in the fermentation residue was due to the sand content, which is generally added by middle men to increase the weight of cassava chips before selling to the factory.

Table 5.1 Characteristics of the ethanol wastewater sample

Parameter	**Unit**	**Value**
pH	-	4.6
COD	mg/l	45,000
Total VFA	mg/l	4,000
Ethanol concentration	mg/l	534
Total solids (TS)	mg/l	12,000
Total phosphorous	mg/l	580
Total nitrogen	mg/l	600
Organic nitrogen (Org-N)	mg/l	208
Ammonium (NH_4^+-N)	mg/l	40
Nitrate (NO_3^--N)	mg/l	350
Nitrite (NO_2^--N)	mg/l	1.6
COD:N:P	-	100:1.33:1.23

Table 5.2 Elemental and chemical compositions of the studied fermentation residue

Elemental composition	**wt%, dry basis**	**mol%**
Carbon	39.66	25.87
Hydrogen	6.02	47.16
Nitrogen	1.52	0.85
Oxygen	53.36	26.11
Sulfur	0.08	0.019
Chemical composition	**wt%, dry basis**	
Starch	39.7	
Hemicellulose	15.5	
Cellulose	23.5	
Lignin	14.9	
Extractives	6.4	
Ash	12.5	

5.4.2 Effects of fermentation residue concentration

5.4.2.1 Organic Removal Results

The organic removal, in terms of COD removal increased greatly with increasing fermentation residue concentration up to 200 mg/l and only slightly increased with further increasing fermentation residue concentration to 1,000 mg/l (Fig.5.1a). Beyond the fermentation residue concentration of 1,000 mg/l, COD removal decreased slightly with further increasing fermentation residue concentration from 1,000 to 1,200 mg/l. The results can be explained that an increase in fermentation residue concentration simply increased the organic loading in the system, leading to increased microbial activity. However, a further increase in

fermentation residue beyond 1,000 mg/l, resulted in an increase in VFA in the system beyond the inhibitory level, affecting microbial activity.

5.4.2.2 Hydrogen Production Results

As shown in Figure 5.1a, the gas production rate has a similar trend to the COD removal. Figure 1b shows two main components of hydrogen and carbon dioxide without methane in the produced gas for all studied fermentation residue concentrations. This implies that the methanogenic activity was completely suppressed by the high COD loading rate operation, causing toxicity from the organic acid accumulation to the methanogens in the studied ASBR [29-30]. Both profiles of hydrogen content and hydrogen production rate mirrored that of COD removal while that of carbon dioxide content showed an opposite trend. The maximum content of hydrogen (40 %) and maximum hydrogen production rate (12.5 l/d) were found at the fermentation residue concentration of 1,000 mg/l. A higher fermentation residue concentration in the alcohol wastewater, which had a higher concentration of all organic compounds, made more organic compound especially starch available for the microbes to digest, causing more hydrogen gas to be produced. However, at a fermentation residue concentration greater than 1,000 mg/l, both hydrogen content and hydrogen production rate decreased because of increasing toxicity of VFA accumulation.

The SHPR, based on either microbial concentration in the ASBR unit or reactor volume, increased with increasing fermentation residue concentration and attained a maximum value of 1,390 ml H_2/g MLVSS d (or 2,880 ml H_2/l d) at a fermentation residue concentration of 1,000 mg/l (Fig.5.1c). It was being consistent with the maximum hydrogen content and hydrogen production rate, and COD removal, as shown in Fig 5.1a and b. When the fermentation residue concentration further increased from 1,000 mg/l to 1,200 mg/l, both SHPR values abruptly decreased, corresponding to the decreases in the hydrogen production rate, hydrogen content, and COD removal.

Both the hydrogen yield of 128 ml H_2/g COD removed and 58 ml H_2/g COD applied were also observed at the fermentation residue concentration of 1,000 mg/l, as shown in Figure 5.1d. With further increasing fermentation residue concentration greater than 1,000 mg/l, hydrogen yield decreased significantly.

