Bioaugmentation for improving acidification recovery of an anaerobic sequencing batch reactor after organic shock load

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abstract

Bioaugmentation was investigated as a method to accelerate the recovery of an anaerobic sequencing batch reactor (ASBR) exposed to the organic shock load. Two sets of ASBRs were subjected to double organic load that influent chemical oxygen demand (COD) concentrations were increased from $4,000 \pm 100$ to $8,000 \pm 150$ mg L^{-1} for a week. Bioaugmented reactor received the enriched butyric acid-utilizing syntrophic culture (10% w/w) and evolutions of microbial community were analyzed by a quantitative polymerase chain reaction. Aftershock load, the performance of acidification was attained with the effluent COD concentrations increased from 70 to 5,300 mg L^{-1} and pH decreasing from 7.1 to 5.1 approximately in two reactors. *Smithella* was badly inhibited by the VFAs accumulation and high H_2 partial pressure. Bioaugmented reactor achieved significantly less time to recover than the non-bioaugmented reactor, 40 and 110 d respectively. The rapid recovery for the bioaugmented reactor was mainly attributed to the added microorganisms containing a large number of *Methanobacteriales* and *Syntrophomonas*, released the feed-back inhibition and resulted in a rapid recovery of *Smithella*, thus accelerated the degradation of propionic acid. Therefore, bioaugmentation is a promising approach for promoting the recovery of the acidified anaerobic reactors caused by the organic shock load.

Keywords: ASBR; Bioaugmentation; Acidification; Recovery; Butyric acid metabolic pathway; *Smithella*

1. Introduction

Microbiologically, anaerobic digestion usually follows four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [1–3]. A delicate balance usually exists between these steps, especially, acidogenesis grows much faster than methanogens, resulting in a volatile fatty acids (VFAs) accumulation and even an acidified system [4,5]. However, the shock load for industrial wastewater occurs frequently, such an acidification problem would become more serious in anaerobic reactors suffering from the overload where a drastic pH drop inhibits the activity of methanogen.

Anaerobic sequencing batch reactor (ASBR) has gained increasing attention in recent years given the merits including constructional and operational simplicity, the flexibility of use and efficient control of effluent quality [6–8]. In addition, ASBR does hold some kinetic advantages over continuous systems such as excellent bio-flocculation abilities, a more powerful driving force of biological reactions and lower investments [9,10]. Nevertheless, due to the intermittent feeding mode, the ASBR will be more fragile or easily acidified after the organic overload, which limits the improvement of organic load and hinders the popularization and application of this technology. Systematic and reliable methods are required to control the adverse responses and speed up the recovery of an acidified ASBR aftershock load. One possible strategy is bioaugmentation.

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Bioaugmentation is defined as the practice of adding specific strain or a consortium of microorganisms to a system to provide a means for enhancing the desired activity and improving the performance of wastewater treatment systems [11–13]. For anaerobic systems, especially, bioaugmentation has made great contributions in the reduction of start-up period [14], the improvement of the flocculation of activated sludge and the efficiency of anaerobic digestion such as CH_4 production [15,16]. However, bioaugmentation in reducing the VFAs accumulation and high H_2 partial pressure due to a shock event is rarely reported. Of the four anaerobic steps, acetogenesis and methanogenesis are the most crucial, in which acetogenesis and methanogens tend to form special constructions and interrelate in what is called a "syntrophic interaction" [17]. Propionic and butyric acid are the most critical intermediates in the syntrophic reactions and their degradations were regarded as the rate-limiting steps because of the thermodynamic restrictions [18]. Therefore, a more practical and efficient means may be to aim at the key intermediates which are called "syntrophic flora" to improve the performance of the anaerobic digestion [19].

The major metabolic pathway of the anaerobic digestion may be different in all kinds of reaction modes, and the metabolic pathway can also be changed when the external environment changes. It is generally believed that glucose fermentation in an ASBR is mainly mediated by the propionic acid metabolism pathway [20]. When subjected to the organic shock load, the metabolic pathway tends to convert from propionic acid to butyric acid for the decrease of the H_2 accumulation [21–23]. Therefore, bioaugmentation butyric acid metabolism pathway may decrease the recovery period of an acidified system. In this study, butyric acid-utilizing syntrophic culture was enriched and used for bioaugmentation to speed up the recovery of an acidified ASBR. The evolutions of the microbial population were also monitored to understand acetogen and methanogen communities in the periods of acidification and recovery.

