Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.868836

53 (2015) 2675–2685
March

Taylor & Francis
Taylor & Francis Group

Simultaneous production of bio-hydrogen and methane from soybean protein processing wastewater treatment using anaerobic baffled reactor (ABR)

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Received 5 June 2013; Accepted 18 November 2013

ABSTRACT

Bio-hydrogen (H_2) and methane (CH_4) co-production from soybean protein processing wastewater (SPPW) was examined using a four-compartment anaerobic baffled reactor (ABR) with the active reactor volume (34 L) under continuous flow condition in this present study. At steady state, the ABR achieved H_2 yields of 25.67 L/d, specific hydrogen production rate of anaerobic activated sludge was 0.28 L/g MLVSS d, CH_4 yields of 13.89 L/d, and chemical oxygen demand (COD) removal of 95% when operated at the organic loading rate of 1.9–2.6 kg COD/m^3 d, hydraulic retention time of 48 h, and temperature of (35 ± 1) °C, respectively. The results showed that the niches of the bio-hydrogen-producing phase and the methane-producing phase in the ABR are different. A high alkalinity in the methanogenic compartment of the ABR was able to secure the pH neutral and methane generation. In general, the ABR proved to be a stable, reliable, and effective process for energy recovery and stabilization treatment of SPPW.

Keywords: Anaerobic baffled reactor (ABR); Soybean protein processing wastewater; Hydrogen production; Methane production; Specific hydrogen production rate

1. Introduction

Energy and environmental security are major problems facing our global economy [1]. The rapid consumption of carbon-containing fossil resources such as oil, coal, and natural gas causes an accelerated release of the bound carbon as CO_2. The resulting increase of the CO_2 concentration in the earth's atmosphere is generally acknowledged as the major cause of global warming and associated climate change [2]. Furthermore, the depletion of fossil resources will cause a shortage of energy carriers in the long term. Considering the energy security and the global environment, there is a pressing need to develop nonpolluting and renewable energy source [3].

Hydrogen is considered as one of the most promising fuels for generalized use in the future, mainly because it is an energy efficient, low polluting, and renewable fuel [4]. The most known industrial methods for producing hydrogen include steam reformation of natural gas, coal gasification, and splitting water with electricity typically generated from carbonaceous fuels [5–9]. In order for H_2 to become a more sustainable source of energy, it should be

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produced through biological routes using wastes [10–13]. Anaerobic fermentation processes to produce hydrogen have been extensively studied over the last decade and show promise for renewable hydrogen production [14–17]. Most effective ways to enhance H_2 production from the anaerobic culture is to restrict or terminate the methanogenesis process by allowing H_2 to become an end product in the metabolic flow [18,19].

An anaerobic baffled reactor (ABR) was developed for the treatment of wastewaters by McCarty in 1981. This process uses a series of vertical baffles to force wastewater to flow under and over them as it passes from the influent to the effluent. The bacteria within the reactor gently rise due to flow characteristics and gas production in each compartment, and settle [20–23]. The most significant advantage is its ability to separate acidogenesis and methanogenesis longitudinally, allowing the reactor to behave as a two-phase system without the associated control problems and high costs. Therefore, organic waste can be converted to hydrogen and volatile fatty acids (VFAs) by fermentative bacteria in the front compartment of the ABR, and the VFAs are further converted to methane by methanogenesis in the subsequent compartment of the ABR [24–26]. Recent years, the study of biological hydrogen production using ABR had been reported [27,28]. ABR is considered superior to continuous stirred tank reactor and up-flow anaerobic sludge blanket (UASB) because of its ability to retain large amounts of biomass in the reactor. Moreover, the ABR has a higher stability to organic and hydraulic shock loads. The advantages make a shift in bacterial population, and allow increased protection against toxic materials and a high resistance to changes in the environmental parameters, such as pH and temperature. The ability to separate acidogenesis and methanogenesis longitudinally down the reactor can permit to control bacterial population especially H_2-producing bacteria to dominate in the reactor [29].

On the other hand, Soybean protein is a kind of main foodstuff additive using soybean as the raw material, and its processing is large industrial consumer of water as well as large producers of wastewater. The effluent from a soybean processing plant has an organic strength of about 10–20 g/L of chemical oxygen demand (COD). Anaerobic process is the appropriate method for treatment and energy recovery from high-strength wastewaters such as soybean protein processing wastewater (SPPW) [30,31].

So far, however, little information is available regarding energy recovery from SPPW in the ABR. Therefore, an attempt was made to investigate the feasibility of hydrogen and methane co-production from SPPW using an ABR in the present study.