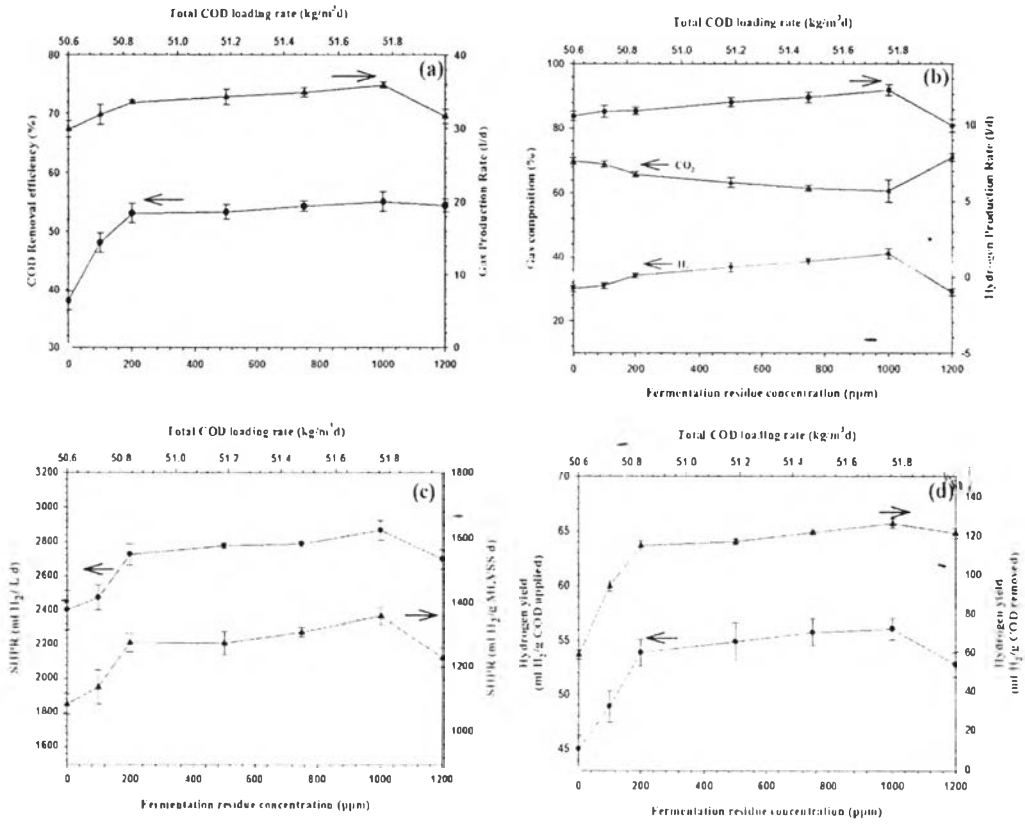

Figure 5.1 Effect of fermentation residue concentration on (a) COD removal and gas production rate, (b) gas composition and hydrogen production rate, (c) specific hydrogen production rates and (d) hydrogen yields when the ASBR system was operated with the ethanol wastewater at constant COD loading rate of 50.6 kg/m^{3}d, pH 5.5, and 55 °C.

5.4.2.3 Volatile Fatty Acid (VFA) Results

Figure 5.2 shows that the total VFA concentration increases steadily with increasing fermentation residue concentration and attains the highest value of 12,000 mg/l as acetic acid at the highest fermentation residue concentration of 1,200 mg/l. The results reveal that the total VFA concentration directly affects the process performance, in terms of both COD removal and hydrogen production [31]. Since the studied ASBR was operated at a constant pH of 5.5, the optimum pH for acidogenic fermentation, the system could withstand a remarkably high level of total VFA of up to 10,800 mg/l [19]. The addition of NaOH to maintain the system pH of

5.5 could reduce the undissociated form of organic acids, leading to lower VFA the toxicity as compared to a higher concentration of undissociated acids at a lower pH [32].

The major components of produced VFA are acetic acid (HAc), propionic acid (HPr), butyric acid (HBu), and valeric acid (HVa) with a high ethanol concentration, as shown in Figure 5.2. All organic acids increased significantly with increasing fermentation residue concentration up to 200 mg/l. Beyond the fermentation residue concentration of 200 mg/l, the concentration of each produced organic acid increased slightly with further increasing fermentation residue concentration from 200 mg/l to 1,200 mg/l. All organic acids are associated with hydrogen evolution while the formation of propionic acid is derived from the consumption of hydrogen, as shown in the following equations [33-34].

$$\text{Glucose} \longrightarrow \text{butyrate} + 2CO_2 + 2H_2 \quad (2)$$

$$\text{Glucose} + 2H_2O \longrightarrow 2\text{acetate} + 2CO_2 + 4H_2 \quad (3)$$

$$\text{Glucose} + 2H_2 \longrightarrow \text{propionate} + 2H_2O \quad (4)$$

The ethanol concentration increased with increasing fermentation residue concentration and attained the maximum value of 4,300 mg/l at a fermentation residue concentration of 1,000 mg/l (Fig.5.2), which was consistent with the highest hydrogen production performance (Fig.5.1). It sharply decreased with further increasing fermentation residue concentration from 1,000 mg/l to 1,200 mg/l. The ethanol concentration profile mirrored both of COD removal and hydrogen content in the produced gas. The organic substrate can be converted to both hydrogen and ethanol, as shown below [19,35].

$$\text{Glucose} \longrightarrow H_2 + \text{ethanol} \quad (5)$$

It is interesting to point out that in the present work, the alcohol concentration (3,800-4,200 mg/l) in the effluent was extremely high, which is similar to other works using alcohol wastewater [35-36]. The high ethanol concentration was a result of the alcohol wastewater being contaminated by yeast

cells, which further metabolized the glucose produced by the thermophiles to ethanol.

