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Effects of hydraulic retention time and influent alkalinity on the performance of bio-electrochemical system assisted anaerobic baffled reactor

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ABSTRACT

The effect of hydraulic retention time (HRT) and influent alkalinity on the performance of bio-electrochemical system assisted anaerobic baffled reactor (BES-ABR) was investigated in this study. The results indicated that as the HRT and influent alkalinity increase, COD removal efficiencies were enhanced correspondingly. The maximum COD removal efficiency of 97.4% was obtained at HRT of 48 h and COD of 4,000 mg/L. The maximum COD removal efficiency of 87.5% was obtained at influent alkalinity of 1,300 mg $CaCO_3$/L and COD of 5,000 mg/L. Analysis of microbial community suggested that electrogenic micro-organisms were responsible for the better performance and stability of the system. The establishment and performance of this novel and efficient partial two-phase separation of BES-ABR strongly depended on the regulation of HRT and influent alkalinity.

Keywords: Alkalinity; Anaerobic baffled reactor; Bio-electrochemical system; Hydraulic retention time; Stability

1. Introduction

Anaerobic baffled reactor (ABR), which could be regarded as a suite of upflow anaerobic sludge bed reactors, is a typical multi-phase and high-efficient anaerobic process initially designed by McCarty in 1981 [1]. ABR was initially compartmentalized horizontally by the partition plate while each compartment was then divided into two parts by vertical baffles, forcing the wastewater to flow under and over them as it passed from inlet to outlet [2]. In terms of improving the efficiency and stability of the reactor, ABR has been experienced several innovations in the past 30 years. The reactor was constantly modified in order to increase solids residence time and enhance the contact between micro-organisms and organic matter, then periodic anaerobic baffled reactor, carrier anaerobic baffled reactor, step-feed anaerobic baffled reactor, hybrid MAB-ABR, and modified anaerobic baffled reactor were built up in succession [3–6]. Compared to those well-established anaerobic reactors, ABR has many advantages due to its special characteristics, such as simple configurations to minimum operation and maintenance costs, forward-flowing process to achieve higher treatment efficiency and the partial

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phase separation of different micro-organisms to enhance the stability of the process [7–11].

The configuration of ABR made it possible to separate hydrolysis, acidogenesis, and methanogenesis horizontally down the reactor, increasing the utilization of organic carbon source to large extent. However, acidogens and methanogens still could not grow simultaneously under the optimum conditions, though acidogensis and methanogenesis occur in different compartments horizontally down the reactor. Fermentative acidogens grow faster than methanogens, which are more sensitive to micro-environment, such as ORP (oxidation–reduction potential) substrate and pH. As a result, the organic acids produced in acidogensis could not be utilized by methanogens in time, limiting the further utilization of organic carbon source [12–14]. The accumulation of volatile fatty acids (VFAs) not only inhibits the activity of methanogens but also hold up the reaction of hydrolysis and acidogenesis, interrupting the anaerobic metabolisms or leading to the anaerobic systems fail [15–17].

With the rapid development of microbial fuel cell (MFC) and microbial electrolysis cell (MEC), bio-electrochemical system has been widely researched to the anaerobic digestion of wastewater [18]. Several research have shown that the efficiency of microbial electrocatalysis process was much higher than that of conventional anaerobic treatment methods [19,20]: Yang and Rabaey reported that the rate of decolorization of azo dyes in bio-electrocatalysis was five times higher than traditional anaerobic treatment technology, while the removal of nitrobenzene was seven times higher [19]. Mohanakrishna et al. observed that the removal of distillery wastewater was enhanced (COD, 72.84%) compared to the traditional fermentation process (29.5% improvement) in single-chamber MFC [20]. MEC hydrogen production process has several advantages over traditional anaerobic reactors, such as the ability to use more fermentable substrates and high hydrogen yields [21,22].

In addition, the growth rate of electrogenic micro-organisms was much higher than methanogens. Caccavoet al. reported that the growth rate of *Geobacter sulfurreducens* with acetate as an electron donor reached the highest at the fifth hour of 9.8×10^7 cells/mL, much higher than that of methanogens [23]. The electrogenic micro-organisms have the capacity to use a wider variety of electron donors compared to methanogens, including acetic, formic, propionic, H_2, lactic, butyrate, and so on [24,25]. The optical growth of electrogenic micro-organisms was at 32–35˚C and pH 5.5–7 [26]. Moreover, methanogens have a low growth rate and a high susceptibility to external conditions, and were more sensitive to environmental variation and operating parameters [27]. So compared to methanogens, the ecological niche of electrogenic micro-organisms was wider.

Zou developed a novel process of an ABR combined with MECs and proposed a new anaerobic metabolic pathway degrading high-strength organic matters more efficiently accompanied with pure hydrogen and methane production [28]. In this BES-ABR system, the first compartment served for fermentation and hydrolysis, while the last three compartments acted as three single compartment MEC reactors. The novel process degraded organic matters via the collaboration of hydrolysis and fermentation bacteria, syntrophic acetogens, and electrogenic micro-organisms, getting rid of the dependence of methanogens in conventional anaerobic reactors and having a higher wastewater treatment efficiency.

In this study, the authors explored the effects of hydraulic retention time (HRT) and influent alkalinity on the performance of BES-ABR system in order to obtain the optimal operating parameters. In addition, the distribution and composition of microbial community was well investigated to explore the relationship between microbial and efficiency of the reactor, which would provide theoretical guidance for the establishment and regulation of novel process.