2. Materials and methods

2.1. Enrichment culture

The butyric acid-utilizing syntrophic culture was enriched in a 0.9 L serum bottle at 35 C± 1°C with a magnetic stirrer of 200 rmp in batch feed mode. The seed sludge was obtained from a laboratory-scale ASBR fed with glucose. The mixed liquor volatile suspended solids (MLVSS) of the enricher-reactor (ER) was 12,574 mg L^{-1}. Feed solution for the ER contained 14 mL L^{-1} of butyric acid as the sole substrate and bicarbonate were used as the buffer. The solution also comprised trace elements as follows (in mg L^{-1}): $MgSO_4·4H_2O$ (9), $CaCl_2·2H_2O$ (8), $FeCl_3·4H_2O$ (2), $CoCl_2·6H_2O$ (2), $MnCl_2·4H_2O$ (0.5), $ZnCl_2$ (0.05), H_3BO_3 (0.05), $NiCl_2·6H_2O$ (0.05), $CuCl_2·2H_2O$ (0.03), 36% HCl (0.001 mL L^{-1}). The pH of the feed substrate was kept at 7.0 ± 0.1 to keep ER neutral. The average hydraulic retention time (HRT) was kept at 10 d, feed exchange rate 1/2 (450 mL, chemical oxygen demand (COD) about 10,000 mg L^{-1}), and the pH_2 in the ER was less than 10^{-4} atm. That the relative abundance of *Syntrophomonas* (butyric acid degraders) was greater than 10% was chosen to define a successful enrichment.

2.2. Experimental setup

Two sets of laboratory-scale ASBR systems (the bioaugmented and non-bioaugmented reactors) were set up to investigate the effect of bioaugmentation on accelerating recovery of an acidified system. Initial MLVSS concentrations of two digesters were 11,574 and 12,046 mg L^{-1} respectively. COD:N:P was maintained at 300:5:1 in the influent substrate. Trace elements were the same as those in the ER. Bioreactors were operated with 4,000 ± 100 mg L^{-1} of influent COD concentrations and at 35 ± 1°C and 300 rmp. HRT was maintained at 24 h and a cycle was 8 h. After 2 weeks of adoption two reactors worked well. Then they were exposed to the double organic load (8,000 ± 150 mg L^{-1}) for a week and showed an acidification performance respectively. Bioaugmented reactor received 10% w/w of the enriched butyric acid-utilizing syntrophic culture by adding activated sludge mixture from the ER, while the non-bioaugmented reactor just restored influent COD concentrations. The performances of the bioaugmented and non-bioaugmented reactors were detected, and the evolutions of the microbial communities were analyzed.

2.3. Analytical methods

COD was measured by standard methods (APHA et al., [24]). The pH was determined using a pH meter with a glass electrode. The volume of CH_4 produced was quantified via the sodium hydroxide displacement method. Specific methanogenic activity (SMA) values against formic, acetic, propionic and butyric acid were analyzed at 36°C in serum bottles [25].

H_2 was detected with a Shimadzu (Japan) GC2010 gas chromatograph equipped with a thermal conductivity detector (stainless column at 80°C, injection temperature 80°C, N_2 as a carrier gas, with Porapak Q packing, Shimadzu C-R3A Chromatopac Integrator, Japan).

VFAs (formic, acetic, propionic and butyric acid) concentrations were quantified by a high-performance liquid chromatography (HITACHI HPLC-2130, Tokyo, Japan; C18 reverse phase column 250/4.6 mm; UV detector; wavelength 210 nm; 5 m particle size; flow rate-1.0 mL min^{-1}; mobile phase phosphate buffer solution (90%, pH = 2.0) and methanol (10%); sample injection 10 μL).

2.4. DNA extraction

DNA was extracted with a Soil Genomic DNA Kit (CoWin Biosciences, Beijing, China) according to manufacturer's protocols. The extracted DNA samples were stored at −20°C for subsequent assays and DNA concentrations were analyzed in duplicates with a NanoDrop 2000c spectrophotometer.

2.5. Quantitative polymerase chain reaction

To investigate the evolutions in microbial populations during the process performance, quantitative polymerase chain reaction (qPCR, ABI7500Fast) was used to determine the abundance of the functional genes. All qPCRs were conducted in a mixture with a total volume of 25 μL solution containing 10 ng of template DNA, 500 nM of each forward and

reverse primer, UltraSYBR Mixture (Low ROX) 1×. The qPCR program used was as follows: 10 min at 95°C (predenaturation), 40 cycles of 15 s at 95°C (denaturation), 1 min at 60°C (annealing), followed by melting curve analysis for SensiMix (Beijing, China). Primers were obtained according to previously published literature as stated in Table 1. The specificity of each PCR assay was confirmed by melting curve analysis (procedure: 95°C 15 s, 60°C 1 min, 95°C 15 s, 60°C 15 s). The $2^{-\Delta\Delta CT}$ method was used to calculate relative changes in gene expression determined from qPCR experiments.

3. Results and discussion

3.1. Culture for bioaugmentation

The butyric acid-utilizing syntrophic culture was enriched successfully after 50 d and demonstrated variations of maximum SMA against formic, acetic, propionic and butyric acid in Table 2. The higher SMA against acetic and butyric acid was achieved while the SMA against propionic acid had no significant difference compared with before enrichment. The reduction of nutrient type led to a decrease in the biomass of activated sludge.