2. Materials and methods

2.1. Bioreactor

Schematic details of the experimental setup including bioreactor used in this study are depicted in Fig. 1. The ABR was contained four equal rectangular compartments with 40 cm internal length, 10 cm width, and 50 cm height. Each compartment was further divided into two parts by slanted edge (45°) baffles to encourage mixing within each compartment, and within each compartment downcomer and upcomer regions were created. The liquid flows were alternatively upwards and downwards between compartment partitions. This provided effective mixing and contacting between the wastewater and biomass at the base of each upcomer. It implied the feeding solution contacted with the active biomass during upflow and it was retained with the reactor providing the homogenous distribution of wastewater. The width of the downcomer and upcomer were 2 and 8 cm, respectively. The liquid sampling ports were at 100 mm away from each top of the compartments. The sludge sampling ports were at the bottom of each compartment. The influent feeding was pumped by a peristaltic pump. A sedimentation tank with a volume of 1.5 L was attached to the last compartment for controlling water level and trapping solids. The trapped solids were discharged from the reactor periodically. Gas was collected via porthole in the top of the reactor separately. During the experimental period, the volumes of biogas for each compartment were measured daily by a waterlocks and wet gas meters. The waterlocks and wet gas meters had been filled with water with pH 3.0 in order to prevent the biogas dissolution. The reactor was wrapped by electrothermal wire and the temperature was maintained at 35 ± 1 ℃.

2.2. Soybean protein processing wastewater

SPPW, which was used in this investigation, was obtained from a local soybean protein processing

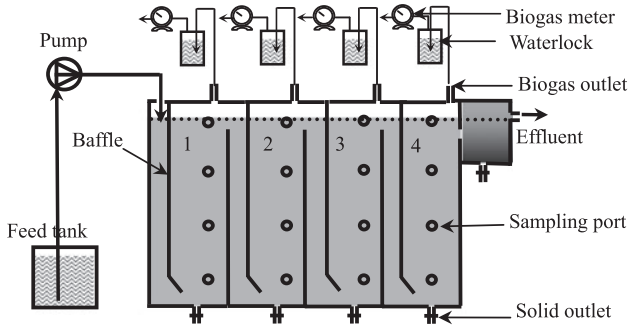

Fig. 1. Schematic details of experimental setup.

plant, and the main characteristics of the SPPW are shown in Table 1. Because the wastewater was able to provide sufficient nitrogen and phosphorus for anaerobic micro-organisms, no additional nitrogen and phosphorus were added and the ABR influent COD: N: P was maintained at 125–250: 80:1. In addition, the microelement solution of 1.0 mL/L was added, which contained (in mg/L): H_3BO_3, 50; $ZnCl_2$, 23; $CuCl_2$, 10; $MnSO_4{\cdot}H_2O$, 50; $AlCl_3$, 50; $CoCl_2{\cdot}6H_2O$, 50; and $NiCl_2$, 50. The raw wastewater was diluted using tap water to required strength.

2.3. Parent mixed cultures

The first and second compartments of the ABR were inoculated with excess sludge taken from a secondary settling tank in a local wastewater treatment plant. The brownish slurry-like sludge was first washed for five times with water, and was then sieved to remove stone, sand, and other coarse matters. The ratio of mixed liquor volatile suspend solid (MLVSS) to mixed liquor suspend solid (MLSS) was 0.66 in the inoculated sludge. The sludge concentrations of the first and second compartments after inoculation were 6.08 g MLVSS/L. The third and fourth compartments of the ABR were inoculated with anaerobic granular sludge from an operating laboratory scale upflow anaerobic sludge blanket reactor treating food wastewater for the past three years, and the sludge concentrations of the third and fourth compartments were 10.30 g MLVSS/L.

2.4. Analytical methods

COD, pH, alkalinity (ALK), MLVSS, MLSS, and oxidation-reduction potential (ORP) were performed according to standard methods [32]. The hydrogen and methane composition was analyzed by a gas chromatograph (GC, Agilent 4890D) equipped with a thermal conductivity detector and a 2 m × 3 mm (i.d.) stainless steel column packed with TDX-01 (80–100 mesh). The temperatures of the injector, detector, and column were kept at 120, 120, and 80˚C, respectively. Nitrogen was used as a carrier gas at a flow rate of 10 mL/min. In the present experiment, the gas phase consisted of H_2 or CH_4, and CO_2. Therefore, by determining the total gas volume and the H_2 or/and CH_4 composition, we could calculate the CO_2 volume and content.

The separation and quantitative determination of the composition of soluble metabolites was performed by another GC (Agilent 4890D) with a flame ionization detector and a 2 m stainless steel column packed with Porapak GDX103 (60/80 mesh). The liquid samples were first centrifuged at 10,000 rpm for 10 min, then acidified with formic acid and filtered through a 0.45 μm membrane, and finally measured for free acids. The operating temperatures of the injector, detector, and column were 200, 220, and 180˚C, respectively. Nitrogen was used as the carrier gas at a flow rate of 3.0 mL/min. The sample injection volume was 1.0 mL. COD, pH, ALK, ORP, VFAs, biogas yield, and its constituents were measured or monitored daily.