2. Materials and methods

2.1. The configuration of the BES-ABR system

The BES-ABR was constructed from plexiglass with a working volume of 3.46 L and internal dimensions of 24 cm long, 8 cm wide and 18 cm deep. The reactors were rectangular, containing four equal compartments. Each compartment was then divided into down-comer region and riser-comer region by 45˚ angled baffles with the length of 4.80 and 1.20 cm, respectively. Three sample ports were placed in each compartment at identical location. The sample for biogas was in the top of the compartment, whereas the sample for liquid was 2.5 cm apart from the top and the sample for sludge was 3.0 cm apart from the bottom of the compartment. The well-enriched anode films and cathodes were then fixed at each side of the last three compartments and a working potential of 0.9 V was used. At the beginning of the operation, N_2 was fluxed for 20 minutes to each compartment to maintain anaerobic environment. The diagram of the reactor is shown in Fig. 1.

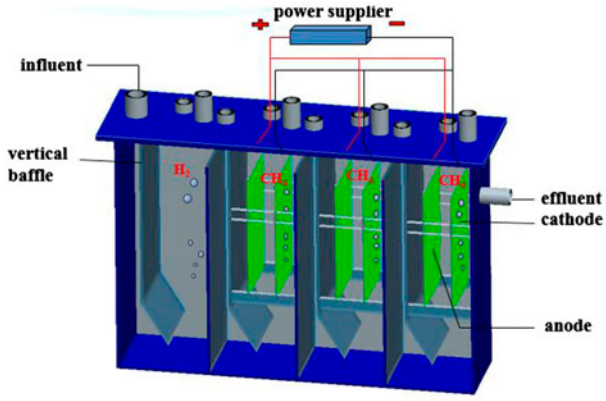

Fig. 1. The diagram of the BES-ABR.

2.2. Operation of BES-ABR system

The BES-ABR was operated continuously by providing the glucose as sole carbon source at different flow rates using a peristaltic pump. Trace elements solution with 1 mL/L was then supplemented to maintain the growth of microbes, which was composed of H_3BO_3 50 mg/L, $CuCl_2$ 30 mg/L, $MnSO_4{\cdot}H_2O$ 50 mg/L, $AlCl_3$ 50 mg/L, $CoCl_2{\cdot}6H_2O$ 50 mg/L, $NiCl_2$ 50 mg/L, and $ZnCl_2$ 50 mg/L. The C:N:P ratio was maintained at 500:5:1 with the addition of NH_4Cl and KH_2PO_4.

The BES-ABR reactor used in this study was proviously used for hydrogen and methane production [28]. After that, the HRT was successively adjusted by peristaltic pump to 12, 24, 36, and 48 h with the COD of 4,000 and 5,000 mg/L (respectively) to investigate the effects of HRT. The alkalinity of the influent was adjusted to 1,000, 1,100, 1,200, and 1,300 mg $CaCO_3$/L by addition of $NaHCO_3$ with the COD of 5,000 mg/L to investigated the effects of influent alkalinity.

The operation parameters of BES-ABR are shown in Table 1. The operating temperature was maintained at 35˚C by heating wire.

2.3. Analysis methods

The physicochemical parameters including COD, pH, and alkalinity were determined accordingly as explained in the American Public Health Association [29]. Biogas and VFA were measured by the gas chromatograph FULI GC9790II, with nitrogen as the carrier gas and hydrogen for burning of the flame. VFA were measured with a flame ionization detector using a capillary column (AT FFAP 30 m × 0.32 mm × 0.50 μm), and the temperature of the column, injector, and detector were 150, 210, and 220˚C, respectively. The sample size used was 5 mL. H_2, CH_4, and CO_2 were monitored with a thermal conductivity detector equipped with a stainless steel column (TDX-01 2 m × 3 mm), and the temperature of the column, injector, and detector were 120, 120, and 150˚C, respectively. The COD, pH, and alkalinity were measured every two days, and the VFA was measured at the end of each condition.

2.4. 16S rDNA gene amplification and denaturing gradient gel electrophoresis (DGGE)

2.4.1. DNA extraction and detection

Total genomic DNA was extracted from 0.5 g granules sampled in the four compartments and from the 9 mm^2 anode film with 3.00 mm wide and 3.00 mm long in the last three compartments using the Soil DNA Kit (OMEGA BIO-TECH) according to the guidelines of the company. The concentration of extracted DNA was measured by Ultramicro ultraviolet spectrophotometer Nanodrop 1000.

2.4.2. The amplification of 16S rDNA in V3 region

The amplification of 16S rDNA in V3 region was performed with the primers 341 F (5′-CCTACGGGAGGCAGCAG-3′) and 517 R (5′-ATTACCGCGGCTGCTGG-3) [30]. Thermal cycling of PCR consisted of 3 min pre-denaturing at 94˚C, 1 min denaturing at 94˚C, 0.75 s of annealing at 54˚C, and extension at 72˚C for 1.50 min with 30 cycles, followed by 5 min at 72˚C for final extension. The PCR reaction mixture (50 μL) contained 0.6 μL forward and reverse primer (each) (10 μmol/L), 5 μL DNA templates, and 25 μL of Thermo Scientific Dream Taq Green PCR Master Mix (2×).

2.4.3. Denaturing gradient gel electrophoresis (DGGE)

Obtained PCR products were subjected to denaturing gradient gel electrophoresis (DGGE), conducted with Dcode Universal Mutation Detection system (Bio-Rad Laboratories, Hercules, CA, USA) according to the method previous described [31]. Then the products were loaded on 8% (w/v) polyacrylamide gels in 1 × TAE solution, and the denaturing gradient ranging from 40 to 60% during DGGE analysis for amplifications of V3-16S rDNA. The electrophoresis lasted 7 h at 150 V and 60˚C, then the specific bands in the gel were cut off, diffused in PCR-grade water, and sent out to be sequenced. At last the bands sequences were analyzed by software Quantity One. The similarity of the assembled sequences was analyzed with the NCBI Blast within the GenBank database.