Variations in the community structure of bacteria and archaea from the ER were characterized using qPCR. *Syntrophomonas*, butyric acid oxidizers, were the most enrichment due to the feed advantage, the relative abundance significantly increased from 0.43% ± 0.004% to 18.84% ± 1.68%

after 50 d enrichment (Fig. 1a). It is generally believed that *Methanosaeta* spp. (K_s = 20 mg L^{-1}, μ_{max} = 2–4 g COD (g VSS d)$^{-1}$) have a better affinity of acetic acid while lower specific growth rate compared with *Methanosarcina* spp. (K_s = 400 mg L^{-1}, μ_{max} = 6–10 g COD (g VSS d)$^{-1}$). When acetic acid concentration is more than 70 mg L^{-1}, *Methanosarcina* spp. are more competitive than *Methanosaeta* spp., vice versa [31]. However, in our study, the fastest reduction occurred in *Methanosarcina* spp. while *Methanosaeta* spp. achieved an increase even though the concentrations of acetic acid were always more than 70 mg L^{-1} in the ER. Similarly, Angenent et al. [32] also found that when the acetic acid concentration was 600 mg L^{-1}, filamentous microorganisms were dominant in granular sludge in an anaerobic membrane bioreactor. Moreover, *Methanobacteriales* kept growing from 13.24% ± 1.86% to 26.28% ± 1.75% as the predominant community in the process of enrichment (Fig. 1b).

3.2. Bioaugmented ASBR performances

3.2.1. Chemical oxygen demand

Before the shock load was introduced, two digesters required about 14 d to attain the average quasi-steady condition in terms of the COD removal efficiency and the CH$_4$ production, were about 98% (effluent COD 120 ± 60 mg L^{-1}) and 410 mL in one cycle respectively (Figs. 2a and 3b), approximately equal to the stoichiometric CH$_4$ production

Table 1
Primer sequences used in this study

Microbial population	Primer	Sequence (5′–3′)	Source
Archaea	ARC-787F	ATTAGATACCCSBGTAGTCC	[26]
	ARC-1059R	GCCATGCACCWCCTCT	
Methanococcales	MCC-495F	TAAGGGCTGGGCAAGT	[26]
	MCC-832R	CACCTAGTYCGCARAGTTTA	
Methanobacteriales	MBT-857F	CGWAGGGAAGCTGTTAAGT	[26]
	MBT-1196R	TACCGTCGTCCACTCCTT	
Methanomicrobiales	MMB-282F	ATCGRTACGGGTTGTGGG	[26]
	MMB-832R	CACCTAACGCRCATHGTTTAC	
Methanosarcina spp.	MSC-450F	TAGCAAGGGCCGGGCAAGA	[27]
	MSC-589R	ATCCCGGAGGACTGACCAAA	
Methanosaeta spp.	MST-387F	GATAAGGGRAYCTCGAGTGCY	[28]
	MST-573R	GGCCGRCTACAGACCCT	
Bacteria	BAC-338F	ACTCCTACGGGAGGCAG	[26]
	BAC-805F	GACTACCAGGGTATCTAATCC	
Syntrophobacter	SBC-695F	ATTCGTAGAGATCGGGAGGAATACC	[29]
	SBC-844R	TGRKTACCCGCTACACCTAGTGMTC	
Smithella	SMI-732F	GRCTTTCTGGCCCDATACTGAC	[29]
	SMI-831R	CACCTAGTGAACATCGTTTACA	
Pelotomaculum	PEL-622F	CYSDBRGMSTRCCTBWGAAACYG	[29]
	PEL-877R	GGTGCTTATTGYGTTARCTAC	
Syntrophomonas	SMS-637F	TGAAACTGDDDDTCTTGAGGGCAG	[29]
	SMS-757R	CAGCGTCAGGGDCAGTCCAGDMA	
Authentic acetogens	FTHFS-F	TTYACWGGHGAYTTCCATGC	[30]
	FTHFS-R	GTATTGDGTYTTRGCCATACA	

