Chemical Engineering Journal 160 (2010) 322–332

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Hydrogen production from glucose-containing wastewater using an anaerobic sequencing batch reactor: Effects of COD loading rate, nitrogen content, and organic acid composition

Thammanoon Sreethawong[a,b], Tharathip Niyamapa[a], Hannarong Neramitsuk[a], Pramoch Rangsunvigit[a,b], Malinee Leethochawalit[c], Sumaeth Chavadej[a,b,*]

[a] The Petroleum and Petrochemical College, Chulalongkorn University, Soi Chula 12, Phyathai Road, Pathumwan, Bangkok 10330, Thailand
[b] Center for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand
[c] Excellent Center of Waste Utilization and Management, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 83 Moo 8, Thakham, Bangkhuntien, Bangkok 10150, Thailand

ARTICLE INFO

Article history:
Received 7 January 2010
Received in revised form 4 March 2010
Accepted 16 March 2010

Keywords:
Hydrogen production
Anaerobic sequencing batch reactor
Glucose
COD loading rate
COD:N ratio

ABSTRACT

In this work, the process performance of an anaerobic sequencing batch reactor (ASBR) for the hydrogen production from glucose-containing wastewater was studied. The anaerobic sludge taken from a brewery wastewater-treating anaerobic unit was pretreated by boiling for 15 min before being added into the ASBR unit as the seeding sludge. The ASBR unit was operated at a temperature of 37 °C, a hydraulic reaction time (HRT) of 24 h, and different chemical oxygen demand (COD) loading rates from 10 to 50 kg m^{-3} d^{-1} without and with pH control at 5.5. The results showed that at the optimum COD loading rate of 40 kg m^{-3} d^{-1} and pH 5.5, the produced gas was found to contain 44% H_2 and 56% CO_2, and no methane in the produced gas was detected at all operating conditions. At these optimum conditions, the highest hydrogen yield was 1.46 mol H_2/mol glucose consumed. Additionally, the main organic components in the liquid effluent were acetic and butyric acids. Under the optimum COD-to-nitrogen (COD:N) ratio of 100:2.4, the system with pH controlled at 5.5 gave the highest specific H_2 production rate of 7.44 l H_2 l^{-1} d^{-1}, corresponding to the highest butyric acid to acetic acid ratio.

1. Introduction

As known, the global warming problem is mainly derived from the excessive emission of greenhouse gases, especially CO_2, released from the combustion of petroleum-based fuels [1]. A potential means of solving the global warming problem is to replace the petroleum-based fuels with alternative energy resources, which, in turn, can reduce the emission of CO_2 to the atmosphere. Hydrogen, which is ultimately derived from renewable energy sources, is environmentally friendly, gives high energy yield, and can be produced by less energy-intensive processes [1]. Hydrogen is considered as the cleanest burning fuel because it produces only water as a product when being combusted, and it does not produce CO_2. Moreover, hydrogen has high energy content per unit mass (122 kJ/g), which can be directly used in fuel cells for generating electricity [2,3].

Hydrogen can be produced from various processes, and most hydrogen is currently being produced from fossil fuel reforming or water electrolysis. Nearly 90% of hydrogen is commercially produced from the steam reforming of natural gas or light oil fractions [1]. However, these processes are energy-intensive and not environmentally friendly [2]. Biological hydrogen production using fermentative bacteria is a promising means of supplying energy for the future because it is a non-polluting and non-energy-intensive process. This is because it can be operated at ambient temperature and at atmospheric pressure. It is even much more attractive if biomass residues and wastewaters are used as the raw material in the biological hydrogen production [4].

Biological hydrogen production processes can be classified as the biophotolysis of water using algae and cyanobacteria, the photodecomposition of organic compounds by photosynthetic bacteria, and the anaerobic decomposition of organic compounds. The anaerobic biological hydrogen production, or dark fermentation process, is the most effective process compared with light-driven processes because it does not rely on the availability of light sources and transparency of the mixed liquor of the wastewater stream [5,6], and the anaerobic decomposition of organic compounds also provides an acceptable evolution rate of hydrogen [1].

* Corresponding author at: The Petroleum and Petrochemical College, Chulalongkorn University, Soi Chula 12, Phyathai Road, Pathumwan, Bangkok 10330, Thailand. Tel.: +66 2 218 4139; fax: +66 2 218 4139.
E-mail address: sumaeth.c@chula.ac.th (S. Chavadej).

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doi:10.1016/j.cej.2010.03.037

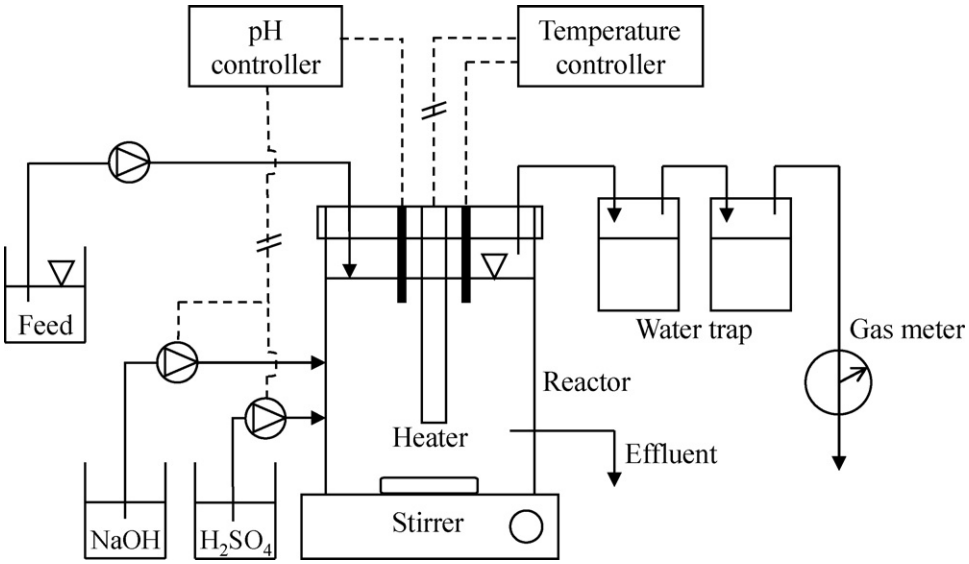

Fig. 1. Schematic of the ASBR system used in this work.

Moreover, this dark fermentation process or anaerobic fermentation provides dual benefits in the environmental aspect along with sustainable energy generation, when it is commonly applied to treating several industrial wastewaters [4]. The anaerobic biohydrogen production comprises two main steps. The first step is hydrolysis, in which particulate organic materials are enzymatically converted to soluble compounds by hydrolytic bacteria. The second step is acidogenesis, in which amino acids, sugars, and fatty acids are further degraded by hydrogen-producing bacteria, and the main products of the fermentation are H_2, CO_2, and organic acids [7]. In addition, methanogenesis can be occurred simultaneously by methanogenic bacteria, which consume the end products from the acidogenesis (i.e. H_2, CO_2, and organic acids) to form CH_4 via both organic acid utilization by acetoclastic methanogens and H_2 utilization (with CO_2) by hydrogen-consuming methanogens [8]. Therefore, the methanogenesis must be completely inhibited in order to achieve a maximum hydrogen production efficiency.

A number of studies have explored the feasibility of using anaerobic hydrogen-producing bacteria to produce hydrogen from organic wastewaters. Most types of reactors used were anaerobic continuously stirred tank reactors (ACSTRs) [9,10], batch reactors [11–13], and anaerobic sequencing batch reactors (ASBRs) [14,15]. The ASBR processes have been reported to offer distinct advantages when compared with the ACSTR processes, including a high degree of process flexibility and no requirement of a separate clarifier [16].