Table 1
Operation parameters and performance conditions of the BES-ABR

Stage	Days	COD (mg/L)	HRT (h)	Alkalinity (mg $CaCO_3$/L)
Effects of HRT and influent COD on the performance of BES-ABR				
Period 1	1–7	4,000	12	1,300
Period 2	8–13	4,000	24	1,300
Period 3	14–19	4,000	36	1,300
Period 4	20–25	4,000	48	1,300
Period 5	26–31	5,000	12	1,300
Period 6	32–37	5,000	24	1,300
Period 7	38–43	5,000	36	1,300
Period 8	44–49	5,000	48	1,300
Effects of influent alkalinity on the performance of BES-ABR				
Period 9	50–57	5,000	48	1,000
Period 10	58–65	5,000	48	1,100
Period 11	66–73	5,000	48	1,200
Period 12	74–80	5,000	48	1,300

3. Results and discussion

3.1. Effects of HRT and influent COD on the performance of BES-ABR

3.1.1. Effects on COD removal efficiency

The COD removal efficiency was relevant to the HRT and COD concentration. In this study, the total COD removal efficiencies increased with the extending of HRT at the same COD concentration, while the total COD removal efficiencies declined with the increase in COD concentration (as shown in Fig. 2). A maximum COD removal efficiencies of 97.4% was obtained at HRT of 48 h and influent COD of 4,000 mg/L.

When HRT was increased from 12 to 48 h, COD removal efficiencies increased from 58.4 to 97.4% at a COD of 4,000 mg/L (as shown in Fig. 2 Period 1–4), while from 26.4 to 74.6% at the COD of 5,000 mg/L (as shown in Fig. 2 Period 5–8). Similar results have been reported by Kuşçu and Sponza [32], whose research was conducted in a four compartments ABR with a volume of 38.4 L, and their results indicated that the COD removal efficiencies had a significant increase with the extending of HRT at the COD of 3,000–3,500 mg/L. Longer HRT lead to improved COD removal, possibly as a result of the lower flow rate ensuring a better substrate-biomass contact to degrade the COD more efficiently. As previously observed, the BOD removal went from 68 ± 17.5% at a 4 h HRT up to 87.3 ± 2.9% at a12 h HRT, a 22% increase in removal efficiency [33]. However, the results were different from the earlier research in that the COD removal efficiencies were increased when the HRT was decreased from 24–18 h to 12–6 h in a six compartments ABR with a volume of 24 L [34]. This was attributed to that the microbes were hungry at 24 h and when HRT was decreased, it would increase the amount of substrate flowed into the system, ensuring the growth and metabolism of microbes.

When COD concentration increased from 4,000 to 5,000 mg/L, COD removal efficiencies declined from 58.4 to 26.4% at a HRT of 12 h, while from 97.4 to 74.6% at a HRT of 48 h. This probably attributed to that the substrate was not the limiting factor with the COD concentration of 5,000 mg/L, and more VFAs produced in response to the increased COD concentration. The limited biomass in the reactor did not have the ability to decompose the intermediate metabolites timely.

In addition, it was clear from this figure that after the step changes in HRT, the total COD removal efficiency responded primarily by altering the COD removal efficiency of the last three compartments, which was more evident at COD of 4,000 mg/L (as shown in Fig. 2 Period 1–4). But the COD removal efficiency of the first compartment had little fluctuation. As shown in Fig. 3 Period 1–4, the total COD removal efficiency was the lowest at HRT of 12 h about 58.4%, and the COD removal efficiencies of the four compartments were 34.7, 8.8, 14.1, and 18.7%, respectively. This may attributed to that the intermediate metabolites produced in the first compartment was almost not decomposed by micro-organisms in the last three compartments. Similar results have been obtained by Wu et al. [35], whose study indicated that the electrogenic micro-organisms had a longer generation time and they could not degrade VFA effectively

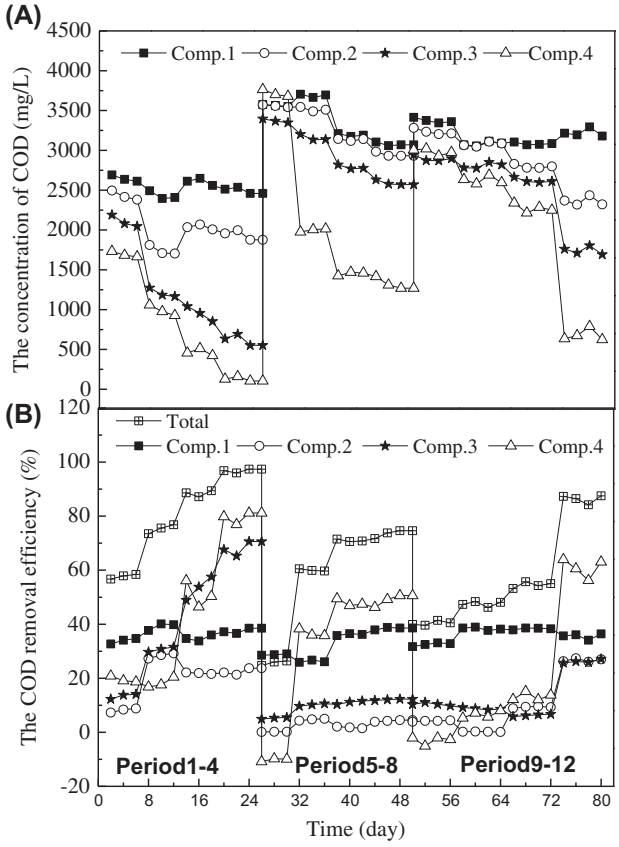

Fig. 2. The COD concentration (A) and removal efficiencies (B) of the BES-ABR.