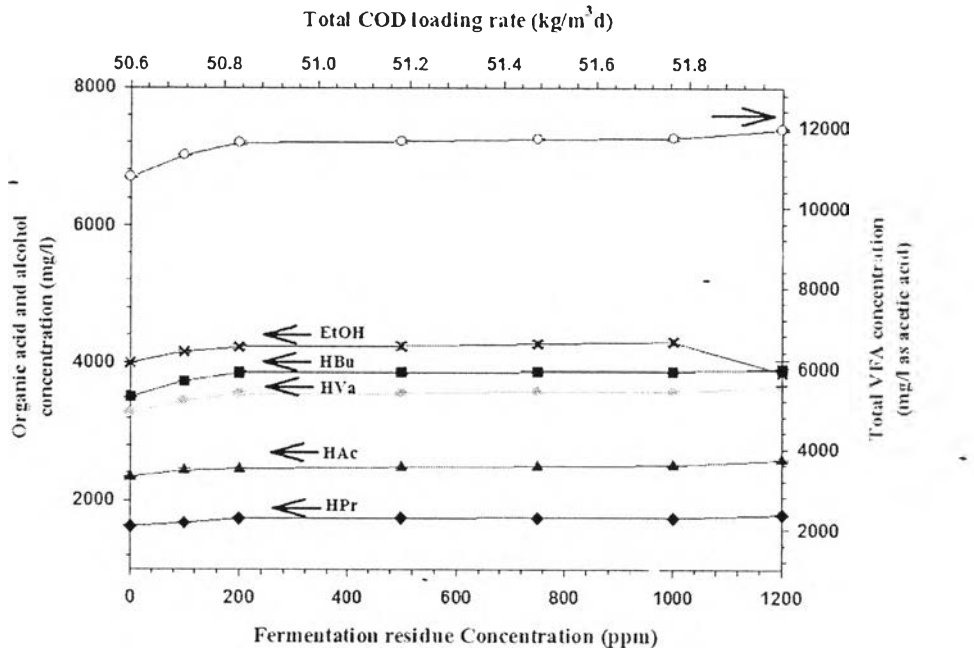

Figure 5.2 Total VFA, VFA composition and ethanol concentration at different fermentation residue concentrations when the ASBR system was operated with the ethanol wastewater at a constant COD loading rate of 50.6 kg/m^{3}d, 55 °C, and pH 5.5.

5.4.2.4 Microbial Concentration and Microbial Washout Results

The microbial concentration, along with the accumulated fermentation residue in the ASBR bioreactor, in terms of MLSS and MLVSS, decreases steadily with increasing fermentation residue concentration up to 1,200 mg/l, as shown in Figure 5.3a. Both the thermophiles and accumulated fermentation residue also had a similar trend. The microbial washout profile from the bioreactors, in terms of effluent SS or effluent VSS (Figure 5.3b) and the fermentation residue washout profile had an opposite trend. The toxicity level of organic acids produced by the hydrogen-producing bacteria at pH 5.5 was found to be around 12,000 mg/l, in agreement with the first set of experiments [19]. When the fermentation residue

concentration was increased in the alcohol wastewater, the total VFA increased, suggesting that the degree of VFA toxicity to the microbes increased with increasing fermentation residue content, causing an increase in microbial washout and decrease in microbial concentration in the system. The decrease in microbial concentration eventually caused both the reduction of COD removal and hydrogen production performance as well as an increase in the washout of fermentation residue. When the fermentation residue concentration increased from 100 mg/l to 1,000 mg/l, the microbial concentration in the ASBR bioreactor decreased significantly whereas hydrogen production performance increased. Thermophiles have an ability to hydrolyze both cellulose and hemicellulose, resulting in increased hydrogen production performance, as further discussed later. Conversely, the hydrogen production performance decreased at a high fermentation residue concentration of 1,200 mg/l due to increasing toxicity from VFA accumulation. Under the optimum COD loading rate of 50.6 kg/m^3d of the alcohol wastewater with the optimum fermentation residue concentration of 1,000 mg/l at 55 °C and pH 5.5, the glucose concentration in the effluent was found to be very low (0.3 mg/l), indicating that most glucose produced was immediately converted to hydrogen, carbon dioxide and organic acids by the thermophiles, as well as ethanol by the yeast.