Table 2
Variations of maximum SMA and biomass before and after enrichment

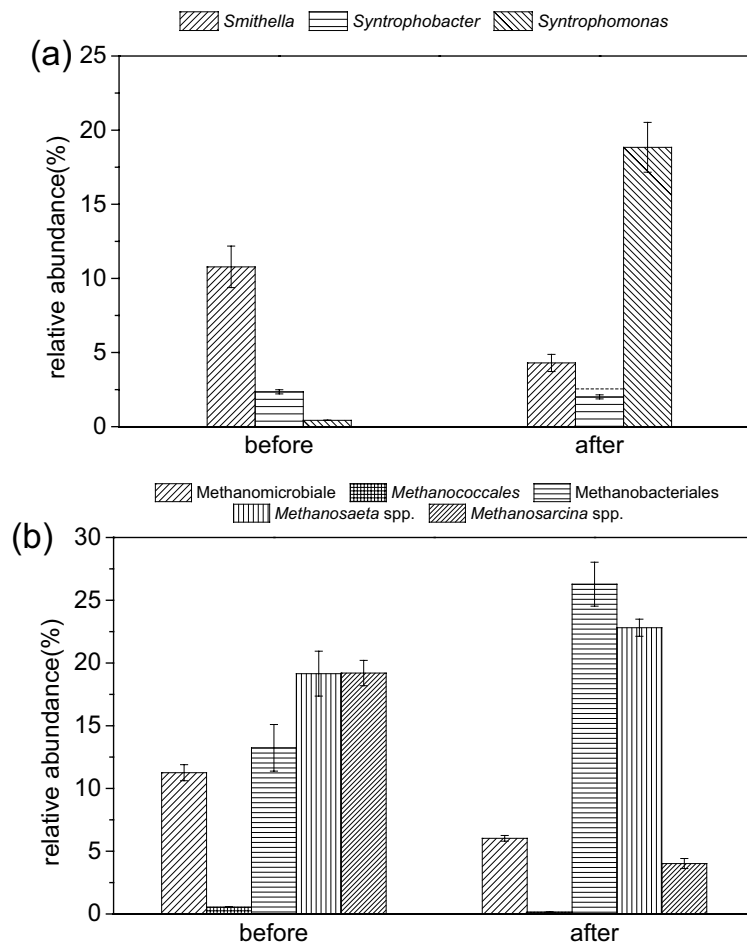|	Maximum SMA (mLCH$_4$·(g VSS d^{-1}))				MLSS (g L^{-1})	MLVSS (g L^{-1})
	Formic	Acetic	Propionic	Butyric		
Before	114	381	294	159	14.04	12.57
After	154	891	329	1,089	11.93	9.20

Fig. 1. Enrichment results by the enricher-reactor. (a) Relative abundance in total bacteria and (b) relative abundance in total archaea.

of 350 mL CH$_4$/g COD removal. With the introduction of organic shock load, two reactors exhibited poor resistance and efficiency. The effluent COD concentrations increased from 70 to 2,600 mg L^{-1} after 2 d (Fig. 2a). Subsequently, organics biodegradation remained failed increasingly in two acidified reactors and more than 5,300 mg L^{-1} of the effluent COD concentrations were detected after 7 d shock load. When the influent COD concentrations were restored to 4,000 ± 100 mg L^{-1} at 21 d, more than 3,400 mg L^{-1} of effluent COD concentrations were measured (Fig. 2a).

By adding the butyric acid-utilizing syntrophic culture, the effectiveness of bioaugmentation was striking that effluent COD concentrations decreased much faster than non-bioaugmented reactor, up to 1,300 mg L^{-1} and average

reduction rate 166.7 mg (L d)$^{-1}$ after 12 d bioaugmentation and kept a continuing decrease in the remaining investigation, for which non-bioaugmented reactor was 2,950 mg L^{-1} and 29.2 mg (L d)$^{-1}$ at the same time (Fig. 2a). The recovery time of the bioaugmented reactor was remarkably less than the non-bioaugmented reactor in terms of the effluent COD concentrations, 40 and 110 d respectively (effluent COD concentrations <600 mg L^{-1}, namely COD removal efficiency >85%, was regarded as the sign of recovery).