in short HRT. The maximum COD removal efficiencies of 97.4% was obtained at HRT of 48 h. At this condition, the intermediate metabolites produced in the first compartment was further decomposed by micro-organisms, and the COD removal efficiencies of the four compartments were 38.5, 23.7, 70.6, and 81.2%, presenting the partial phase separation characteristic and achieving the cascade degradation of the substance.

Furthermore, it was apparent that COD removal efficiency dropped dramatically to 26.4% at HRT of 12 h and COD of 5,000 mg/L, and the COD removal in compartment 1 was 29%, while the last three compartments had no significant function. The removal of COD mainly depended on the high activity of hydrolysis fermentation bacteria in compartment 1. Additionally, the COD removal of the last three compartments were 0.2, 5.5, −9.9% respectively, which suggested that electrogenic micro-organisms could not efficiently degrade the VFA and even some of them lost the activity. The COD removal efficiency of the compartment 2 and compartment 3 were almost the same at different HRT with COD of 5,000 mg/L, while the COD removal efficiency of the first compartment increased a little with the extension of HRT (as shown in Fig. 2 Period 5–8). The function of the last three compartments did not present well on the whole. The COD removal efficiency with COD of 5,000 mg/L were lower than that of 4,000 mg/L at all HRTs.

In this study, the partial phase separation was apparent at lower loadings and longer HRT, and the BES-ABR performed best at HRT of 48 h with COD of 4,000 mg/L, consistent with the ecological niche of electrogenic micro-organisms. It was different from the previous reports that the ABR transformed into two-phase reactor with higher loadings and shorter HRT, and that with lower loadings and longer HRT, the ABR served as a completely mixed reactor did not present partial phase separation [36].

3.1.2. Effects on VFA accumulation

From Fig. 3, it could be observed that the VFA varied according to HRT and influent COD concentration.

Acetate and butyrate were absolutely predominant at all conditions, which indicated that the HRT and influent COD concentration had no significant influence on the fermentation type. Afterwards, acetate and butyrate decreased rapidly in the last three compartments, which indicated that the electrogenic micro-organisms gave priority to consume acetate and butyrate rather than propionate and valeric acid. Similar results had been obtained previously, as the ABR transformed to ABR-BES, the concentration of acetate had a substantial decrease, indicating the exoelectrogens on the anodes consuming acetate efficiently [37].

The total VFA increased accompanied with the increase in influent COD concentration, but decreased longitudinally down the reactor. The hydrolysis and fermentation bacteria in the first compartment degraded the organic matters into VFA, which were further consumed by electrogenic micro-organisms in the last three compartments. At higher loadings, the VFA accumulated and resulted in a decrease in pH to further inhibit the activity of electrogenic micro-organisms, so the COD removal efficiency was lower. This was in good accordance with previous reports that the inefficient degradation of VFA by methanogen at higher loadings lead to the anaerobic system souring investing the effects of HRT on VFA at a influent COD of 3,000 mg COD/L [38].

As the HRT extended, the concentration of total VFA in effluent decreased at both COD concentration. The lower concentration of total VFA in the last three compartments indicated the higher activity of