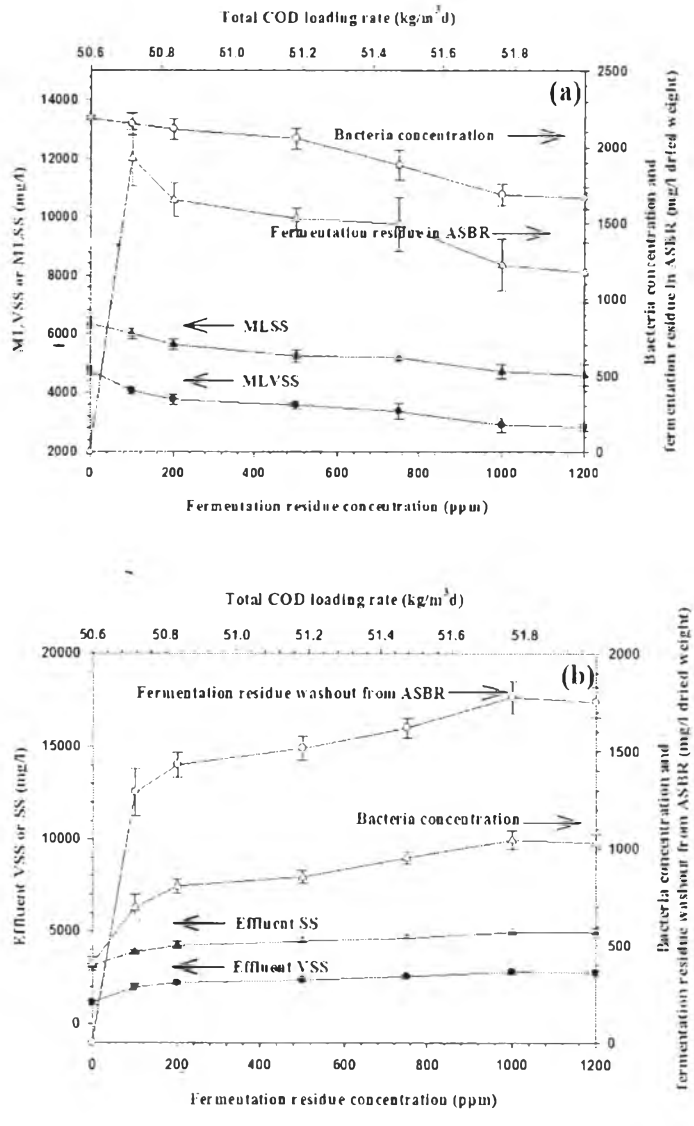

Figure 5.3 Effect of fermentation residue concentration on (a) bacteria concentration in ASBR, fermentation residue in ASBR (mg/l dried weight), MLVSS and MLSS and (b) bacteria concentration washout from ASBR, fermentation residue washout from ASBR (mg/l dried weight), effluent VSS and effluent SS when the ASBR system was operated with the ethanol wastewater at a constant COD loading rate of 50.6 kg/m^{3}d, 55 °C, and pH 5.5.

5.4.2.5 Nitrogen and Phosphorous Results

Both nitrogen and phosphorous are essential nutrients for the growth of microbes [18-19]. Both nitrogen and phosphorous uptakes increased greatly with increasing fermentation residue concentration from 100 mg/l to 1,000

mg/l (Fig.5.4a).With further increasing fermentation residue concentration from 1,000 to 1,200 mg/l both nitrogen and phosphorous uptakes remained almost unchanged. Interestingly, the optimum concentration of added fermentation residue to the alcohol wastewater at 1,000 mg/l gave the highest nutrient uptakes of both nitrogen and phosphorous, corresponding to the highest hydrogen production performance. The nitrogen sources for microbial growth came from ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen, and organic nitrogen [37]. As shown in Fig 4b, the organic nitrogen concentration in the ASBR decreases markedly with increasing fermentation residue concentration while the nitrate-nitrogen concentration slightly decreases. Both ammonium and nitrite nitrogen concentrations remained almost unchanged with increasing fermentation residue concentration in the studied conditions. The results suggest that most organic nitrogen was preferentially consumed by the hydrogen-producing bacteria for their growth under the studied conditions.