2.5. The ABR operation

The investigation was carried out with a constant hydraulic retention time (HRT) and varying influent COD concentration. The ABR was fed with different concentration diluted SPPW (1832–2,400 and 3,868–5,107 mgCOD/L) at constant HRT value (48 h) corresponding to organic loading rate of 0.9–1.2 and 1.9–2.6 kg COD/m^3 d, respectively. The loading rates were only increased when steady-state conditions were obtained for the existing loading condition. When gas production rate, effluent COD, and VSS in the bioreactor became constant, the samples were collected and subjected to the analysis of the following parameters, i.e. feed and effluent COD, effluent total ALK; effluent total VFAs; reactor pH, gas production, and composition.

3. Results and discussion

3.1. Acidogenic compartments of the ABR for bio-hydrogen production

3.1.1. Bio-hydrogen production

After inoculation with the selectively enriched aerobic acidogenic mixed consortia and anaerobic mixed consortia, the ABR was operated initially with

Table 1
Characteristics of SPPW

COD/ (mgL^{-1})	BOD/ (mgL^{-1})	T/˚C	pH	TN/ (mgL^{-1})	TP/ (mgL^{-1})	TSS/ (mgL^{-1})	NH_3–N/(mgL^{-1})	Sugar/%	Protein/ (mgL^{-1})
5,000–16,300	2,250–8,000	25–40	4.2–5	1,700–2,550	125–183	21,400–42,400	71–140	1.2–3.7	14.31–42.4

designed wastewater at OLR of 0.9–1.2 kg COD/m^3 d after adjusting the influent feed pH to 6.7 by $NaHCO_3$ powder for a period of 30 days. Constant COD removal efficiency and gas production were considered as indicators for satisfactory formation of the stability. Subsequently, the bioreactor was shifted to higher OLR 1.9–2.6 kg COD/m^3 d with the same wastewater for a period of 34 days. Experimental data documented the feasibility of fermentative H_2 production along with substrate degradation during operation.

Fig. 2 shows the time course of the hydrogen production in the first and second compartments of the ABR. Hydrogen production rate was stable at 15.05 and 10.62 L/d, respectively. The gas was mainly composed of H_2 and CO_2. The contents of H_2 and CO_2 in gas were 55–58% and 35–40%, respectively. The biomass concentrations of the first and second compartments were 19.27 and 31.35 g MLVSS/L (data not shown), Therefore, the specific hydrogen production rates for the first and second compartments were 0.39 and 0.17 L/g MLVSS d, with the average specific rate of 0.28 LH_2/g MLVSS d in the ABR system. This value is substantially higher than 0.25 LH_2/g MLVSS d from municipal food waste (MFW) in the ABR found by Tawfik et al. [27], 0.25 LH_2/g MLVSS d from organic fraction of municipal solid waste (OFMSW) found by Liu et al. [33] in semi-continuous mesophilic two-stage anaerobic processes, and 0.18 LH_2/g MLVSS d using H_2-producing bacteria from OFMSW under mesophilic conditions obtained by Shin et al. [34]. And it is close to 0.29 LH_2/g MLVSS d from MFW obtained by Han and Shin [35].

3.1.2. VFAs and ethanol in the hydrogen-producing process

The distribution of metabolites is a crucial signal in the assessment of the efficiency of hydrogen production course. Fermentative H_2 production is associated with acid and solvent generation as metabolic intermediates due to the acidogenic metabolism under acidophilic microenvironment. The concentrations of the main metabolic products measured throughout the experiment. The profile of soluble metabolites (VFAs and ethanol) in the first and second compartments at the OLR of 0.9–1.2 and 1.9–2.6 kg COD/m^3 d under steady-state condition is depicted in Fig. 3. In the first

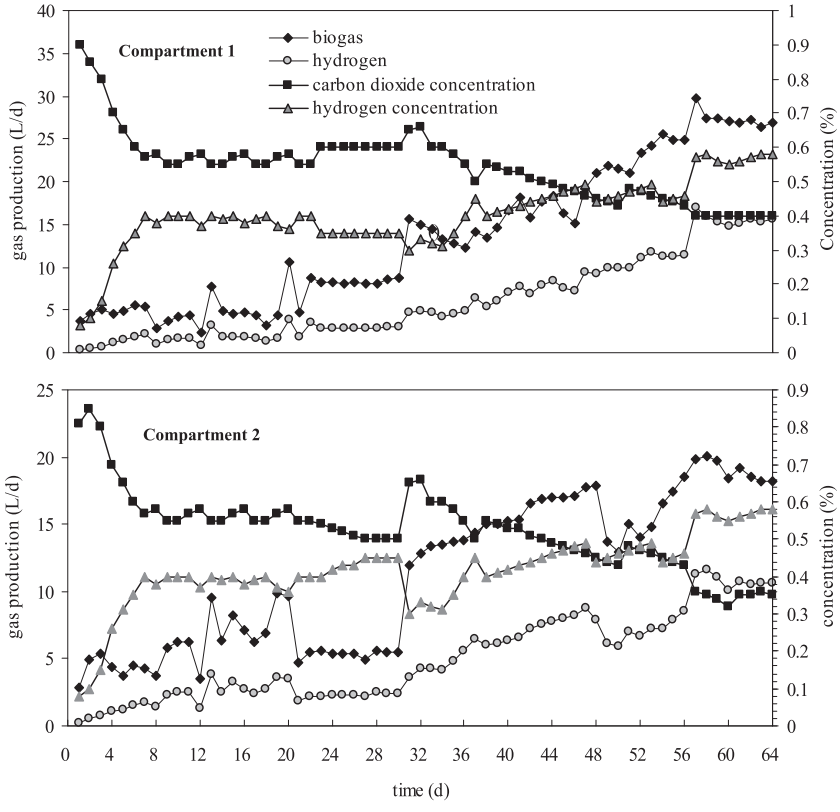

Fig. 2. Variation of gas yields and hydrogen concentration in the hydrogen production process.