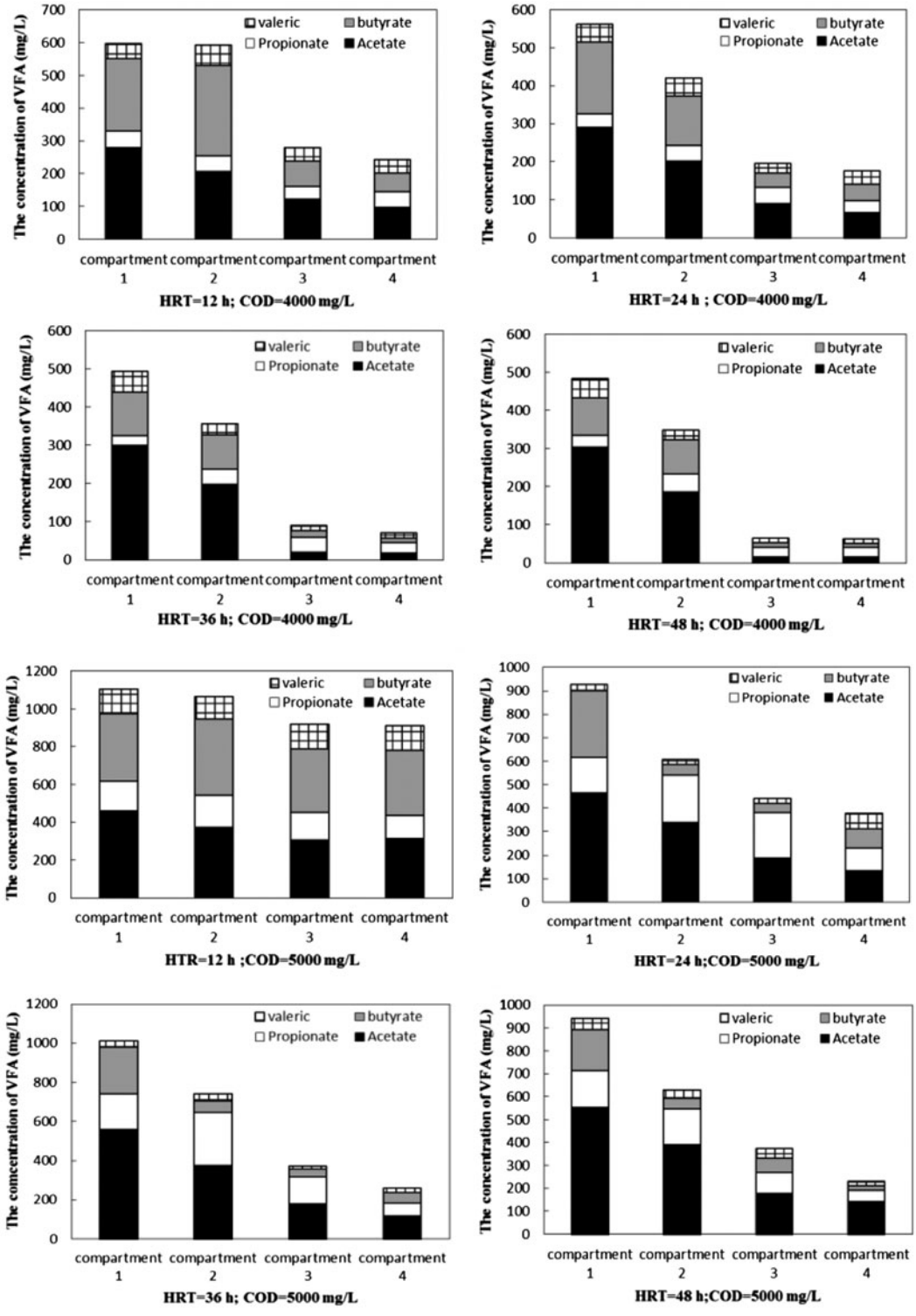

Fig. 3. The profile of VFA at different HRT and COD concentration.

electrogenic micro-organisms at longer HRT. The decrease in VFA was the result of the enhancement of contact between electrogenic micro-organisms and VFA and the better stability of reactor at longer HRT.

3.1.3. Effects on pH

Fig. 4(A) summarized the pH in continuous BES-ABR operation mode. As seen in Period 1–4, the pH increased longitudinally down the reactor at one HRT, and that the pH increased in all compartments with the extending of HRT. The lower pH in the first compartment was due to progressed acid fermentation, which caused an accumulation in VFA concentrations. The VFA produced was further consumed step by step in the last three compartments, resulting in an increase in pH horizontally. At longer HRT, due to the enhancement of contact between electrogenic micro-organisms and VFA, the VFA was degraded sufficiently resulting in the increase in pH.

The pH decreased on the whole when the concentration of influent COD increased to 5,000 mg/L (as seen in Period 5–8). It may attributed to that at higher loadings, much organic matters was degraded into VFA by hydrolysis and fermentation bacteria, and the accumulation of VFA caused the decrease in the pH. This was similar with the previous research that the increase in methanol concentration led to lower pH inside SCMFCs and lower coulombic efficiencies, which might indicate lower activity of electrogenic micro-organisms [39].

At HRT of 12 h with COD of 5,000 mg/L, the pH in the four compartments were 4.97, 4.91, 4.90, and 5.09 respectively, which was not the optimum ecological condition of electrogenic micro-organisms [26]. At this condition, the electrogenic micro-organisms degraded VFA inefficiently, so the minimum removal of COD was presented. At HRT of 48 h with COD of 4,000 mg/L, the pH values in four compartments were 5.75, 7.15, 7.20, and 7.40, respectively. Meanwhile, the contact between electrogenic micro-organisms and VFA was enhanced, resulting in more consumption of VFA. As a consequence, the higher pH maintained high activity of electrogenic micro-organisms and the highest removal of COD was obtained.

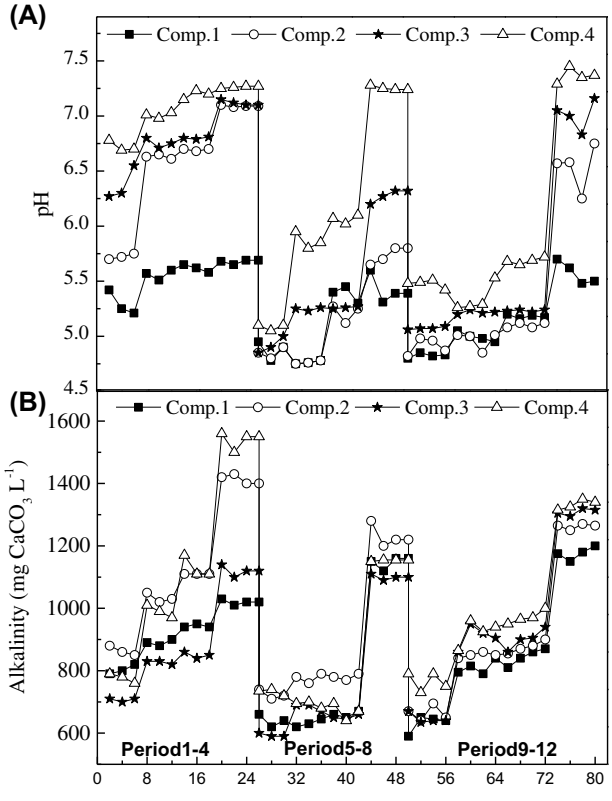

Fig. 4. The pH (A) and alkalinity (B) of the BES-ABR.