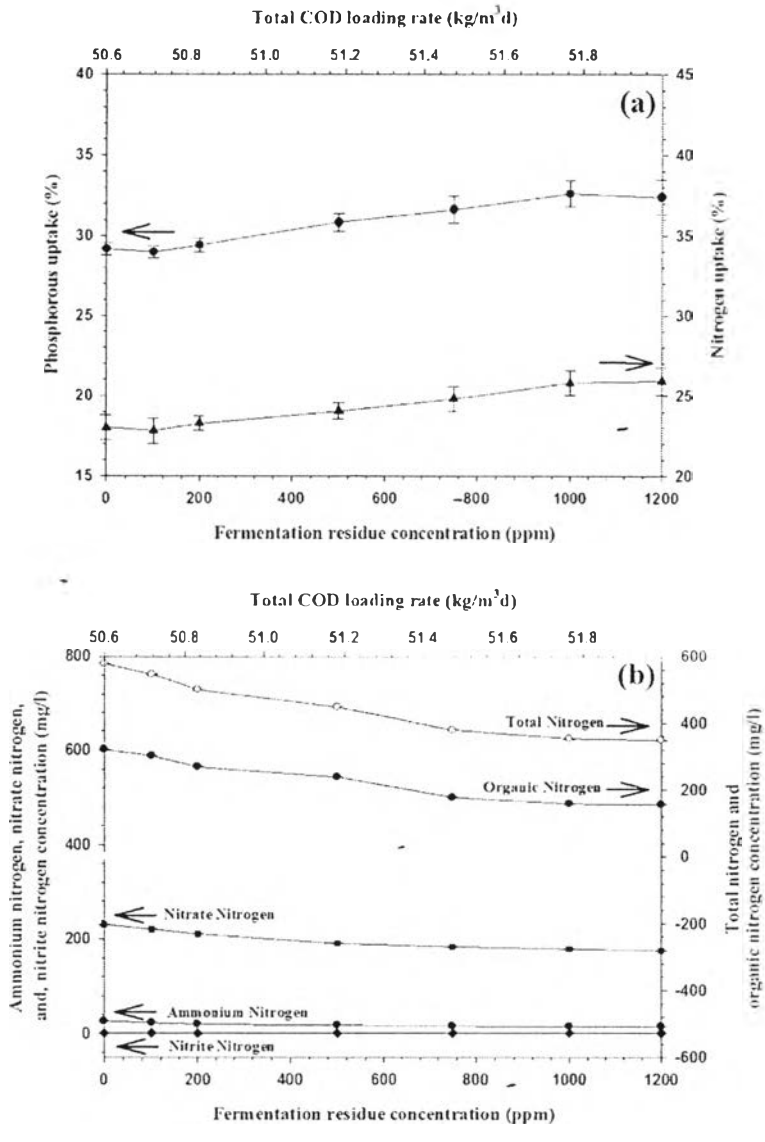

Figure 5.4 Effect of fermentation residue concentration on (a) nitrogen and phosphorous uptakes and (b) total nitrogen, organic nitrogen and inorganic nitrogen concentrations when the ASBR system was operated with the ethanol wastewater at a constant COD loading rate of 50.6 kg/m^{3}d, 55 °C, and pH 5.5.

5.4.2.6 Digestibility Results

Figure 5.5 shows the digestibility of all chemical components of the fermentation residue and the microbial concentration profile in relation to the fermentation residue concentration. The digestibility of cellulose, starch, or

hemicellulose increased steadily with increasing fermentation residue concentration from 100 to 1,000 mg/l and was mostly unchanged with further increasing fermentation residue concentration from 1,000 to 1,200 mg/l, whereas both the lignin and extractive fractions remained almost unchanged. The results suggest that the thermophiles cannot digest lignin or phenolic and oily compounds but they show high ability to degrade both cellulose and hemicellulose. Surprisingly, the digestibility or degradability of both cellulose and hemicellulose were found to be as high as that of starch. The layer of cellulose and hemicellulose with lignin had to be hydrolyzed prior to the hydrolysis of the embedded starch by the extracellular enzymes secreted from the thermophiles. As a result, the digestibility of both cellulose and hemicellulose was as high as that of starch.

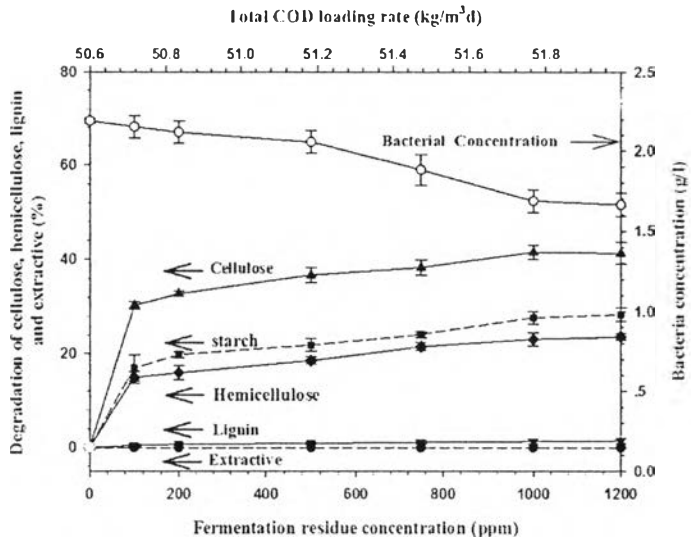

Figure 5.5 Digestibility of fermentation residue and microbial concentration in relation to the fermentation residue concentration when the ASBR system was operated with the ethanol wastewater at a constant COD loading rate of 50.6 kg/m^{3}d, 55 °C, and pH 5.5.