The biological hydrogen production process is greatly influenced by many operational factors, including pH, temperature, oxidation-reduction potential, and nutritional requirements [1,10,17–19]. However, some factors, such as chemical oxygen demand-to-nitrogen (COD:N) ratio, or nutrient supplementation, have not been studied systematically to optimize the process. In this work, the effects of operating parameters (i.e. COD loading rate, pH, and COD:N ratio) on the efficiency of biological hydrogen production from glucose-containing wastewater using an ASBR with mixed culture were extensively investigated. The operating conditions were optimized in order to obtain the highest hydrogen production rate and yield. Moreover, the composition of produced organic acids was analyzed and correlated to the process performance of the studied ASBR system with respect to the hydrogen production in order to obtain a better understanding and to optimize the process.

2. Experimental

2.1. Studied wastewater

Glucose anhydrous (AJAX Finechem) was used as a fermentation substrate because its small molecule can be easily assessed for the process performance of hydrogen production. Supplement nutrients for bacterial growth having compositions of 5.24 g NH_4HCO_3, 0.125 g K_2HPO_4, 0.015 g $MgCl_2{\cdot}6H_2O$, 0.025 g $FeSO_4{\cdot}7H_2O$, 0.005 g $NaHCO_3$, 0.125 mg $CoCl_2{\cdot}5H_2O$, and 6.72 g $CuSO_4{\cdot}5H_2O$ (AJAX Finechem) (per liter of water) were added to the solutions containing different glucose concentrations [20]. The feed solution was freshly prepared and was not used longer than 2 days in order to minimize the fluctuation in the feed composition due to self-biodegradation with time.

2.2. Seed sludge

The seed sludge, which had been collected from the anaerobic treatment plant of the Boonrawd Brewery Co., Ltd., Bangkok, Thailand, was heat-treated by boiling it for 15 min to inactivate any hydrogen-consuming bacteria and to obtain mainly hydrogen-producing bacteria [21]. Analysis of the sludge sample showed that pH and total suspended solids (TSS) of the seed sludge were 6.5 and 35,600 mg l^{-1}, respectively.

2.3. ASBR set-up and operation

The ASBR was constructed of PVC, whose opacity can inhibit the activity of photosynthetic bacteria. The reactor had an inner diameter of 13 cm and a height of 30 cm, and was operated with a working volume of 4 liters. Four sampling ports were located at different heights of the column. A schematic of the ASBR unit is presented in Fig. 1. An electric heater with a thermocouple was used to control the system temperature at 37 °C. The pH of the mixed solution was controlled automatically at 5.5 (in case of pH control) by feeding 1 M NaOH and 1 M H_2SO_4 solutions via diaphragm pumps connected to a pH controller (48PH2, Extech Instruments). For the first start-up, 560 cm^3 of the heat-treated seed sludge was added to the bioreactor. The feed solution having different glucose concentrations was introduced to the bioreactor at any desired total volume of the feed by using a diaphragm pump in order to obtain different COD loading rates, while the system hydraulic retention

Table 1
Operating conditions used in this work.

COD loading rate ($kg\,m^{-3}\,d^{-1}$)	Feed COD concentration ($mg\,l^{-1}$)	Feed glucose concentration ($mg\,l^{-1}$)	COD:N ratio	Temperature (°C)	pH
10	10,000	9,375			
20	20,000	18,750			
30	30,000	28,125	100:2.4	37	Without pH control
40	40,000	37,500			
50	50,000	46,875			
10	10,000	9,375	100:2.4	37	5.5
20	20,000	18,750			
30	30,000	28,125			
40	40,000	37,500			
50	50,000	46,875			
40	40,000	37,500	100:1.4 100:2.4 100:3.3	37	Without pH control
40	40,000	37,500	100:1.4 100:2.4 100:3.3	37	5.5

time (HRT) was kept constant at 24 h. The bioreactor was operated according to a total cycle length of 6 h, consisting of 20 min for feeding, 180 min for reacting, 140 min for settling, and 20 min for decanting. For the feeding and decanting phases, the feed and decant volumes were both adjusted to 1 liter. For each cycle, the total feed volume was controlled by a level probe connected to a diaphragm pump. The liquid in the reactor was homogeneously mixed using a magnetic stirrer at 400 rpm to keep the microbial cells suspended, as well as to provide mixing during the feeding and reacting phases.

2.4. Experimental procedure

In order to investigate the individual effects of three operational parameters (pH, COD loading rate, and COD:N ratio) on the biohydrogen production performance, the following steps were systematically performed. Firstly, the COD loading rate was increased stepwise from 10 to 50 $kg\,m^{-3}\,d^{-1}$ with 10 $kg\,m^{-3}\,d^{-1}$ increments while keeping the temperature and COD:N ratio constant at 37 °C, and 100:2.4, respectively. Secondly, the effect of pH was comparatively investigated by not controlling the system pH and by keeping the pH at 5.5, at which the highest hydrogen production activity was obtained [22–24], at various COD loading rates from 10 to 50 $kg\,m^{-3}\,d^{-1}$. Lastly, to study the effect of COD:N ratio, the COD:N ratio was varied at 100:1.4, 100:2.4, and 100:3.3 (mg/mg) by varying the NH_4HCO_3 amount. Each run was operated both without and with pH control over 2 weeks to ensure that the system reached steady state. The steady state condition was justified when the effluent COD and the gas production rate were nearly invariant with time (with <5% standard deviation from day-to-day analyses). After that, samples of the effluent and produced gas were analyzed and measured. Table 1 summarizes all the operating conditions used for the ASBR system in this work. When the ASBR system reached the steady state under a set of operating conditions, the next set was successively applied for the operation without replacing a new seed sludge.

2.5. Measurements and analytical methods

The gas production rate was measured using a wet gas meter. The composition of the produced gas was analyzed by a gas chromatograph (AutoSystem GC, PerkinElmer) equipped with a thermal conductivity detector and a stainless-steel HayeSep D 100/120 mesh packed column (Alltech). The amount of volatile fatty acids (VFA) in the effluent samples was determined by the distillation and titration method [23]. The organic acid composition of the distilled samples was analyzed by another gas chromatograph (PR2100, Perichrom) equipped with a flame ionization detector and a DB-WAXetr capillary column (J & W Scientific). The mixed liquor volatile suspended solids (MLVSS) and the bioreactor pH were measured according to the standard methods [23]. The COD in the feed or effluent sample was determined by the dichromate method using a COD analyzer (DR/2000, HACH). The amount of glucose in the feed or effluent sample was determined by a glucose assay kit (SIGMA) using a UV–vis spectrophotometer (2550, Shimadzu) [23]. The total nitrogen in the feed solutions was determined by the TNT persulfate digestion method [23]. For each studied condition, the ASBR system was operated to reach steady state before taking data. The experimental data taken from three runs were averaged and used to assess the process performance of the studied ASBR system. As mentioned above, the standard deviation of all experimental data was found to be <5%.

3. Results and discussion

3.1. Effect of COD loading rate on organic removal

Fig. 2 shows the effect of COD loading rate on glucose conversion and COD removal of the two systems without and with pH control. At COD loading rates in the range of 10–40 $kg\,m^{-3}\,d^{-1}$ for both systems (without and with pH control), glucose was converted by more than 98% (Fig. 2a). The high percentage of glucose conversion is due to its small molecules, the smallest of the carbohydrates, so it can easily be consumed by hydrogen-producing bacteria. However, glucose conversion at the highest COD loading rate (50 $kg\,m^{-3}\,d^{-1}$) decreased to 85 and 81.5% for the systems without and with pH control, respectively, suggesting that the system was operated under an overloading condition.

The percentage of COD removal for the system with pH control at 5.5 increased with increasing COD loading rate and reached the maximum value of 80.2% at a COD loading rate of 40 $kg\,m^{-3}\,d^{-1}$ (Fig. 2b), consistent with the increases in H_2 and VFA production, as clearly shown in the following sections. With increasing COD loading rate beyond 40 $kg\,m^{-3}\,d^{-1}$, the COD removal rapidly decreased to 42.1% at a COD loading rate of 50 $kg\,m^{-3}\,d^{-1}$, consistent with the decrease in glucose conversion. In the case without pH control, the system exhibited a similar trend of COD removal efficiency. In a comparison between the systems without and with pH control, both maximum COD removal and optimum COD loading rate increased with pH control.