compartment of the ABR, the dominant metabolic products were ethanol, acetic acid, propionic acid, and butyric acid, revealing the dominance of ethanol, acetic acid, propionic acid, and butyric acid-producing bacteria in the compartment. The total amount of VFAs and ethanol in the hydrogen production compartment was 2833.61 and 1859.63 mg/L (OLR 1.9–2.6 kg COD/m^3 d), respectively. It also can be seen that the compositions of soluble metabolites in the first and second compartments were similar at different OLR condition, the total VFAs concentrations were increased by improving influent COD concentration. For propionic and butyric acids, there existed a similar variation tendency that was the highest concentration occurred in the first compartment (the ratio of propionic acid and butyric acids to total VFAs was over 60%), and then decreased in the second compartment. This indicated that propionic acids and butyric acids was the main intermediate of acidogenic degradation of SPPW in the first compartment. The concentration of acetic acid in the second compartment reached peak value, which was around 963.23 mg/L (OLR 1.9–2.6 kg COD/m^3 d).

Dark fermentative H_2 release is an anaerobic ubiquitous phenomenon. When bacteria grow on organic substrates, hydrolysis of proteins, lipids, and carbohydrates provides building blocks and metabolic energy for growth. Oxidation of such compounds generates electrons which need to be disposed via the production of fermentation products, including VFAs and H_2 [5,10]. There are three hydrogen-producing pathways by fermentation, namely the decarboxylation of pyruvic acid, the regulation of NADH/NAD^+ equilibrium, and Hydrogen-Producing Acetogens (HPA). HPA connect acidogenic fermentation bacteria with methanogens in functional niche. They can convert intermediate products such as ethanol, propionic acid, and butyric acid into acetic acid, H_2, and CO_2 (Reactions 1–3), which can be metabolized directly by methanogens to methane. In the present study, the concentration of ethanol, propionic acid, and butyric acid dropped from the first compartment to the second compartment and the acetic acid concentration increased in the second compartment, this might be caused by the oxidation of HPA. Ethanol, propionic acid, and butyric acid from the first compartment were converted to acetic acid and hydrogen by HPA through reactions 1–3 in the second compartment, thus achieved a higher hydrogen yield in this compartment (Fig. 2).

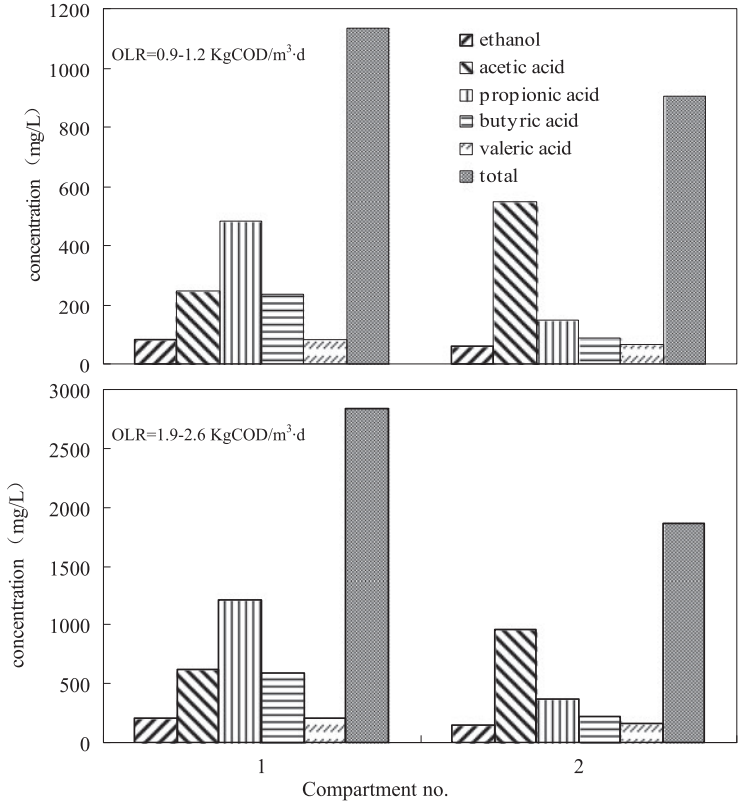

Fig. 3. VFAs and ethanol in the hydrogen production compartments with OLR.