As shown in Table 5.3, the digestibility of various lignocellulosic materials at high temperatures are higher than those at low temperatures [6,38] because under anaerobic condition at high temperatures, facultative and obligatory anaerobic bacteria can produce more effective enzymes to

hydrolyze the lignocellulosic materials [11]. Moreover, the digestibility of pretreated lignocellulosic materials with heat, acid solution, or lime solution was also higher than those of the un-treated ones [6]. Pretreatment can remove the barriers and makes cellulose more accessible to microbial hydrolysis [43]. A continuous system seems to provide higher digestibility of cellulose than a batch system. The studied system showed high digestibility for both cellulose (41.6 %) and hemicellulose (21.8 %), without pretreatment under thermophilic operation.

5.5 Conclusions

Hydrogen production from alcohol wastewater containing fermentation residue using an anaerobic sequencing batch reactor under thermophilic temperature, and a controlled pH of 5.5 was investigated. The ASBR system was operated at the optimum COD loading rate of 50.6 kg/m^3d using alcohol wastewater while the fermentation residue was added at different concentrations in the alcohol wastewater. An optimum fermentation residue concentration of 1,000 mg/l gave the highest hydrogen production performance with a maximum specific hydrogen production rate of 1,390 ml H_2/g MLVSS d and maximum hydrogen yield of 125 ml H_2/g COD removed. The results confirmed that thermophiles have a high ability to digest both cellulose (41.6 %) and hemicellulose (21.8 %) under acidogenic fermentation even though the studied ASBR system was operated at an extremely high COD loading rate of 50.6 kg/m^3d of the alcohol wastewater with a very short HRT of 21 h. The lignin fraction could not be broken down anaerobically.

Table 5.3 Comparison of the digestibility of various lignocellulosic materials under various conditions

Feed stock	Pretreatment	Organic loading rate	System or condition	Temperature (°C)/pH	HRT (h)	Cellulose digestibility (%)	Ref.
cassava residue	no	51.73 kg/m^{3}d	ASBR	55 /5.5	21	41.6	Present study
cellulose	no	3 g/l	CSTR/ anaerobic	37/6.4-6.5	48	71.4	[12]
avical	no	5 g/l	batch/ anaerobic	30 /7.0	240	10	[38]
avical	no	5 g/l	batch/ aerobic	30 /7.0	240	27	[38]
cornstalk	lime	5 g/l	batch/ anaerobic	60 /7.0	60	58.6	[6]
cornstalk	no	5 g/l	batch/ anaerobic	60 /7.0	84	18.3	[6]
wheat straw stillage	hydrothermal	17.1 kg/m^{3}d	UASB	55 /6.8	48	25	[39]
cellulose	no	10 g/l	CSTR/ anaerobic	70 /5.3-5.7	240	64.9	[40]
corn stover	alkali/enzymatic hydrolysis	-	CSTR/ anaerobic	35 /-	720	45	[41]
corn stover	alkali/enzymatic hydrolysis	-	CSTR/ anaerobic	35 /-	960	60	[41]
corn stover	alkali/enzymatic hydrolysis	-	CSTR/ anaerobic	35 /-	1,200	50	[41]
laminaria japonica	no	3.4 g/l	CSTR/ anaerobic	35 /5.5	144	0.19±0.02 FPU/ml*	[42]
laminaria japonica	no	3.4 g/l	CSTR/ anaerobic	50 /5.5	144	0.11±0.03 FPU/ml*	[42]
laminaria japonica	no	3.4 g/l	CSTR/ anaerobic	65 /5.5	144	0.08±0.02 FPU/ml*	[42]

*cellulase activity indicated the hydrolysis efficiency [42]

5.6 Acknowledgements

The authors would like to thank Thailand Research Fund, Thaioil Public Co., Ltd., Thailand and National Research University Project of Thailand, (Project code: EN271A) for providing financial support for this research. The Sustainable Petroleum and Petrochemicals Research Unit under the Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Thailand, and the Innovation Learning Center, Srinakarinwirot University, Bangkok, Thailand, are also acknowledged. In addition, the authors would like to thank Sapthip Lopburi Co., Ltd., Thailand, for kindly providing the seed sludge, fermentation residue and alcohol wastewater used in this study.