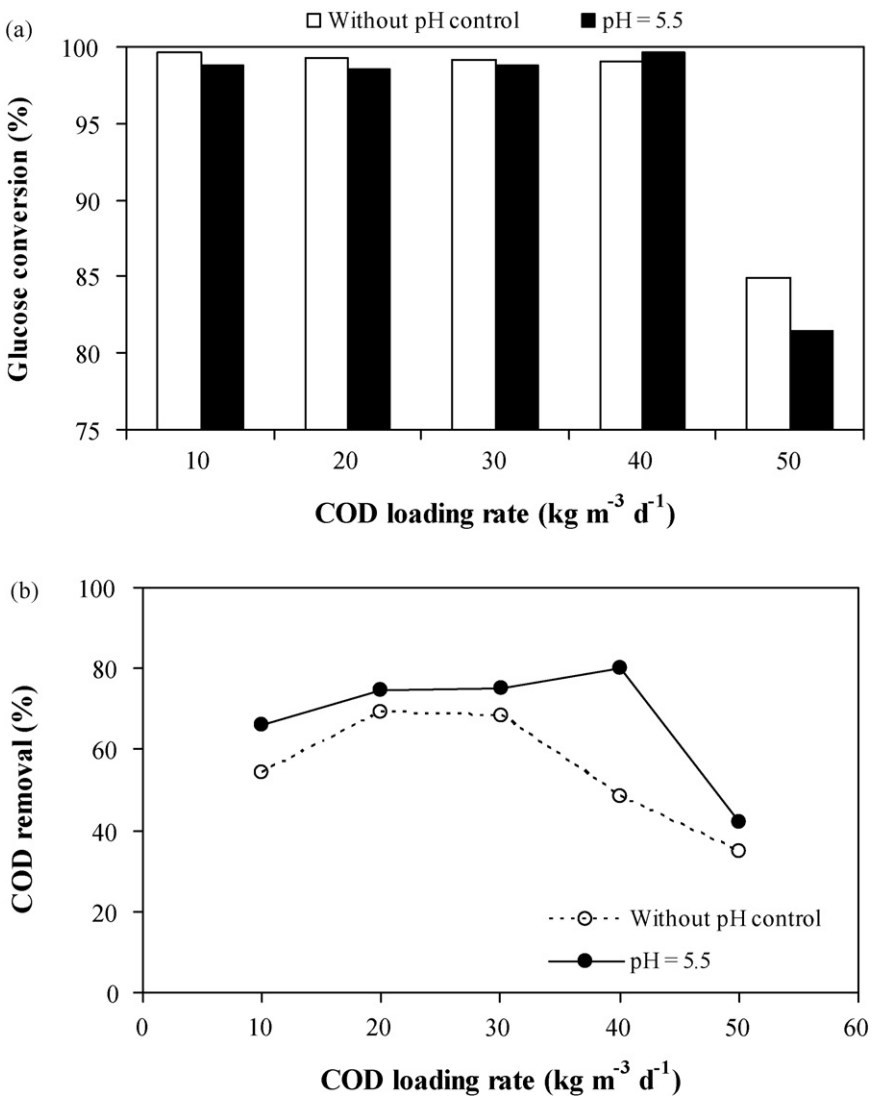

Fig. 2. Effects of COD loading rate and pH on (a) glucose conversion and (b) COD removal at 37 °C and 24 h HRT.

3.2. Effect of COD loading rate on gas production

Fig. 3 depicts the gas production rate, gas composition, specific H_2 production rate, and yield of H_2 production as a function of COD loading rate of the two systems with and without pH control. For the system with pH control, the gas production rate dramatically increased with increasing COD loading rate from 0.41 l h^{-1} at 10 kg m^{-3} d^{-1} to 2.88 l h^{-1} at 40 kg m^{-3} d^{-1} and decreased rapidly to 1.33 l h^{-1} at 50 kg m^{-3} d^{-1} (Fig. 3a). The decrease in gas production rate at high COD loading rates was observed for both the systems with and without pH control. This is due to the toxicity effect of VFA accumulation in the bioreactor, which will be discussed later. The comparative results between the systems without and with pH control at 5.5 show that, at any given COD loading rate in the range of 10–40 kg m^{-3} d^{-1}, the gas production rate increased when the system pH was controlled, especially at a COD loading rate of 40 kg m^{-3} d^{-1}, which is considered to be the optimum COD loading rate for the highest gas production rate (2.88 l h^{-1}). At a COD loading rate of 50 kg m^{-3} d^{-1}, the gas production rate from both the systems without and with pH control decreased substantially because of the overloading effect, as well as the toxicity derived from the excessive amounts of NaOH used for pH adjustment for the case of pH control.

Under the studied conditions, the main components of the produced gas were found to be hydrogen and carbon dioxide (Fig. 3b). Oxygen was detected in trace amounts, which resulted from a leakage of air while transporting the produced gas samples for GC analysis, and can be neglected, and no methane in the produced gas was detected at all operating conditions. This is due to the complete suppression of the methanogenic step at high COD loading rate operation by the toxicity of organic acid accumulation. For the system with pH control, the hydrogen content in the produced gas increased with increasing COD loading rate from 20% at 10 kg m^{-3} d^{-1} to a maximum of 44% at 40 kg m^{-3} d^{-1}; after that, it decreased to 30% at a COD loading rate of 50 kg m^{-3} d^{-1} (Fig. 3b). At a COD loading rate of 50 kg m^{-3} d^{-1}, the hydrogen content from the system without pH control was higher than that from the system with pH control. A possible reason might be the same as explained above for the gas production rate. Moreover, the carbon dioxide percentage showed the opposite trend to the hydrogen percentage, and it reached a minimum of 56% at a COD loading rate of 40 kg m^{-3} d^{-1}.