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (1)$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (2)$$

$$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (3)$$

Fermentation process with the formation of butyrate and acetate is one of the most efficient ways for bio-hydrogen production especially by *Clostridium sp.* [36]. The butyric acid/acetic acid ratio has been considered as a crucial indicator for evaluating the efficiency of hydrogen-producing cultures [37]. The ratio of butyric acid/acetic acid was in the range of 1.6–2.2 was reported for H_2 production from a co-digestion of MFW and kitchen wastewater (KWW) using ABR by Tawfik and El-Qelish [27]. According to the study of Ren et al. [38], ethanol-type fermentation was obtained when the mass percentage of ethanol and acetic acid reached above 60% in the total fermentation end product. Ethanol-type fermentation had a higher hydrogen production ability than mixed acid-, butyric acid-, and propionic acid-type fermentations [39]. In the present study, the ratio of butyric acid/acetic acid and mass percentage of ethanol and acetic acid are lower than the values reported by other researchers [27,36,38]. But the results are similar to the study reported by Li et al. [40], which investigated hydrogen production from diluted molasses by anaerobic fermentation bacteria in an ABR with an effective volume of 27.48 L. The results indicate that hydrogen prodution by HPA is an important pathway in the ABR.

3.2. Methanogenic compartments of the ABR for methane production

3.2.1. Methane production

Experimental data also documented the feasibility of utilizing soluble metabolites bound wastewater as substrate for the subsequent production of biogas (CH_4) and additional reduction of substrate (COD). Bioreactor performance data illustrated significant variation in the CH_4 production and substrate degradation during the operation. Fig. 4 shows the time course of the biogas production in the third and fourth compartments of the ABR throughout the experiment. The stepwise increased OLR caused the fluctuation of biogas yields in each compartment of the ABR. The biogas production rate was stable at 14.80 and 0.63 L/d, respectively. The biogas was mainly composed of CH_4 and CO_2. The contents of CH_4 and CO_2 in biogas were 65–68% and 30–35%, respectively.

3.2.2. Conversion of VFAs in the methane-producing process

The profile of soluble metabolites (VFAs and ethanol) in the methane production process at the OLR of 0.9–1.2 and 1.9–2.6 kg COD/m^3 d under steady-state condition is depicted in Fig. 5. It can be seen that the compositions of soluble metabolites in the third and fourth compartments were similar at different OLR condition. During the stable period at the OLR of 0.9–1.2 kg COD/m^3 d, the concentrations of ethanol, acetic acid, propionic acid, and butyric acid in the fourth compartment were 27.27, 57.12, 68.72, and 23.70 mg/L, respectively. The total amount of VFAs and ethanol was 211.29 mg/L. The compositions of soluble metabolites and total VFAs concentrations did not vary when the OLR was further increased from 1.9 to 2.6 kg COD/m^3 d. The variation observed in soluble metabolites concentration suggested that of VFAs and ethane were consumed under methanogenic microenvironment in the process of CH_4 generation.

VFAs are important mid-products in the anaerobic digestion, and their concentrations affect the efficiency of fermentation. The end fermentation products produced in the acidogenesis phase are very important for the whole system performance because they can affect the OLR, efficiency, and running stability of the methanogenesis phase [41]. The conversion rate from VFAs to acetic acid will affect the methanogenic bacteria quantity, and subsequently affect the degradation rate of acetic acid and methane yield [42]. Siegert and Banks [43] considered that VFA concentrations above 2000 mg/L led to inhibition of cellulose degradation, while VFA concentrations above 4,000 mg/L caused only feeble inhibition of glucose degradation. Wang et al. [41] found that when the highest concentrations of ethanol, acetic acid, and butyric acid were 2,400, 2,400, and 1,800 mg/L, respectively, there was no significant inhibition of the activity of methanogenic bacteria. In the present study, the concentrations of acetic acid, ethanol, and butyric acid are lower than the above-mentioned values. This is a main reason for the high efficiency and running stability of the ABR.

Many researchers have considered that propionic acid accumulation in anaerobic reactor can cause acidification and it has been known as an important factor that restricts processing efficiency and operational stability, but their conclusions have been varied. Barredo and Evison [44] pointed out the methanogenic bacteria quantity would fall according to two distinction indexes as the propionic acid concentration