3.2.2. Volatile fatty acids

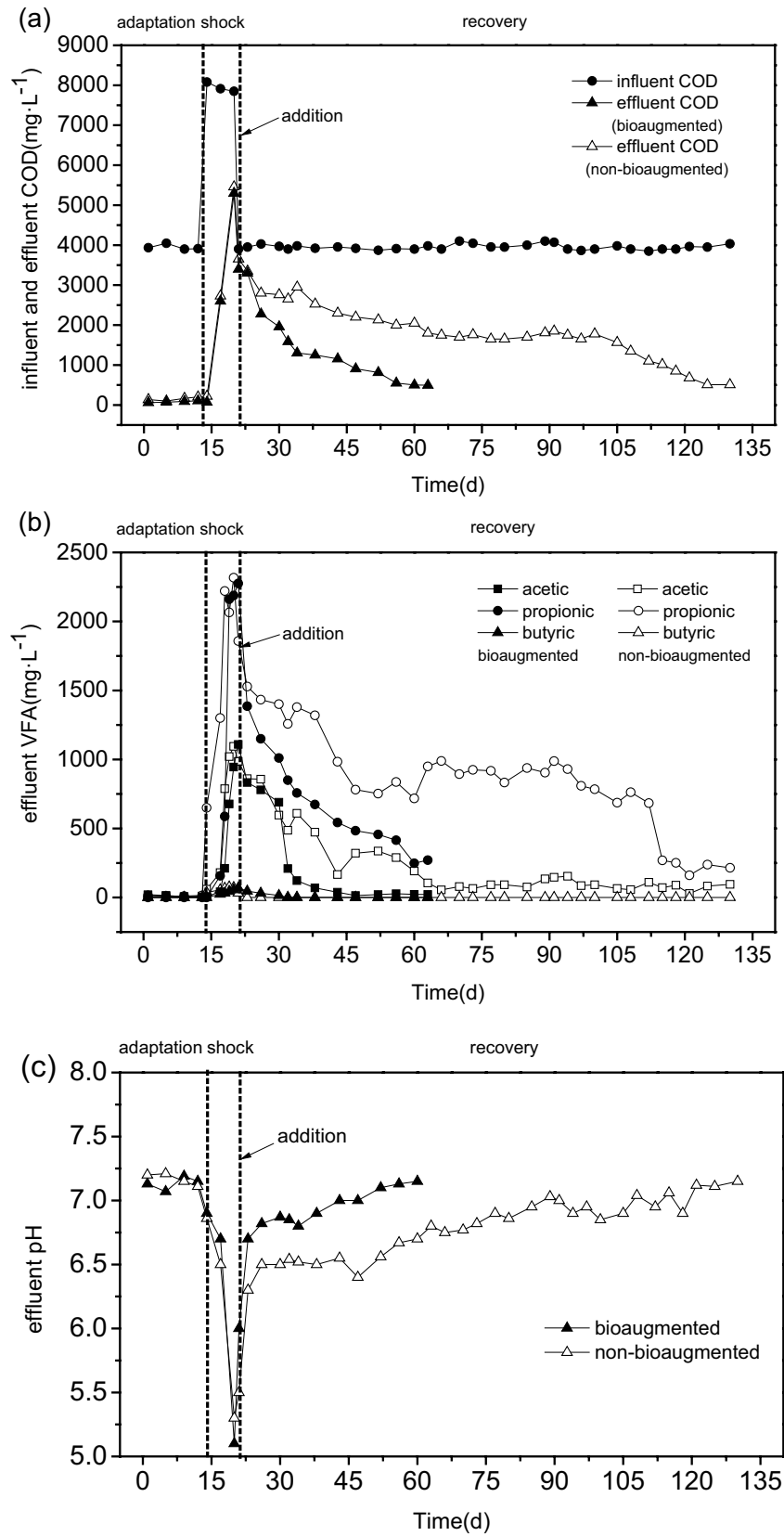Distinct variations of VFAs production signifying the change of microenvironment in two digestors were shown

Fig. 2. Working conditions and overall performance of the bioaugmented and non-bioaugmented reactors. (a) Influent and effluent COD concentrations, (b) effluent VFA concentrations, and (c) effluent pH value. The operation process was divided into three phases: adaption, shock, and recovery. Values are the mean of triplicates.

in Fig. 2b. The concentrations of effluent VFAs exhibited a concomitant rise and around 52.7% of effluent COD was VFAs when subjected to shock load. At the end of the shock phase (day 20), the elevated acetic and propionic concentrations of 1,109 and 2,275 mg L^{-1} respectively were detected, and propionic acid was the most dominant, which indicated that glucose degradation was mostly through propionic acid metabolic pathway under this operating condition. In addition, the stagnation or marked drop of propionic acid concentrations might be due to feedback inhibition of an anaerobic system caused by acidification events. Meanwhile, only a small amount of butyric acid (<30 mg L^{-1}) was detected during the shock phase, which was different from the previous report. Wang [21] found that butyric acid concentrations increased from 35 to 405 mg L^{-1} when an ASBR suffered from a shock load for 11 d. Therefore, the less shock time and shock load might account for the lower butyric acid concentrations in both reactors. And in our other study when the organic loads were increased from 6,000 to 12,000 mg L^{-1} for 10 d in an ASBR, the butyric acid concentrations reached to more than 700 mg L^{-1}.

The addition of *Methanobacteriales* (26.28% ± 1.75%) and *Syntrophomonas* (18.84% ± 1.68%) contributed to the decrease of H_2 partial pressure and more conversions from VFAs to acetic acid, thus a growth of *Methanosarcina* spp. from 14.13% ± 0.70% to 22.49% ± 1.31% was achieved (Fig. 4b) and acetic acid accumulation was reduced (Fig. 2b). After releasing feedback inhibition, the rapid recovery of *Smithella* (propionic acid degraders) was observed (Fig. 4b), which accelerated the decrease of the propionic acid concentrations. After 10 d bioaugmentation, a rapid reduction of the propionic acid concentrations from 2,275 to 1,011 mg L^{-1} was attained. For comparison, the activity of methanogens in the non-bioaugmented reactor was inhibited by the lower pH (<6.5), especially *Methanosaeta* spp. (Fig. 4b), resulting in a low degradation efficiency of acetic acid (Fig. 2b), which hindered the syntrophic balance between the acetogens and methanogens. On days 63, the average concentrations of acetic and propionic acid in the bioaugmented reactor were <25 and <270 mg L^{-1} respectively, while the non-bioaugmented reactor averaged >100 and >900 mg L^{-1} respectively at the same time (Fig. 2b).