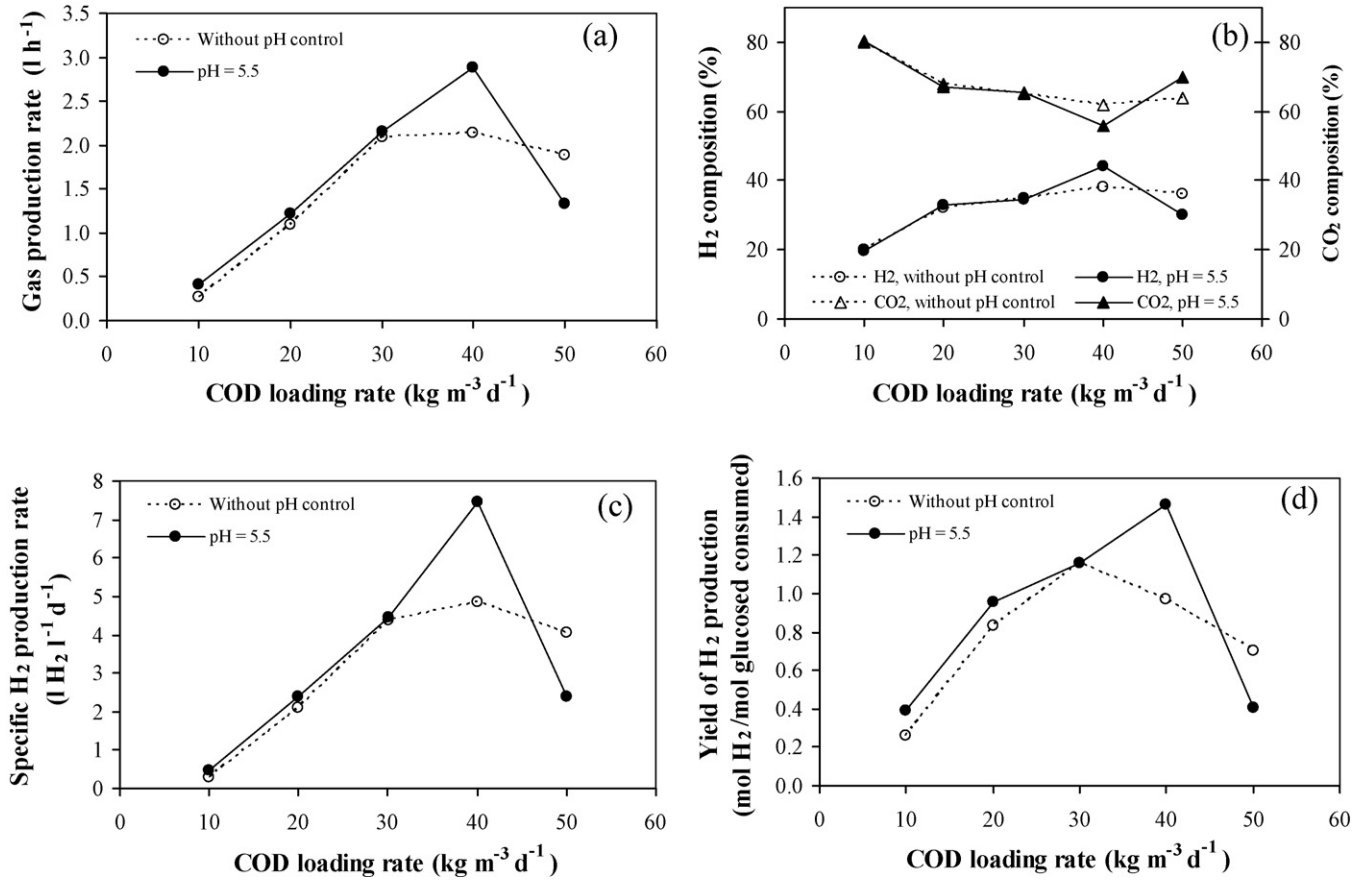

Fig. 3. Effects of COD loading rate and pH on (a) gas production rate, (b) gas composition, (c) specific H_2 production rate, and (d) yield of H_2 production at 37 °C and 24 h HRT.

The specific H_2 production rate, which is calculated from the hydrogen production rate per unit volume of the reactor, was found to be related to the COD loading rate (Fig. 3c). For the system with pH control, the specific H_2 production rate increased with increasing COD loading rate and reached a maximum of 7.44 l H_2 l^{-1} d^{-1} at a COD loading rate of 40 kg m^{-3} d^{-1}, at which the highest gas production rate and highest hydrogen percentage in the produced gas were obtained. Beyond the optimum COD loading rate, the specific H_2 production rate decreased abruptly when the COD loading rate increased from 40 to 50 kg m^{-3} d^{-1} for both systems.

The yield of hydrogen production is defined as the molar ratio of produced hydrogen to glucose consumed. For the system with pH control, the yield of hydrogen increased with increasing COD loading rate from 0.39 mol H_2/mol glucose consumed at 10 kg m^{-3} d^{-1} to reach a maximum of 1.46 mol H_2/mol glucose consumed at the optimum COD loading rate of 40 kg m^{-3} d^{-1} (Fig. 3d). When the COD loading rate further increased to 50 kg m^{-3} d^{-1}, the hydrogen yield decreased drastically. In the case of the system without pH control, the hydrogen yield had a similar trend except that the optimum COD loading rate shifted to 30 kg m^{-3} d^{-1}, corresponding to a maximum hydrogen yield of 1.16 mol H_2/mol glucose consumed. Comparing the two systems, improvement of the process performance, in terms of COD removal and hydrogen production efficiency (hydrogen production rate, specific hydrogen production rate, and hydrogen yield), can be achieved by maintaining the system pH at 5.5. The present results agree well with the work of Fang and Liu [22], which showed an optimum pH at 5.5 for hydrogen production. An explanation of why the system with pH controlled at 5.5 gave the higher process performance will be given in the next section.

3.3. Effect of COD loading rate on VFA concentration and composition, and alcohol concentration

In the acidogenic step, hydrogen can be produced through two basic biochemical reactions, as shown by Eqs. (1) and (2) [10]:

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + 2H_2O \rightarrow \underset{\text{(Acetic acid)}}{2CH_3COOH} + 2CO_2 + 4H_2 \quad (1)$$

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \rightarrow \underset{\text{(Butyric acid)}}{CH_3CH_2CH_2COOH} + 2CO_2 + 2H_2 \quad (2)$$

In this work, VFA is quantified as equivalent acetic acid by using the distillation–titration method [23]. Fig. 4 presents the results of VFA concentration, VFA composition, and alcohol concentration in the bioreactor as a function of COD loading rate of the two systems without and with pH control. For the system without pH control, the VFA concentration in the bioreactor increased from 5,300 to 17,300 mg l^{-1} when the COD loading rate increased from 10 at 40 kg m^{-3} d^{-1}, but it decreased rapidly to 5,800 mg l^{-1} when the COD loading rate further increased to 50 kg m^{-3} d^{-1} (Fig. 4a). A comparison between the two systems shows that the VFA concentration for the system without pH control was much lower than that for the system with pH control. Since the final pH at the steady state of the system without pH control was determined to be 6.67, 5.59, 5.23, 5.23, and 4.98 at the COD loading rates of 10, 20, 30, 40, and 50 kg m^{-3} d^{-1}, respectively. The toxicity of the organic acid accumulation can be reduced by adding NaOH to increase the system pH to 5.5, especially at very high COD loading rates, therefore resulting in higher H_2 production rate, as confirmed experimentally (Fig. 3c). This is due to the fact that the free acid (unionized) form of organic acids is more toxic than the ionized form.