5.7 References

1. Kapdan IK, Kargi F: Bio-hydrogen production from waste materials. *Enzyme Microbe Tech* 2006, 38:569-582
2. Luo G, Xie L, Zou Z, Wang W, Zhou Q: Exploring optimal conditions for thermophilic fermentative hydrogen production from cassava stillage. *Int J Hydrogen Energ* 2010, 35:6161-6169
3. Zhang Q, He J, Tian M, Mao Z, Tang L, Zhang J, Zhang H: Enhancement of methane
   production from cassava residues by biological pretreatment using a constructed microbial consortium. *Bioresource Technol* 2011, 102:8899-8906
4. Luo G, Xie L, Zhou Q, Angelidaki I: Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process. *Bioresour Technol* 2011, 18:8700-8706
5. Mabee WE, McFarlane PN, Saddler JN: Biomass availability for lignocellulosic ethanol production. *Biomass Bioenergy* 2011, 35:4519-4529
6. Cao GL, Guo WQ, Wang AJ, Zhao L, Xu CJ: Enhanced cellulosic hydrogen production from lime-treated cornstalk wastes using thermophilic anaerobic microflora. *Int J Hydrogen Energ* 2012, 37:13161-13166

7. Zhao W, Zhang Y, Du B, Wei D, Wei Q, Zhao Y: Enhancement effect of silver nanoparticles on fermentative biohydrogen production using mixed bacteria. *Bioresource Technol* 2013, 142:240-445
8. Mullia P, Yogeswari MK, Sridevi K: Optimisation and enhancement of biohydrogen production using nickel nanoparticles-A novel approach. *Bioresource Technol* 2012, 141:212-219
9. Kim SK, Lee T: Degradation of lignocellulosic materials under sulfidogenic and methanogenic conditions. *Waste Manage* 2009, 29:224-227
10. Lynd LR, Weimer PJ, Zyl WH, Pretorius IS: Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev.* 2002, 66:506-577
11. Cheng CL, Lo YC, Lee KS, Lee DJ, Lin CY, Chang JS: Biohydrogen production from lignocellulosic feedstock. *Bioresource Technol* 2012, 102:8514-8523
12. Pavlostathis SG, Miller TL, Wolin MJ: Cellulose fermentation by continuous cultures of ruminococcus albus and methanobrevibacter smithii. *Appl Microbiol Biotechnol* 1990, 33:109-116
13. Lo YC, Lu WC, Chen CY, Chang JS: Dark fermentative hydrogen production from enzymatic hydrolysate of xylan and pretreated rice straw by Clostridium butyricum CGS5. *Bioresour Technol* 2010, 101:5885-5891
14. Fan YT, Zhang YH, Zhang SF, Hou HW, Ren BZ: Efficient conversion of wheat straw wastes into biohydrogen gas by cow dung compost. *Bioresour Technol* 2006, 97:500-505
15. Su H, Cheng J, Zhou J, Song W, Cen K: Improving hydrogen production from cassava starch by combination of dark and photo fermentation. *Int J Hydrogen Energ* 2009, 34:1780-1786
16. Sigurbjornsdottir MA, Orlygsson J: Combined hydrogen and ethanol production from sugars and lignocellulosic biomass by thermoanaerobacterium AK54, isolated from hot spring. *Appl Energy* 2012, 97:785–791
17. Kim DH, Lee DY, Kim MS: Enhanced biohydrogen production from tofu residue by acid/base pretreatment and sewage sludge addition. *Int J Hydrogen Energ* 2011, 36:13922-13927