3.2.3. Effluent pH

During the period of the shock load was introduced, the effluent pH in two digestors gradually reduced from 7.1 to 5.1 by the VFAs accumulation which corresponded to the non-removed COD (Fig. 2c). In the bioaugmented reactor, marked variations were detected that the effluent pH increased from 5.1 to 6.0 after the first day of adding the enriched culture while for the non-bioaugmented reactor was from 5.3 to 5.5 comparatively (Fig. 2c). Subsequently, the effluent pH of the bioaugmented reactor showed a rapid and continuous improvement, which depended on the higher organics utilization efficiency, less VFAs accumulation as well as the conversion of a metabolic pathway. The bioaugmented reactor required approximately 70 d less to achieve the effluent pH 7.2 compared to non-bioaugmented reactor (Fig. 2c).

3.2.4. H_2 partial pressure

Anaerobic digesters usually show the acidified performances due to the overload resulting in the unbalance of the syntrophic interaction between acetogenesis and methanogenesis. The syntrophic metabolism of VFAs and alcohols mainly depends on the interspecies H_2 transfer (IHT), in which H_2 served as the electron carrier [33]. Based on the IHT, the high H_2 partial pressure will disturb this syntrophic balance. Generally speaking, only when the H_2 partial pressure is less than 10^{-4} atm, the process of acetogenesis can proceed smoothly, according to the thermodynamics theory [17,34,35]. After one-week shock load, the H_2 partial pressure in two reactors significantly rose, peaking at approximately 3.8×10^{-3} atm (approximately 380 Pa) in one cycle (Fig. 3a), which was about 2.5-fold higher than the shock load was not introduced (1.5×10^{-3} atm).

Approximately 90 cycles (23 d) after the shock load, the effect of bioaugmentation was apparent in terms of H_2 partial pressure of bioaugmented reactor vs. non-bioaugmented reactor (Fig. 3a). After bioaugmentation, *Methanobacteriales* (H_2-utilizing methanogens) showed a rapid increase from 32.45% ± 1.77% to 45.24% ± 3.91% (Fig. 4b) and played an important role in reduction of H_2 partial pressure, which decreased 31.6% from 3.8×10^{-3} to 2.6×10^{-3} atm (Fig. 3a). Compared to the bioaugmented reactor, a decrease of 11.1% in the non-bioaugmented reactor was measured at the same time, which might result from *Methanomicrobiales* (H_2-utilizing methanogens) badly inhibited in an acidified system (Fig. 4b). The bioaugmented reactor required 210 cycles (70 d) less to reach to the initial level aftershock load in comparison to non-bioaugmented reactor.

3.2.5. CH_4 production

The CH_4 productions in two reactors were similar before and during the shock load. Following the shock load on day 21, CH_4 production appeared a major disappearance which decreased from 410 to 110 mL in a cycle (Fig. 3b). Daily CH_4 production began to increase after the shock event with the restoration of influent organics. In the bioaugmented reactor, the recovery of *Methanobacteriales* and *Methanosarcina* spp. (Fig. 4b) promoted the CH_4 production and began to increase after adding enriched microorganisms for 10 cycles (Fig. 3b). Compared to the bioaugmentation, the CH_4 production of non-bioaugmented reactor began to increase after 70 cycles. The bioaugmented reactor required 100 cycles to attain more than 330 mL CH_4 production in one cycle while the non-bioaugmented counterpart was 300 mL after 310 cycles (Fig. 3b).