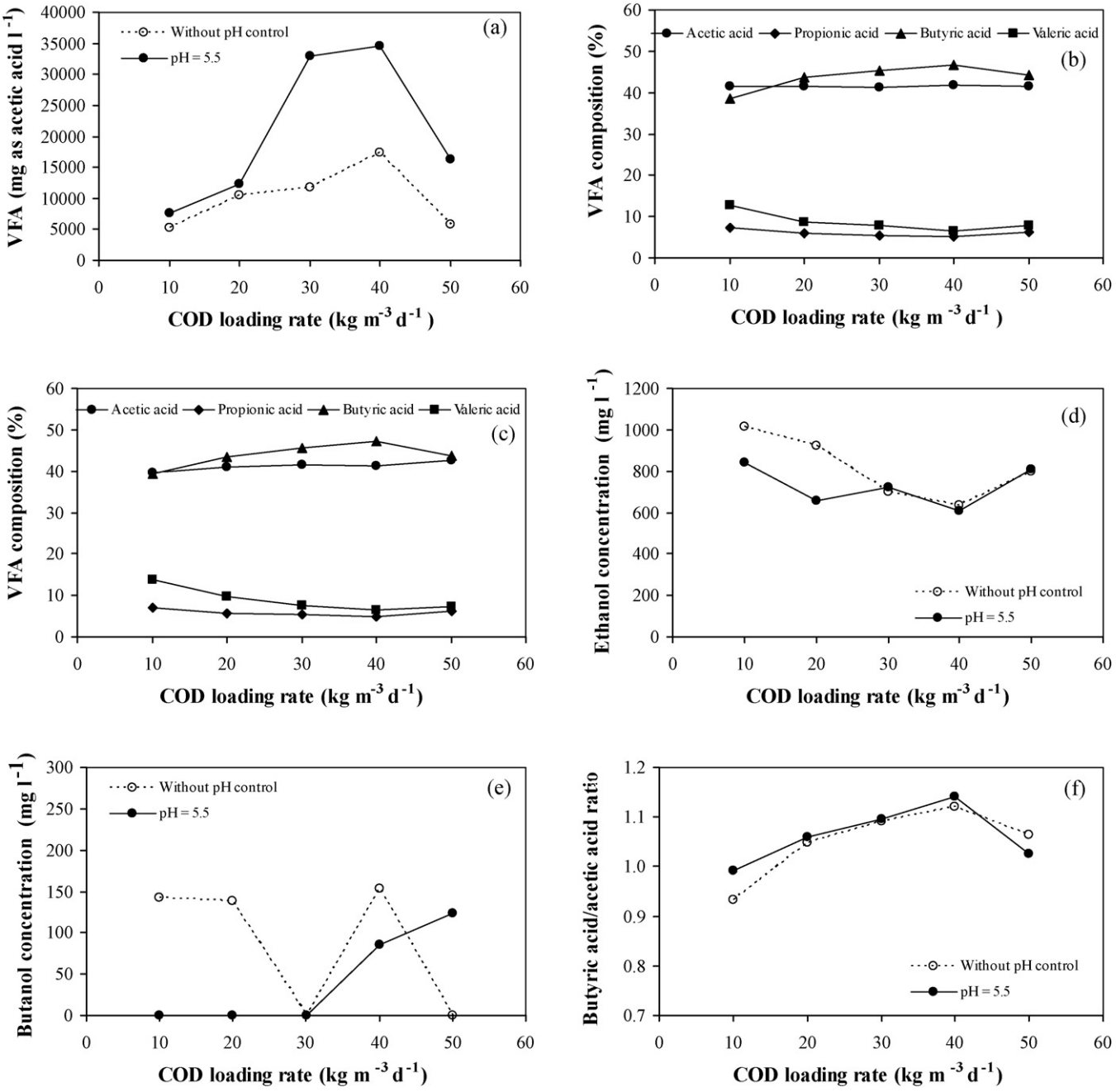

Fig. 4. Effects of COD loading rate and pH on (a) VFA production, (b) VFA composition for the system without pH control, (c) VFA composition for the system with pH control, (d) ethanol concentration, (e) butanol concentration, and (f) butyric acid/acetic acid ratio at 37 °C and 24 h HRT.

Apart from the production of organic acids under the studied conditions, only ethanol and butanol were produced, and higher molecular weight alcohols were not detected. The distribution of organic acids, ethanol, and butanol in the bioreactor is dependent on the COD loading rate and solution pH (Fig. 4b–e). Under the studied conditions, both butyric and acetic acids were found to be the main products of the acidogenic fermentation whereas small amounts of valeric and propionic acids were produced. For the system without pH control, the percentages of butyric and acetic acids increased with increasing COD loading rate and reached a maximum at the optimum COD loading rate of 40 kg m^{-3} d^{-1} (Fig. 4b). At this point, the acetic and butyric acids were 41.7 and 46.8%, respectively. Beyond this optimum COD loading rate, they decreased with increasing COD loading rate. The percentages of propionic and valeric acids, as well as the ethanol concentration, in both the systems decreased slightly with increasing COD loading rate. As shown in Eqs. (1) and (2), hydrogen production is favored when both butyric and acetic acids are predominant. Hydrogen, however, can be consumed during the propionic acid production via Eq. (3):

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + 2H_2 \rightarrow \underset{\text{(Propionic acid)}}{2CH_3CH_2COOH} + 2H_2O \quad (3)$$

Thus, to maximize the biohydrogen production, the production of propionic acid should be avoided or minimized [25]. From the results shown in Fig. 4b and c, for any given COD loading rate, the propionic acid concentration is much lower than the concen-

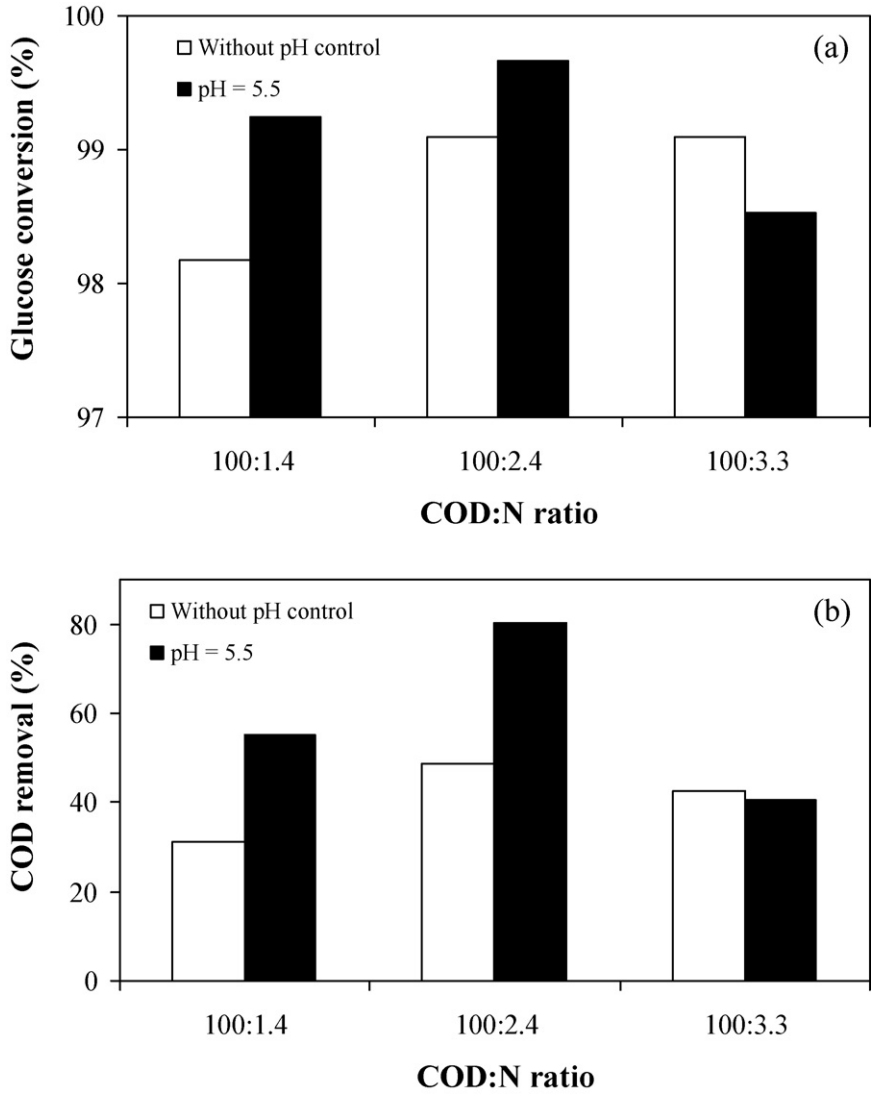

Fig. 5. Effects of COD:N ratio and pH on (a) glucose conversion and (b) COD removal at a COD loading rate of 40 kg m^{-3} d^{-1}, 37 °C, and 24 h HRT.

trations of acetic and butyric acids, suggesting that the rates of hydrogen production by Eqs. (1) and (2) are much higher than the rate of hydrogen consumption by Eq. (3). For the system with pH control (Fig. 4c), the trend of VFA composition is similar to that of the system without pH control. The maximum percentages of butyric and acetic acids were found at a COD loading rate of 40 kg m^{-3} d^{-1} for both systems, and at this optimum COD loading rate, the systems had the lowest concentrations of valeric acid and ethanol. For the produced alcohols, the variation of ethanol concentration for the system with pH control (in the range of 600–850 mg l^{-1}) was found to be less than that for the system without pH control (in the range of 600–1050 mg l^{-1}) (Fig. 4d), whereas the butanol concentration for both systems was found with much smaller amounts only in the range of 0–150 mg l^{-1} (Fig. 4e).