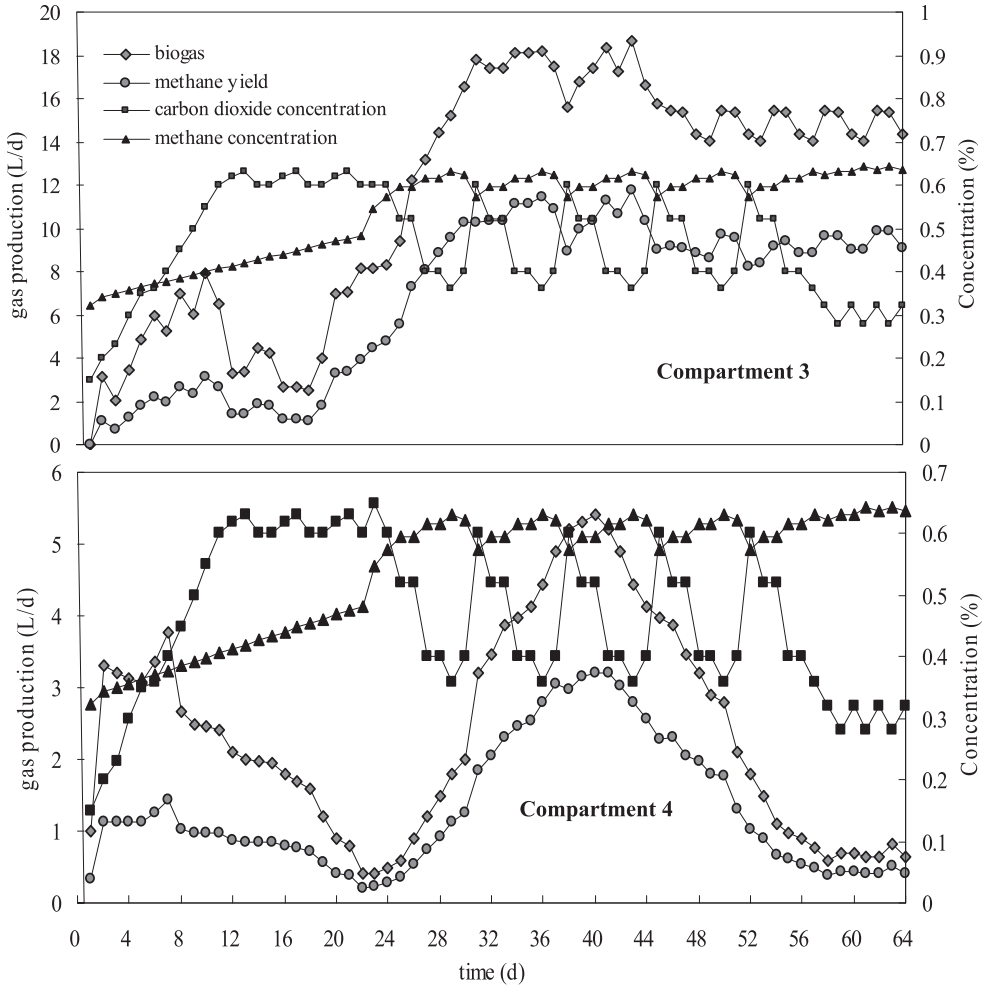

Fig. 4. Variation of biogas yields and gas concentration in the methane production process.

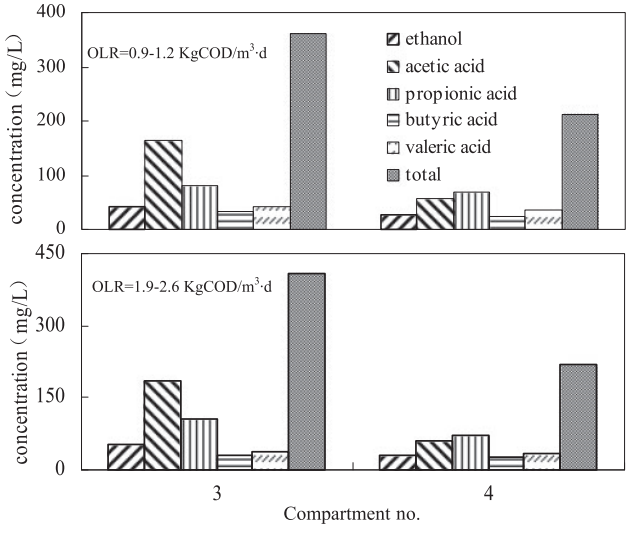

Fig. 5. VFAs and ethanol in the methane production compartments.

increased. Yeole et al. [45] found that when the pH was 7 and the propionic acid concentration was 5,000 mg/L, the methane yield decreased to 22–38% and indicated that the inhibition would be greatly strengthened when pH was decreased. Demirel and Yenigun [46] concluded that propionic acid would inhibit methanogenic bacteria growth when its concentration was above 951 mg/L, while adding butyric acid could improve the inhibition to some extent. However, Pratap et al. [47] increased the propionic acid concentration to 2,750 mg/L and no inhibition appeared. Wang et al. [41] found that when the propionic acid concentration was increased to 900 mg/L, significant inhibition appeared, the bacteria concentration decreased from 6×10^7 to $0.6–1 \times 10^7$/mL and their activity would not reconvert. These effects resulted in the accumulation of ethanol and VFAs, and the total methane yield consequently became very low (<321 mL). An optimization analysis showed that ethanol, acetic acid, propionic acid, and butyric acid at

concentrations of 1,600, 1,600, 300, and 1,800 mg/L, respectively, led to the maximum accumulative methane yield of 1,620 mL and the maximum methanogenic bacteria concentration of 7.3×10^8/mL. In the present study, the propionic acid did not been accumulated in the ABR during the operation. After increasing the OLR, the propionic acid concentrations increased in each compartment. Yet, the propionate acid concentration in the compartment 4 is lower than the compartment 3, and the propionate acid concentration in the compartment 3 is lower than the compartment 2. This indicates that the activity of methanogenesis is high in the ABR system.