3.1.4. Effects on alkalinity

Fig. 4(B) summarized the alkalinity in continuous BES-ABR operation mode. Alkalinity in the same compartment increased with the extending of HRT while the alkalinity in the same compartment decreased at higher loading. This result was consistent with the earlier observations that alkalinity was decreased when the HRT was decreased from 24–18 h to 12 h with a six-compartment ABR with a volume of 24 L [34]. At HRT of 12 h with influent COD of 5,000 mg/L, the alkalinity in these four compartments were 640.4, 693.8, 723.1, and 822.0 mg $CaCO_3$/L, respectively. Lower alkalinity may attribute to the accumulation of VFA, resulting in a weaker buffering capability of the reactor and the minimum removal of COD. At HRT of 48 h with influent COD of 4,000 mg/L, the alkalinity in the four compartments was 1,048.6, 1,235.4, 1,426.05, and 1,674.3 mg $CaCO_3$/L, respectively. The increase in alkalinity could be caused by the fact that the electrogenic micro-organisms consumed lots of VFA, resulting in a better buffering capability of the reactor.

3.2. Effects of influent alkalinity on the performance of BES-ABR

3.2.1. Effect on COD removal efficiency

Influent alkalinity was corresponding to the buffering capacity. When the alkalinity increased, the buffering capacity raised too, enhancing the stability of reactor [40]. The results (as shown in Fig. 2 Period 9–12) indicated that when the influent alkalinity was

increased from 1,000 to 1,300 mg $CaCO_3$/L, the total COD removal efficiencies increased from 40.5 to 87.5% correspondingly at a COD of 5,000 mg/L. In addition, the increase in COD removal efficiencies were mainly due to the last compartment, while the effects of influent alkalinity on the first compartment was negligible. It may attributed to the fact that hydrolysis and fermentation bacteria in the first compartment have a high growth rates and were less sensitive to environmental variation and operating parameters. When the influent alkalinity increased from 1,000 to 1,300 mg $CaCO_3$/L, the COD removal efficiencies of the last compartment increased from −2.7 to 63.1, which was not consistent with the previous study [41]. Their research indicated that the influent alkalinity in the range of 25–50% mg $NaHCO_3$/(mg COD) had little influence on the COD removal efficiencies and the stability of reactor. The different results probably attribute to the configuration of reactor and the characteristic of microbial community, and higher influent alkalinity could well maintained the growth of electrogenic micro-organisms in the last three compartments, achieving better COD removal efficiencies.

3.2.2. Effects on VFA accumulation

The concentration of VFA could reflect the performance of anaerobic reactor [42]. The effect of influent alkalinity on VFA (Fig. 5) showed that acetate and butyrate were absolutely predominant at all conditions, indicating that influent alkalinity had little influence on the type of anaerobic fermentation. With the increase in influent alkalinity, the total concentration of VFA in the same compartment decreased. This is possibly as a result of better ecological environment maintained at higher influent alkalinity and the enhanced activity of micro-organisms. At the same time, the concentration of VFA was decreased longitudinally down the reactor. The best performance of this BES-ABR was achieved at an influent alkalinity of 1,300 mg $CaCO_3$/L, with the concentration of VFA 786.1 and 224.7 mg/L in the first and last compartments. The concentration of VFA in the last two compartments had no significant difference with the influent alkalinity of 1,000, 1,100, and 1,200 mg $CaCO_3$/L, revealing that the activity of electrogenic micro-organisms was inhibited.

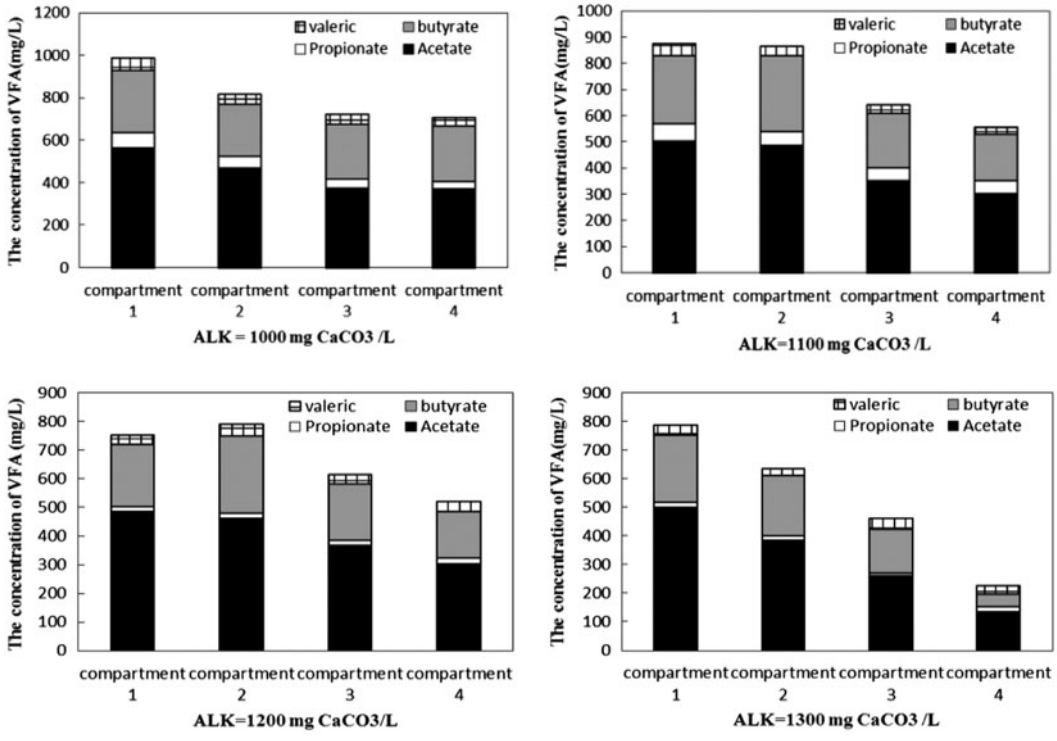

Fig. 5. The profile of VFA at different influent alkalinity.