As shown in Fig. 4f, the ratios of butyric acid to acetic acid (B/A) of both systems had a similar trend; it increases with increasing COD loading rate and reaches a maximum at the optimum COD loading rate of 40 kg m^{-3} d^{-1}. Beyond this optimum COD loading rate, the B/A ratio decreased with increasing COD loading rate. The highest specific H_2 production rate and B/A ratio were simultaneously achieved at a COD loading rate of 40 kg m^{-3} d^{-1}. The B/A ratio was also reported to increase with respect to the hydrogen production [3]. Hence, the B/A ratio can be used to indicate the optimum COD loading rate for the hydrogen production.

3.4. Effect of nitrogen content on organic removal

Nutrient supplementation has been used for improving the treatment of wastewater containing relatively resistant organic wastes, including highly concentrated lipid wastes [26]. Nitrogen is classified as a macronutrient, which is one of the necessary nutrients for bacterial growth [27]. The results of the glucose conversion and COD removal for both systems without and with pH control at various COD:N ratios under the optimum COD loading rate of 40 kg m^{-3} d^{-1} are shown in Fig. 5. For any given operating conditions, glucose was degraded by higher than 98%, and the COD:N ratio of 100:2.4 (stoichiometric ratio or optimum ratio for the organic decomposition activity and growth of anaerobic bacteria [28,29]) gave the highest glucose conversion for both systems (Fig. 5a). The slight decrease in glucose conversion at the ratio of 100:1.4 for both the systems possibly results from insufficient nitrogen for bacterial metabolism. Interestingly, in the case of the system with pH control at the lowest COD:N ratio (100:3.3), or the highest nitrogen-to-COD ratio, the glucose conversion decreased as compared with that at the stoichiometric COD:N ratio (100:2.4).

The percentage of COD removal for the system without pH control rapidly decreased from 48.6 to 31.3% when changing COD:N ratio from 100:2.4 to 100:1.4 (Fig. 5b). This trend was also observed

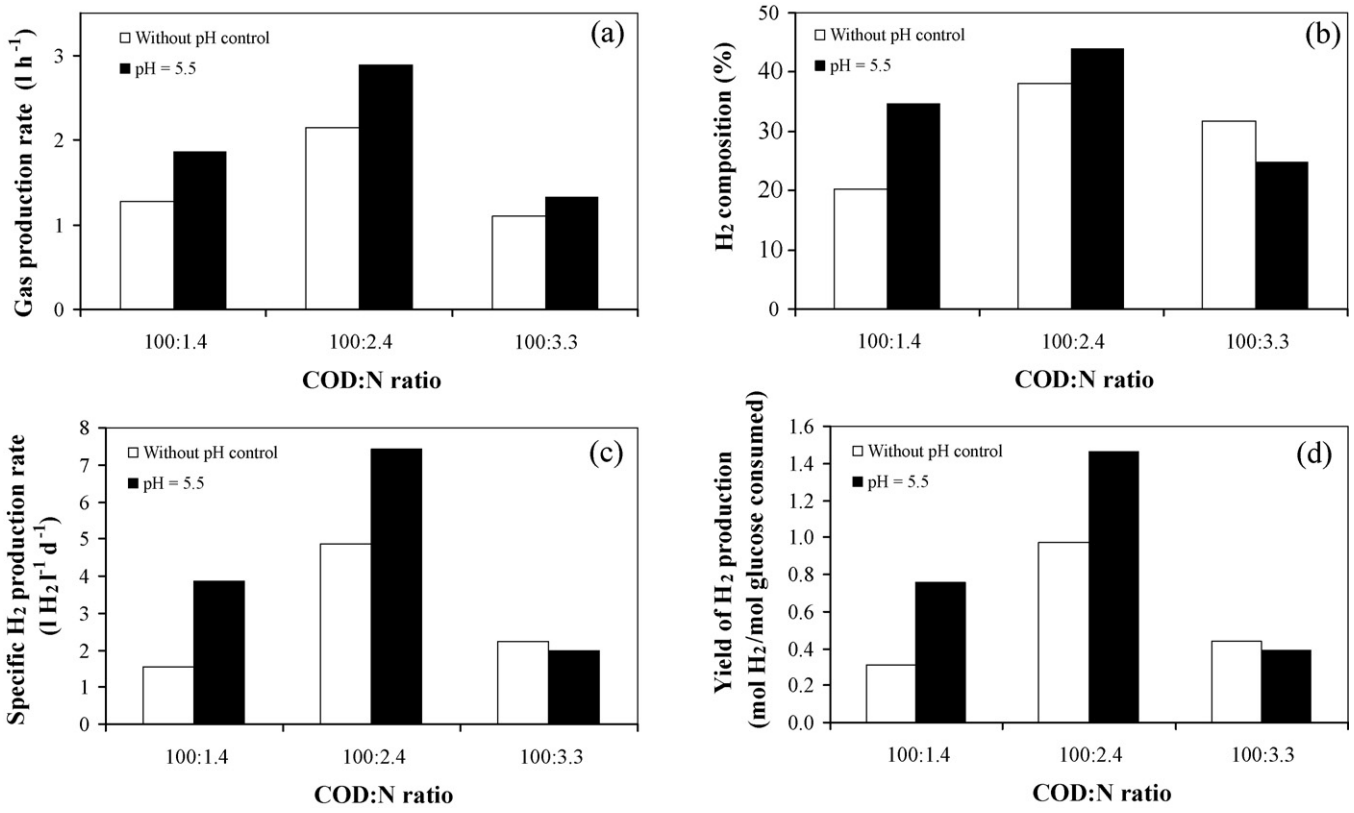

Fig. 6. Effects of COD:N ratio and pH on (a) gas production rate, (b) H_2 composition, (c) specific H_2 production rate, and (d) yield of H_2 production at a COD loading rate of 40 kg m^{-3} d^{-1}, 37 °C, and 24 h HRT.

for the system with pH control, in which the maximum COD removal (80.2%) was obtained at the stoichiometric COD:N ratio of 100:2.4. A possible explanation is the insufficiency of nitrogen for bacterial metabolism at a COD:N ratio of 100:1.4 [27]. Because the hydrogen production reached a maximum at the COD:N ratio of 100:2.4 for the system with pH control (as shown next in Fig. 6), this led to the high COD removal. The increase in the percentage of COD removal when the pH was controlled can be explained by the aforementioned reason whereby the toxicity due to the organic acid accumulation is reduced by adding NaOH to decrease the free acid form, which is more toxic than the ionized form. A further increase in nitrogen at the COD:N ratio of 100:3.3 (greater than the stoichiometric ratio) showed a decrease in the organic removal for both glucose and COD. This can be explained in that, at a COD:N ratio of 100:3.3, the nitrogen content in the feed increased to 1,320 mg l^{-1} in terms of ammonium-nitrogen, which is close to the reported toxicity level of ammonium-nitrogen of 1,500 mg l^{-1} [30].

3.5. Effect of nitrogen content on gas production

Fig. 6 shows the results of gas production rate, H_2 content, specific H_2 production rate, and H_2 yield as a function of COD:N ratio. For the system without pH control, the gas production rate increased from 1.28 to 2.14 l h^{-1} when the COD:N ratio was changed from 100:1.4 to 100:2.4, and rapidly decreased to 1.10 l h^{-1} at a COD:N ratio of 100:3.3 (Fig. 6a). This trend was also observed for the system with pH control, in which the gas production rate increased from 1.86 to 2.88 l h^{-1} when the COD:N ratio was changed from 100:1.4 to 100:2.4, and greatly decreased to 1.33 l h^{-1} at a COD:N ratio of 100:3.3. The aforementioned explanation of the effect of nitrogen content on the organic removal can be used for that on the hydrogen production. The addition of nitrogen to the feed with insufficient nitrogen was also reported to enhance the hydrogen production by stimulating the microflora in the bioreactor and improving their degradation activity [31]. In this present work, a comparison between the systems without and with pH control shows that the gas production rate increased when the system pH was controlled, at all operating conditions, especially at a COD:N ratio of 100:2.4, at which the gas production rate increased to 2.88 l h^{-1}. A possible reason might be the same as explained above for the effect of COD loading rate.