3.3. Microbes evaluations

Microbial community evolutions were evaluated as shown in Fig. 4 and two reactors showed similar acidification characteristics. *Smithella* were badly inhibited and approximately reduced by 60% from 7.56% ± 1.35% to 2.98% ± 0.56% following the shock load on day 21 (Fig. 4a). Comparatively, the relative abundance of *Methanosarcina* spp. was detected a decrease of only 27% from 19.52 ± 1.17 to 14.13% ± 0.88%
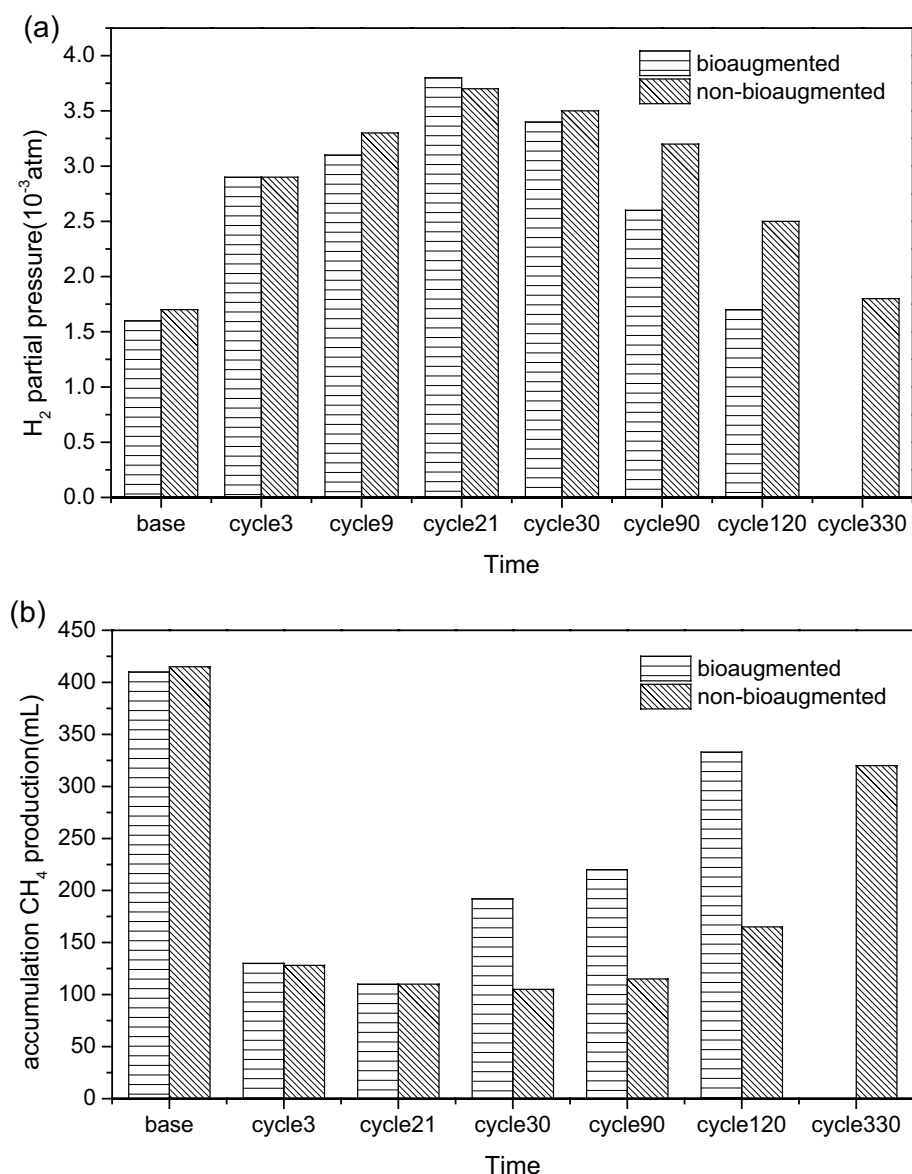
Fig. 3. Results of H_2 partial pressure and accumulation CH_4 production in one cycle by the bioaugmented and non-bioaugmented reactors. (a) H_2 partial pressure and (b) accumulation CH_4 production. Values are the mean of triplicates.

(Fig. 4b). This may be explained by the following two reasons. First, *Methanosarcina* spp. usually employ both aceto-clastic and hydrogenotrophic methanogenesis metabolic pathway in an anaerobic system [36], a large number of intermediates (acetic acid and hydrogen) can provide abundant nutrients for the growth of *Methanosarcina* spp. While excessive propionic acid accumulation leads to a feedback inhibition on *Smithella*, thus hindering the rate of proliferation. In addition, methanogens can adapt to low pH conditions by decreasing the pH progressively [37]. It should be noted that more than 300% growth of *Syntrophomonas* was observed in two digestors during the shock phase, indicating that the metabolic pathway started to convert from propionic to butyric acid in an acidified ASBR even if the butyric acid concentrations were small (Fig. 2b) due to the utilization of acetogen. Furthermore, *Pelotomaculum* was not

detectable and unlikely to occur (Fig. 4b), their determination could be neglected in this environment during the evaluation phases.