3.3. Wastewater treatment process performance evaluation

Performance of the biogas recovery from high-strength wastewater process was also evaluated for substrate degradation potential as COD removal efficiency. The concentrations of COD at influent and effluent in each compartment and the COD removal efficiencies during various operations for the entire length of the study are shown in Fig. 6. The ABR in the first 30 days was run at lower level of OLR and mainly finished the seed sludge culturing. The adaptation period is very important since the bacteria population used as seed is going to be exposed to the anaerobic environment of ABR system. After the acclimatization of the anaerobic activated sludge in 24 days, the ABR was subjected to a steady-state operation and the removal of total COD from the wastewater was remarkable (above 92%); in the later experimental period, the COD removal increased continually when the volume loading rate enhanced, basically about 95%. In the ABR system, the first compartment where acidogenic bacteria were dominant, COD was removed through the cytogenesis and gas releases (mainly CO_2 and H_2) (Fig. 2), while a significant amount of COD was converted to liquid intermediate products (e.g. ethanol, butyrate, and propionate) and stayed in the system; the substrates in second compartment were converted into acetic acid and hydrogen by acetogen and COD was mainly removed through the conversion of intermediate products (e.g. acetic acid) to methane by the methanogenic microbes in the third and fourth compartments. It realized phase separation in the ABR could convert substrate in depth. It is the main reason of achieving high COD removal rate in this ABR system.

The results obtained in the present study are different from many reported studies. Tawfik and El-Qelish [27] found that increasing the OLR from 29

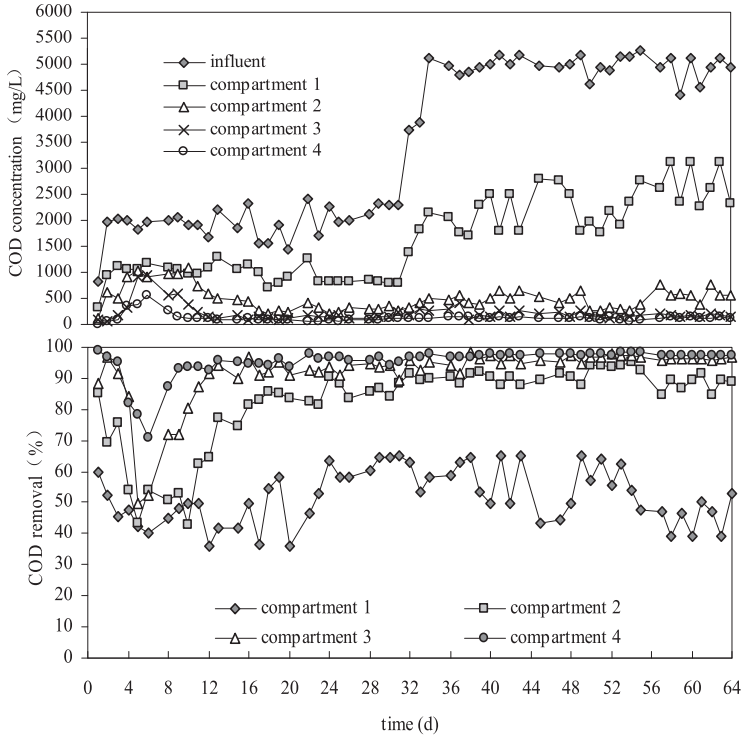

Fig. 6. COD concentration and removal contribution of each compartment in ABR.

to 36 kg COD/m^3 d leads to a significant drop in the H_2 production from 6.0 ± 0.52 to 5.4 ± 0.87 L H_2/d when studying the hydrogen production from MFW and KWW in a mesophillic ABR at constant HRT of 1.6 days, respectively. Further increase in OLR up to 47 kg COD/m^3 d resulted a H_2 production of 5.3 ± 1.04 L/d. Similar trends have been reported in other studies [48,49] where the rate of H_2 production was substantially dropped at high OLR. Ren et al. [50] found that H_2 yield increased up to 16.83 m^3H_2/d with OLR at the range of 3.11–68.21 kg COD/m^3 d, but significantly dropped to 7.38 m^3H_2/d at higher OLR of 85.57 kg COD/m^3 d. It indicated that high OLR will cause the accumulation of VFAs, these affected not only the hydrogen yields but also the COD removal efficiency and running stability.