From the gas composition results, H_2 and CO_2 were found to be the main components of the produced gas at different COD:N ratios. Again, a small amount of oxygen was detected as a result of air leakage while injecting the gas samples into the GC. No methane in the produced gas was detected at any of the operating conditions. For the system without pH control, the hydrogen percentage in the produced gas increased with increasing COD:N ratio from 20% at a COD:N ratio of 100:1.4 to 38% at a COD:N ratio of 100:2.4 (Fig. 6b). For the system with pH control, the percentage of hydrogen reached a maximum of 44% at a COD:N ratio of 100:2.4. However, when the COD:N ratio was adjusted to 100:3.3, the percentage of hydrogen in the produced gas decreased to 32% for the system without pH control and to 25% for the system with pH control, due to the toxicity of ammonium-nitrogen in the system. The optimum COD:N ratio for anaerobic fermentation was reported at 100:2.2, as mentioned above, which is relatively close to the condition exhibiting a maximum percentage of hydrogen production at a COD:N ratio of 100:2.4 in this work.

The specific H_2 production rate shown in Fig. 6c was calculated from the gas production rate and the H_2 content. From the results, the COD:N ratio had a significant influence on the specific hydrogen production rate. The specific hydrogen production rate reached a maximum of 7.44 l H_2 l^{-1} d^{-1} at the stoichiometric COD:N ratio of 100:2.4 for the system with pH control, at which the highest hydrogen percentage in the produced gas was also achieved.

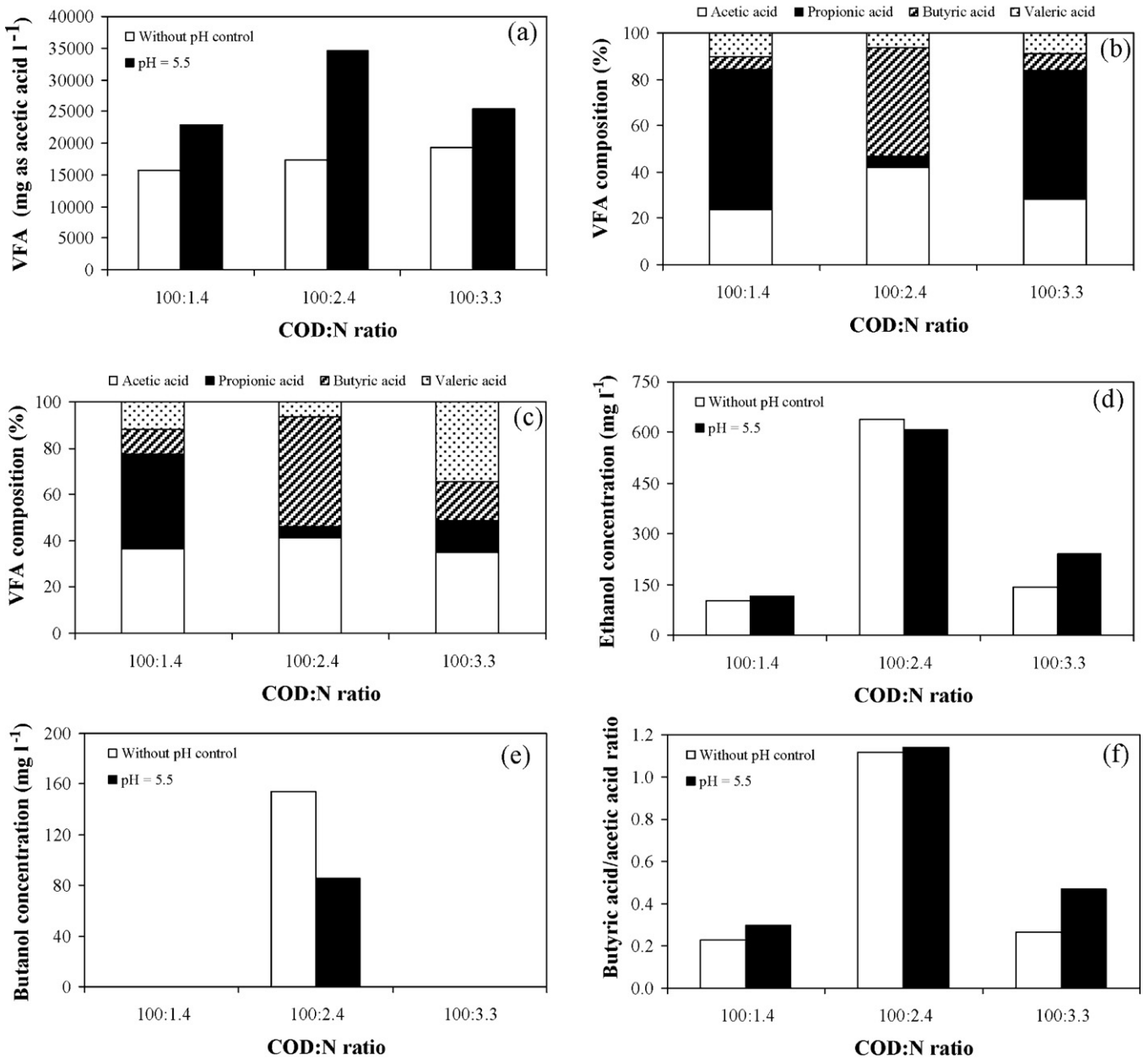

Fig. 7. Effects of COD:N ratio and pH on (a) VFA production, (b) VFA composition for the system without pH control, (c) VFA composition for the system with pH control, (d) ethanol concentration, (e) butanol concentration, and (f) butyric acid/acetic acid ratio at a COD loading rate of 40 kg m^{-3} d^{-1}, 37 °C, and 24 h HRT.

For the system with pH control, the highest yield of hydrogen (1.46 mol H_2/mol glucose consumed) was obtained at the stoichiometric COD:N ratio of 100:2.4 (Fig. 6d). The decrease in the yield of hydrogen at the COD:N ratio of 100:1.4 or 100:3.3 agrees well with the increases in the contents of valeric and propionic acids (as shown next in Fig. 7) because the hydrogen produced is believed to be further utilized to form these acids, as exemplified in Eq. (3). To maximize the hydrogen yield, the system should have high

Table 2
Comparative results of this work and other published works.

Substrate	Reactor	Maximum H_2 yield (mol H_2/mol substrate consumed)	HRT (h)	SHPR[a] (l H_2 l^{-1} d^{-1})	Reference
Glucose	ASBR[b]	1.46	24	7.44	This work
Sucrose	ASBR[b]	2.6	8	10.08	[12]
Sugar wastewater	ACSTR[c]	2.59	12	0.69	[7]
Winery wastewater	UASB[d]	2.14	2	3.81	[31]
Glucose	Batch	2.1	12	3.60	[32]

[a] SHPR, specific hydrogen production rate.
[b] ASBR, anaerobic sequencing batch reactor.
[c] ACSTR, anaerobic continuously stirred tank reactor.
[d] UASB, upflow anaerobic sludge blanket.