3.2.3. Effects on pH

The effect of different influent alkalinity on pH in the four compartments is presented in Fig. 4(A) Period 9–12. The pH increased in one compartment correspondingly to the increment in influent alkalinity. The best performance of this BES-ABR was observed at influent alkalinity of 1,300 mg $CaCO_3$/L, with the pH in the four compartments 5.47, 6.67, 7.07, and 7.4, respectively. The range of pH was close to that of traditional ABR. Similar results had been obtained that the pH of the five compartments of ABR were 5.70, 6.60, 7.10, 7.25, and 7.40 at 1.5 kg COD/(m^3 d) treating algae-rich wastewater at stable stage [43]. Hence, it can be concluded that pH in different compartments was suitable both for hydrolysis fermentation bacteria and electrogenic micro-organisms, ensuring the collaboration between them. The most optimal performance of this BES-ABR was achieved with a COD reduction of 87.5% and ideal profiles of pH and VFA in the four compartments at the influent alkalinity of 1,300 mg $CaCO_3$/L. At this influent alkalinity, hydrolysis fermentation bacteria could provide optimal ecological environment. The lowest influent alkalinity was 1,300 mg $CaCO_3$/L for the good performance of this BES-ABR at COD of 5,000 mg/L with pH 5.5 in the first compartment and between 6.5 and 7.5 for the last three compartments.

3.2.4. Effect on alkalinity

As shown in Fig. 4(B) Period 9–12, the alkalinity was increased in one compartment correspondingly to the increment in influent alkalinity and the alkalinity rose gradually longitudinally down the reactor. The good performance of this BES-ABR was achieved at the influent alkalinity of 1,300 mg $CaCO_3$/L with a COD of 5,000 mg/L. At this influent alkalinity, the alkalinity in the four compartments was 750, 920, 1,000, and 1,350 mg $CaCO_3$/L, respectively. The results indicated that it was difficult to maintain a good performance of this BES-ABR when no extra alkalinity was provided.

3.3. Analysis of microbial communities at different HRT

3.3.1. The diversity of microbial communities

For further learning of the microbial communities at different HRT in different compartments, the spatial distributions and abundances of bacteria in each compartment were investigated and the results are presented in Fig. 6. The fingerprints in b-1–b-4 were much more than that of a-1–a-4. It indicated the species diversities at HRT of 48 h were significantly higher than that of 12 h and this was well agreed with a better removal of COD at longer HRT. Similar results had been obtained by Han et al., whose research indicated that a shorter HRT supported fewer microbial species due to the fewer available carbon source [44]. Moreover the higher species diversity resulted in better stability and performance of the reactor, consistent with the higher alkalinity at longer HRT. There were two possible explanations for this result. First, a shorter HRT might result in the washout of microbial species, so the degradation of the COD had a decrease. Second, a shorter HRT might lead to a weak interaction between substrate and micro-organisms, which had a negative effect on the removal of COD. Additionally, the bacterial communities in the last three compartments varied to a large extend under the two HRTs, while it had no significant difference in the first compartment. Hence, it can be concluded that the succession of bacterial communities in the last three compartments was responsible for the performance of the BES-ABR at different HRTs.

3.3.2. The homology of microbial communities

According to the intensity of various bands on the DGGE fingerprints, a total of 12 bacteria typical bands (1–12) were excised, re-amplified, purified, and sequenced as shown in Table 2. The bands 1, 2, and 3, mainly served as functional community in traditional anaerobic digestion, existed in all four compartments at HRT of 12 h, while disappeared in the last three compartments at HRT of 48 h. So those three bacteria were replaced by other community and did not express as the dominant bacterial communities at HRT of 48 h in the last three compartments. Bands 4 and 5, proved to be H_2-producing bacteria, were only appeared in the first compartment. Band 6 to band 12 proved to have high electrical activity. However, the bands 6, 7, and band 8 only appeared in the last three compartments, and their intensity was much weaker at HRT of 12 h compared to HRT of 48 h, which indicated that the electrogenic micro-organisms had higher activity at HRT of 48 h. Band 9 to band 12 presented only in the last three compartments at HRT of 48 h, indicating those four bacteria were inhibited at HRT of 12 h, which was responsible for the lower removal efficiency of COD. More research was needed to identify the function and characteristic of those microbes, further to optimize the performance of BES-ABR.