18. Poontaweegeratigarn T, Chavadej S, Rangsunvigit P: Hydrogen production from alcohol wastewater by upflow anaerobic sludge blanket reactors under mesophilic temperature. Int. J. Chem. Biol. Eng. 2012, 6:305-308
19. Intanoo P, Rangsunvigit P, Namprohm W, Thamprajamchit B, Chavadej J, Chavadej S: Hydrogen production from alcohol wastewater by an anaerobic sequencing batch reactor under thermophilic operation: Nitrogen and phosphorous uptakes and transformation. *Int J Hydrogen Energ* 2012, 37:11104-11112
20. Argun H, Kargi F, Kapdan IK, Oztekin R: Biohydrogen production by dark fermemtation of wheat powder solution: Effects of C/N and C/P ratio on hydrogen yield and formation rate. *Int J Hydrogen Energ* 2008, 33:1813-1819
21. Hawkes FR, Hussy I, Kyazze G, Dinsdale R, Hawkes DL: Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. *Int J Hydrogen Energ* 2007, 32:174-184
22. Lin CY, Hung WC: Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. *Int J Hydrogen Energ* 2008, 33:3660-3667
23. Kapdan IK, Kargi F: Bio-hydrogen production from waste materials. *Enzyme Microb Technol* 2006, 38569–38582
24. Cheng J, Su H, Zhou J, Song W, Cen K: Hydrogen production by mixed bacteria through dark and photo fermentation. *Int J Hydrogen Energ* 2011, 36:450-457
25. Lee KS, Hsu YF, Lo YC, Lin PJ, Lin CY, Chang JS: Exploring optimal environmental factors for fermentative hydrogen production from starch using mixed anaerobic microflora. *Int J Hydrogen Energ* 2008, 32:1565-1572
26. Hwang JH, Choi JA, Shanab RA, Bhatnagar A, Min B, Song H, Kumar E, Choi J, Lee ES, Kim YJ, Umg S, Lee DS, Joen BH: Effect of pH and sulfate concentration on hydrogen production using anaerobic mixed microflora. *Int J Hydrogen Energ* 2009, 34:9702-9719
27. Eaton AD, Clesceri LS, Rice EW, Greenberg AE: Standard Methods for The Examination of Water and Wastewater. *American Public Health Association, American Water Works Association & Water Environment Federation*, Washington, DC, 2005.

28. Lin L, Yan R, Liu Y, Jiang W: In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: Cellulose, hemicelluloses and lignin. *Bioresource Technol* 2010, 101:8217-8223.
29. Sreethawong T, Niyamapa T, Neramitsuk H, Rangsunvigit P, Leethochawalit M, Chavadej S: Hydrogen production from glucose-containing wastewater using an anaerobic sequencing batch reactor: Effects of COD loading rate, nitrogen content and organic acid composition. *Chem Eng J* 2010, 160:322-332
30. Fang HH, Liu H: Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource Technol* 2002, 82:87-93
31. Lee J: Biological conversion of lignocellulosic biomass to ethanol. *J Biotechnol* 1997, 56:1-24
32. Appels L, Baeyens J, Degrève J, Dewil R: Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog Energ Conbust* 2008, 34:755-781
33. Hawkes FR, Dinsdale R, awkes DL, Hussy I: Sustainable fermentative hydrogen production: Challenges for process optimization. *Int J Hydrogen Energ* 2002, 27:1339-13347
34. Han SK, Kim SH, Shin SH: UASB treatment of wastewater with VFA and alcohol generated during hydrogen fermentation of food waste. *Process Biochem* 2005, 40:2897-2905
35. Guo WQ, Ren NQ, Wang XJ, Xiang WS, Meng ZH, Ding J, Qu YY, Zhang LS: Biohydrogen production from ethanol-type fermentation of molasses in an expanded granular sludge bed (EGSB) reactor. Int J Hydrogen Energ 2008, 33:4981-4988
36. Limwattanalert N., 2011. Hydrogen production from ethanol wastewater by using upflow anaerobic sludge blanket reactor. M.S. thesis, The Petroleum and PetrochemicalCollege, Chulalongkorn University.
37. Gao DW, An R, Tao Y, Li J, Li XX, Ren NQ: Simultaneous methane production and wastewater reuse by a membrane-based process: Evaluation with raw domestic wastewater. *J Hazard Mater* 2011, 186:383-389

38. Song N, Cai HY, Yan ZS, Jiang HL: Cellulose degradation by one mesophilic strain *Caulobacter sp.* FMC1 under both aerobic and anaerobic conditions. *Bioresource Technol* 2013, 131:281-287
39. Kaparaju P, Serrano M, Angelidaki I: Optimization of biogas production from wheat straw stillage in UASB reactor. *Appl Energy* 2010, 87:3779-3783
40. Gadow SI, Jiang H, Hojo T, Li YY: Cellulosic hydrogen production and microbial community characterization in hyper-thermophilic continuous bioreactor. *Int J Hydrogen Energ* 2013, 38:7259-7267
41. Yue Z, Chen R, Yang F, MacLellan J, Marsh T, Liu Y, Liao W: Effect of dairy manure and corn stover co-digestion on anaerobic microbes and corresponding digestion performance. *Bioresource Technol* 2013, 128:65-71
42. Shi X, Kim DH, Shin HS, Jung KW: Effect of temperature on continuous fermentative hydrogen production from *Laminaria japonica* by anaerobic mixed culture. *Bioresource Technol* 2013, 144:225-231
43. Balat M: Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. *Energy Convers. Manage.* 2011, 52:858-875