Archaeal community emphasized the excellent tolerance and crucial role of *Methanobacteriales* that were little influenced when the shock load was introduced, and the relative abundance increased by 41.4% after adding enriched microorganisms for 10 d. While for the non-bioaugmented reactor, an increase of 3.8% was obtained correspondingly (Fig. 4b). In the process of bioaugmentation, the growth of *Methanosarcina* spp. (from 14.13% ± 0.71% to 17.58% ± 1.28%) was detected in the high concentrations of acetic acid (>70 mg L^{-1}) in comparison to *Methanosaeta* spp. (from 12.17 ± 0.78 to 11.33% ± 0.59%), which accorded with previous studies [31]. Similar results were also detected in the non-bioaugmented reactor. Due to the bioaugmented reactor releasing
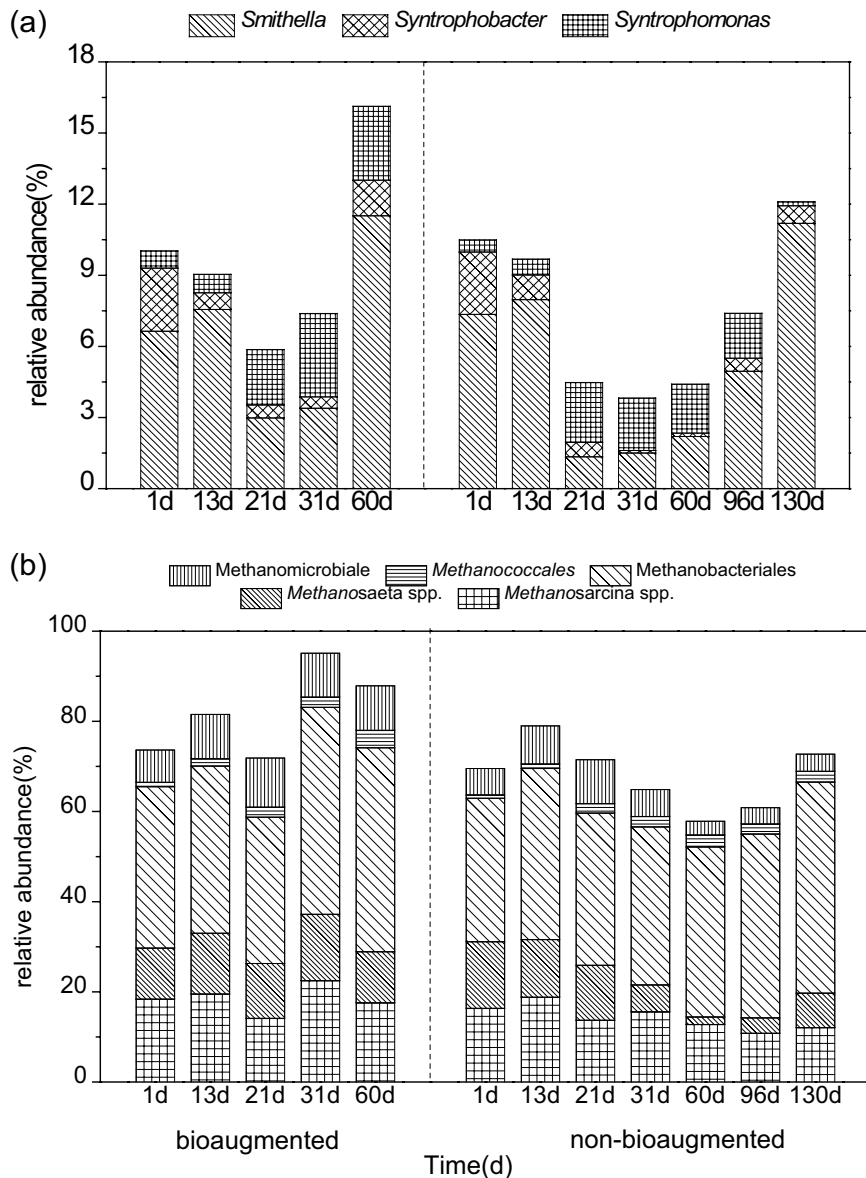
Fig. 4. Variations of the microbial community in the bioaugmented and non-bioaugmented reactors. (a) Relative abundance in total bacteria and (b) relative abundance in total archaea. Values are the mean of triplicates, error bars are not listed.

feedback inhibition more quickly, *Smithella* achieved a rapid growth from 2.98% ± 0.56% to 11.51% ± 0.86% after 40 d bioaugmentation (Fig. 4b), which significantly accelerated the recovery of an acidified system. Comparatively, for the non-bioaugmented reactor, it took approximately 110 d for *Smithella* to increase from 1.34% ± 0.06% to 11.19% ± 0.30%. In addition, we found that *Methanosarcina* spp. and *Methanobacteriales* grew fast (21–31 d) before *Smithella* began to increase (31–60 d). It might be deduced that the activity of acetogenesis could not recover quickly until the methanogenesis process was smooth. When the abundance of *Syntrophomonas* were compared in two reactors, bioaugmentation strategy realized a marked increase from 0.78% ± 0.09% to 3.12% ± 0.48% while the non-bioaugmented reactor decreased from 0.67% ± 0.08% to 0.17% ± 0.01% (Fig. 4b), indicating that butyric acid metabolic pathway was

enhanced and the effect of bioaugmentation lasted for the rest of experiments without any further addition.

4. Conclusions

Bioaugmentation with the butyric acid-utilizing syntrophic culture accelerated the recovery of an acidified ASBR subjected to a week organic shock load. The addition of *Syntrophomonas* and *Methanobacteriales* was beneficial to decrease VFAs accumulation and H_2 partial pressure, which accelerated the recovery of *Smithella* and remarkably reduced the recovery time of an acidified system. The recovery efficiency of the bioaugmented reactor was about 3-fold (40 and 110 d) faster than the non-bioaugmented reactor. A better bioaugmentation effect could be achieved by the only inoculation once.

Acknowledgment

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