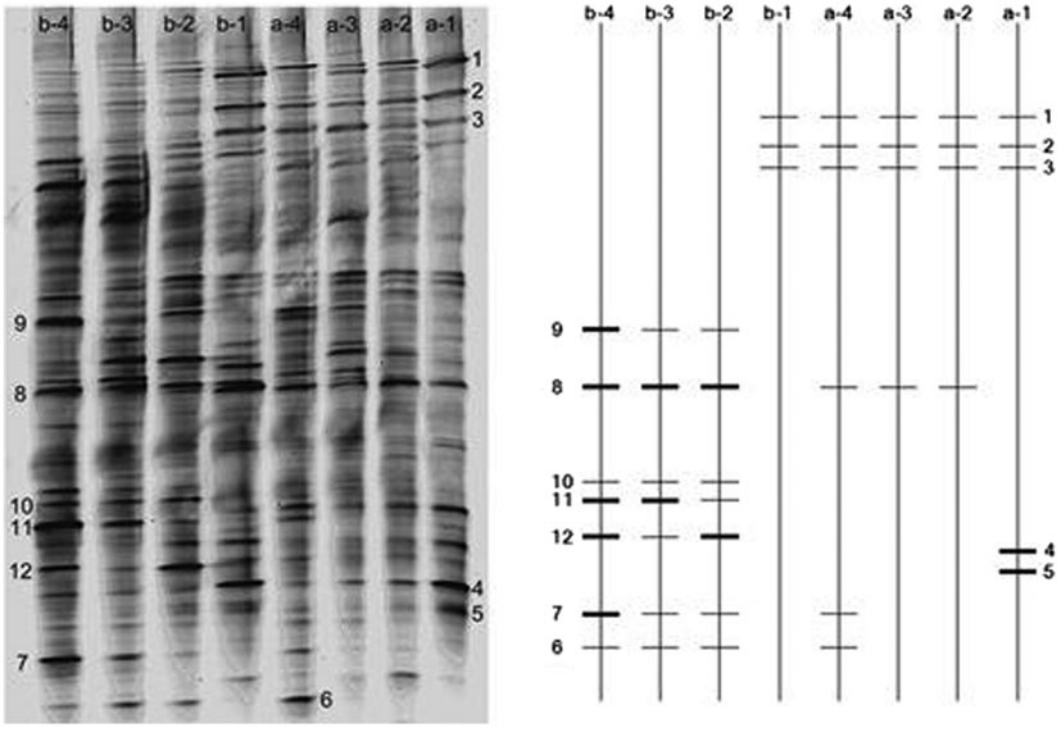

Fig. 6. PCR-based DGGE fingerprints of 16S rDNA at different HRT (a-1–a-4 and b-1–b-4 were the samples from compartment 1 to compartment 4 at HRT of 12 and 48 h, respectively).

Table 2
BLAST results of excised bands

Band no.	Identified bacterium	Similarity (%)	Length (bp)	Genebank no.
1	Uncultured bacterium	96	132	EU670685.1
2	*Pectobacterium* sp.	95	1,388	GQ205111.1
3	Uncultured *Bacteroides* sp.	96	841	GQ332238.1
4	Uncultured bacterium	97	526	DQ464500.1
5	Uncultured *clostridium* sp.	98	151	AM396265.1
6	*Clostridium favososporum*	95	825	JQ897403.1
7	Uncultured bacterium isolate	98	169	KF680911.1
8	*Citrobacter* sp. Z7	97	1,405	JX185134.1
9	*Spirochaetaceae* bacterium	95	1,504	JX828432.1
10	Uncultured *Clostridium* sp.	96	182	FR870457.1
11	Uncultured bacterium	96	173	KF680918.1
12	Uncultured *Firmicutes* bacterium	99	196	KC770783.1

3.3.3. Clustering pattern analysis of 16S rDNA fingerprints

Fig. 7 shows the UPGMA analysis results based on DGGE. It can be found that the a-1 and b-1 showed relatively high similarity (similarity index, 0.84), indicating the bacterial communities in compartment 1 stayed relative stable at different HRT. In addition, both a-1 and b-1 had lower similarity (similarity index, 0.21) with the last three compartments, revealing a partial two-phase separation of different micro-organism performed well. Furthermore, the similarity index of the last three compartments at different HRT was only 0.4, demonstrating the change in HRT had a significant influence on the composition of bacterial communities in the last three compartments, which agreed well with the overall trend of the profile of COD.

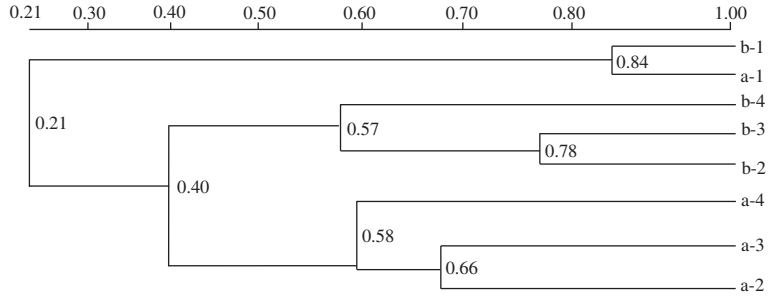

Fig. 7. Cluster analysis obtained from the DGGE profiles based on the averaged similarity Matrix at different HRT (a-1–a-4 and b-1–b-4 were the samples from compartment 1 to compartment 4 at HRT of 12 and 48 h, respectively).

4. Conclusion

The performance of this novel BES-ABR strongly depended on the regulation of HRT and influent alkalinity. When alkalinity was increased from 1,000 to 1,300 mg $CaCO_3$/L, COD removal efficiencies were increased from 40.5 to 87.5% at a COD of 5,000 mg/L, indicating BES-ABR held some promise for high-strength wastewater treatment. When HRT was increased from 12 to 48 h, COD removal efficiencies were increased from 58.4 to 97.4% at a COD of 4,000 mg/L, while from 26.4 to 74.6% at the COD of 5,000 mg/L. Furthermore, the influence of HRT and influent alkalinity was more evident on electrogenic micro-organisms in the last three compartments, and the increased abundance of electrogenic micro-organisms was responsible for the better performance and stability.

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