3.4. Ecological factors characteristic of the hydrogen and methane co-production process

ALK, pH, and OPR were also monitored during process operation in each compartment of the ABR (Fig. 7). Inlet pH of feed in the ABR was adjusted to 6.7 prior to feeding. During the stable operation period of the OLR 1.9–2.6 kg COD/m^3 d, the pH and ORP in each compartment of the ABR were 5.65, 6.89, 7.24, 7.23 and −265, −301, −307, −308 mV, respectively. Ren's [51] study showed that fermentation type in the acidogenic phase can be transformed by changing pH, ORP. Typical butyric acid-type, propionic acid-type, and ethanol-type fermentations occur at the conditions of pH above 6, about 5.5 and below 4.5, respectively. However, Eh ≥ −100 mV always leads to propionic

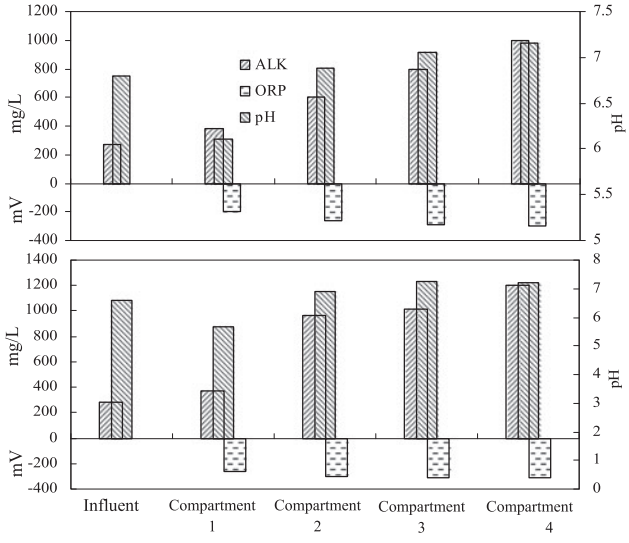

Fig. 7. The variation of pH, ALK, and ORP in the ABR for energy recovery.

acid-type fermentation at pH range 5–6. At pH about 5, either butyric acid-type or propionic acid-type fermentation can dominate, depending on the ORP conditions. In the present study, the first and second compartments of the ABR are mixed-acid fermentation (Fig. 3). The suitable pH and OPR value in the second compartment provided a favorable condition for HPA.

The ALK of wastewater is a measure of its capacity to neutralize acids and is due primarily to the salts of weak acids. If the acid concentrations (H_2CO_3 and VFA) exceed the available ALK, the ABR will "sour". This will be severely inhibiting the microbial activity, especially the methanogens. In the methane fermentation phase, the pH value is elevated, being controlled by the bicarbonate buffering system. During the stable operation period of the OLR 1.9–2.6 kg COD/m^3 d, the ALK value in each compartment of the ABR was 370, 960, 1,010, and 1,200 mg/L, respectively. ALK/COD ratio in the anaerobic reactors may cause minimum pH in the anaerobic reactor to fall below 6.2 which can lead to failure of the system. Souza et al. [52] and Moosbruger et al. [53] found that an ALK/COD ratio 0.5 in the influent decreased the pH to 6.6, which is considered as the lower limit value recommended for anaerobic digestion processes. In our study, these ratios were 0.68 and 0.76 in the third and fourth compartment of ABR, respectively, which were higher than the lower limit value. Behling et al. [54] reported that, if an UASB reactor is stable, the total VFAs/ALK ratio should be between 0.4 and 0.8. In our study, total VFAs/ALK ratios were 0.51 and 0.18 in the third and fourth compartment of ABR, respectively, which indicated that the ABR provided an optimum buffering capacity to convert effectively the VFA and ethanol to methane. In addition, the ALK in the hydrogen production process helps to resist changes in pH caused by the addition of acids as a measure of the stability of the digestion process. The effluent pH and OPR in the second compartment of the ABR also provided a favorable microenvironment for methanogenic bacteria of the third and fourth compartments. Therefore, the ABR achieved a high methane yield and COD removal efficiency.

4. Conclusions

The results of this study indicate that the ABR is a suitable process for producing bio-hydrogen and methane simultaneously from SPPW. At steady state, the ABR achieved specific hydrogen production rate of 0.28 L/g MLVSS d, CH_4 yields of 13.89 L/d, and COD removal of 95% when operated at the organic loading rate (OLR) of 1.9–2.6 kg COD/m^3 d, HRT of 48 h and temperature of (35 ± 1)°C, respectively.

During the stable operation period of the OLR 1.9–2.6 kg COD/m^3 d, the pH, ALK, and ORP in each compartment of the ABR were 5.65, 6.89, 7.24, 7.23; 370, 960, 1,010, 1,200 mg/L, and −265, −301, −307, −308 mV, respectively. A high ALK in the methanogenic compartments of the ABR was able to secure the pH neutral and methane generation. Overall, this work demonstrates that bio-hydrogen production can be very efficiently coupled with a subsequent step of methane production, and SPPW can be an ideal feedstock for the proposed gaseous biofuel production process.

Acknowledgments

This research was supported by the knowledge innovation program of the chinese academy of sciences (Grant No. KZCX2-EW-402-02), the Ningbo Natural Science Foundation (Grant No. 2013A610177), the key project of fujian provincial science & technology program (Grant No. 2012Y0069), and the national natural science foundation of china (Grant No. 21276248, 50808152).

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