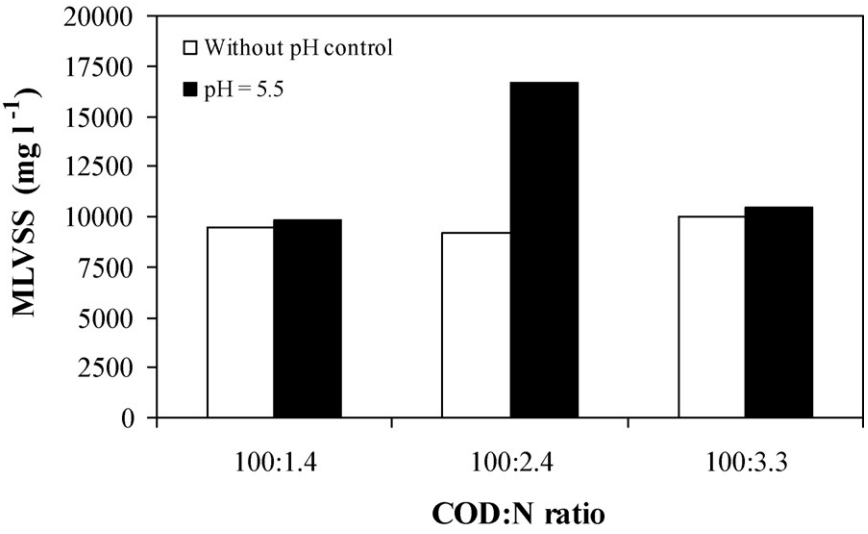

Fig. 8. Effects of COD:N ratio and pH on MLVSS at a COD loading rate of 40 kg m^{-3} d^{-1}, 37 °C, and 24 h HRT.

contents of butyric and acetic acids as the fermentation end products, not propionic and valeric acids. The decrease in the hydrogen yield under either deficient or excess N condition in the present work agrees well with the work of Argun et al. [32], who reported that high nitrogen concentrations inhibited hydrogen formation by dark fermentation probably by shifting the metabolic pathway of microflora.

Comparative maximum hydrogen yields between this work under the optimum conditions and other published works using other types of bioreactors are presented in Table 2. It can be seen that this work gave a low hydrogen yield compared with the others. This is because the experiment was operated at higher HRT as compared to the other work. At a high HRT, the hydrogen production decreases because the produced hydrogen can react with glucose to produce propionic acid (Eq. (3)). When considering the specific hydrogen production rate, a comparatively high value was achieved in this work compared with the others, implying that the ASBR system can provide a higher specific hydrogen production rate than other bioreactor types. Moreover, the ASBR system can be operated at a higher COD loading rate with a smaller bioreactor size due to the high specific hydrogen production rate. To improve the hydrogen yield, the ASBR system operated at a lower HRT will be further studied in our future work. Moreover, the number of cycles per day in the ASBR operation will be further investigated to improve the hydrogen production efficiency.

3.6. *Effect of nitrogen content on VFA concentration and composition, and alcohol concentration*

The experimental results of VFA concentration, VFA composition, and alcohol concentration for the systems without and with pH control at all investigated COD:N ratios are depicted in Fig. 7. For the system with pH control, the VFA concentration increased from 22,900 to 34,600 mg as acetic acid l^{-1} with increasing N content in terms of COD:N ratio from 100:1.4 to 100:2.4 and decreased to 25,500 mg as acetic acid l^{-1} when the COD:N ratio was changed to 100:3.3 (Fig. 7a). On the other hand, for the system without pH control, the VFA concentration slightly increased with increasing COD:N ratio to reach a maximum of 19,300 mg as acetic acid l^{-1} at a COD:N ratio of 100:3.3. The slight increase in VFA concentration with decreasing COD:N ratio for the system without pH control can be explained in that the system pH did not significantly vary (the system pH was found to be 5.38, 5.23, and 5.27 at COD:N ratios of 100:1.4, 100:2.4, and 100:3.3, respectively). The same explanation for the effect of N content on the COD removal can be used for describing the effect of nitrogen content on the VFA concentration. The comparison between the systems without and with pH control showed that the VFA concentration in the system with pH control was higher than that in the system without pH control at any given COD:N ratio. As mentioned above, the pH control at 5.5 can decrease the toxicity of the accumulated VFA, since it reduces the VFA in the free acid form, which is more toxic than the ionized form. As a consequence, the system with pH control has a higher VFA concentration. Therefore, both the specific H_2 production rate and the yield increase since H_2 production always accompanies the VFA production (Eqs. (1) and (2)).

For the system without pH control, propionic acid was the main product of the anaerobic fermentation at the COD:N ratios of 100:1.4 and 100:3.3 (Fig. 7b). These results corresponded well to the decrease in specific hydrogen production rate (Fig. 6c) because hydrogen was utilized to form propionic acid according to Eq. (3). The lowest propionic acid concentration was found at a COD:N ratio of 100:2.4, at which a maximum specific hydrogen production rate of 7.44 l H_2 l^{-1} d^{-1} was obtained. For the system with pH control, the main components of the VFA product were propionic and acetic acids at a COD:N ratio of 100:1.4, and acetic and valeric acids at a COD:N ratio of 100:3.3, whereas butyric and acetic acids were the main components at a COD:N ratio of 100:2.4 (Fig. 7c), the same as those for the system without pH control. The present results suggest that the high concentrations of propionic and valeric acids correspond to the decrease in hydrogen production at deficient and excess N contents. For the ethanol and butanol productions (Fig. 7d and e), the highest ethanol and butanol concentrations were found to correspond to the stoichiometric COD:N ratio of 100:2.4, which provided a maximum hydrogen production, whereas the other COD:N ratios produced much less amounts of both alcohols or even did not produce butanol in the bioreactor. The results imply that the ethanol and butanol productions can be possibly used as an indicator to determine the optimum N content in the system.

As shown in Fig. 7f, the B/A ratios exhibit the same trend as the hydrogen production. For any given COD:N ratio, the B/A ratio of the system with pH control was slightly higher than that of the system without pH control, and the maximum B/A ratio was achieved at a COD:N ratio of 100:2.4 for both systems. From the present results, it can be concluded that both conditions, i.e. nitrogen deficiency and nitrogen surplus, promote propionic acid production, for which hydrogen is simultaneously consumed, leading to the decrease in hydrogen production. Hence, the propionic acid concentration

must be minimized in the operation of acidogenic fermentation to maximize hydrogen production.

3.7. Effect of nitrogen content on microbial concentration

The concentration of hydrogen-producing bacteria can also be quantified by using MLVSS [23]. The experimental data of the MLVSS at various COD:N ratios are shown in Fig. 8. For the system without pH control, the microbial concentration, in terms of MLVSS, only slightly changed with varying COD:N ratio. However, for the system with pH control, the MLVSS significantly increased with adjusting COD:N ratio from 100:1.4 to 100:2.4, and then decreases with further adjusting to 100:3.3. The highest microbial concentration was found to correspond to the stoichiometric COD:N ratio of 100:2.4. The present results also confirm that a pH of 5.5 is suitable for the growth of hydrogen-producing bacteria, as mentioned above [22].

4. Conclusions

In this work, biological hydrogen production from glucose-containing wastewater by dark fermentation using an ASBR system was found to be dependent on several factors, including COD loading rate, pH, and COD:N ratio. For the ASBR system with pH control at 5.5, the results showed that pH control could enhance the hydrogen production by reducing the toxicity from the accumulation of VFA produced from the acidogenesis process. The maximum hydrogen production was achieved at a COD loading rate of 40 kg m^{-3} d^{-1} under pH control at 5.5, 37 °C, and 24 h HRT. The main components of the produced gas were hydrogen and carbon dioxide. Additionally, the main organic acids in the bioreactor were found to be acetic and butyric acids. An insufficient amount of nitrogen in the feed could cause a decrease in both organic removal and hydrogen production efficiency because nitrogen is necessary for bacterial growth and metabolism. A stoichiometric COD:N ratio of 100:2.4 was found to be optimum for the biohydrogen production. At this condition, the hydrogen yield was 1.46 mol H_2/mol glucose consumed, and the highest specific hydrogen production rate was 7.44 l H_2 l^{-1} d^{-1}. The highest hydrogen efficiency was found to correspond to the highest ratio of butyric acid to acetic acid.

Acknowledgments

This work was supported by Thaioil Public Co., Ltd., Thailand and the Sustainable Petroleum and Petrochemicals Research Unit, Center for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, Thailand.

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