Biochemical Engineering Journal 121 (2017) 163–170

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Selective acetate production with CO_2 sequestration through acetogen-enriched sludge inoculums in anaerobic digestion

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ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 7 January 2017
Accepted 6 February 2017
Available online 7 February 2017

Keywords:
Acetogen
Acetate
Anaerobic digestion
CO_2 sequestration

ABSTRACT

In order to investigate the role of acetogens in anaerobic digestion, acetogens-enriched sludge was used as inoculum for anaerobic digestion. Acetogens were successfully enriched from raw sludge with sodium formate and the *fhs* gene copy number was up to 6.08×10^9 copies/mL. By using acetogens-enriched sludge as inoculum, the substrate utilization rate and acetate percentage in volatile fatty acids (VFAs) were significantly improved. The yield of acetate was 1.77 times higher than the yield in the control fermentation study without addition of acetogens-enriched sludge. With CO_2 sparging in the headspace of the reactors, the acetate yields were greatly enhanced due to the acetogenesis reaction. The microbial community structure shifted significantly after three batches of anaerobic fermentation with acetogens-enriched inoculums. The dominated terminated restriction fragment shifted from 519 bp (71.81%) to 483 bp (21.2%), and 519 bp probably represents *Granulicatella* genus while 483 bp represents *Clostridium, Eubacterium, Methylophilus, Nannocystis,* respectively. The findings in this study provide a sound basis for development of a potential enhanced anaerobic digestion process to increase acetate yield while mitigating greenhouse gas (CO_2) emission.

1. Introduction

Anaerobic digestion has been exploited as a worldwide environmental sustainable technology for bioenergy recovery for over a century. It can generate methane [1], hydrogen and even high value added chemicals from the organic wastes such as sewage sludge and wastewater [2–4]. In recent years, volatile fatty acids (VFAs) production from the organic wastes has become more and more attractive, since VFAs can be used as carbon source to improve the biological nitrogen and phosphorous removal in wastewater treatment process and used as a sort of platform chemicals in industries [5–7]. Among these VFAs, acetate is one of the most important one [8].

CO_2 is a greenhouse gas generated from the organic components in sewage sludge during anaerobic digestion. Its sequestration potential during anaerobic digestion is an area of special research interest [9,10]. CO_2 and methane are common products in the anaerobic digestion of organic wastes. With the generation of VFAs instead of CO_2 and CH_4 from organic wastes, the discharge of greenhouse gas is mitigated and the organic substances are transformed into useful chemicals. So far, VFAs production has been widely reported by inhibition of methanogenesis and use of heat-treated sludge inoculums, etc [11–13].

Currently, there are many studies to investigate the mechanism of VFAs production or to enhance the VFAs production from different solid wastes. Several pre-treatment strategies, such as thermal, chemical or enzymatic treatment have been verified effectively to improve the VFAs yields by anaerobic digestion [14,15]. Furthermore, the thermal-alkalinity pretreatment and some specific inhibitors to methanogenesis have been reported as effective methods to selectively accumulate the acetate in the consequent acidogenic digestion [16]. Bajon Fernández et al. reported the calcium- and/or magnesium-rich natural ores ($CaSiO_3$ or Mg_2SiO_4) can be used to capture the CO_2 and then to enhance the CH_4 production [17,18]. However, there is little information about the simultaneously selective acetate production and CO_2 emission reduction during the anaerobic digestion, which is an very important strategy to realize the efficient and green chemicals production from organic wastes.

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http://dx.doi.org/10.1016/j.bej.2017.02.008
1369-703X/

Acetogenic bacteria are strictly anaerobic microorganisms, most of which are able to form acetate from H_2 plus CO_2 via the acetyl-CoA pathway, making a great contribution to the global carbon cycle [19–21]. Acetogens constitute a very phylogenetically diverse bacteriological group and can use diverse electron donors and acceptors. Considering their physiological and phylogenetic diversities, it is hard to clearly describe their diverse in situ functions in reactors or natural habitats [22]. Patil et al. enriched a mixed microbial community from an anaerobic environment on H_2:CO_2 for acetate production [23]. As reported by Salomoni et al. [24], an increase in CO_2 contents in the headspace of anaerobic reactors improved the activity of acetogenic bacteria. Nie et al. set up a novel coupling fermentation system which combined syntrophic fermenters and acetogens to enhance the acetate accumulation [25]. In addition to sodium formate, acetogenic communities were enriched with syngas substrate from thermophilic anaerobic sludge. Acetogens are a group of obligatory anaerobic bacteria which reduce CO_2 to acetic acid via the acetyl-CoA pathway. However, the quantitative contribution of the acetogens on the CO_2 consumption and acetate accumulation is still not clear.

In view of the above findings, we hypothesize that acetogen-enriched sludge inoculums could selectively enhance acetate production associated with CO_2 sequestration and its emission mitigation from anaerobic digestion, which will provide additional benefits to organic waste treatment by means of anaerobic digestion. Therefore, the objectives of this study were, (1) to enrich acetogens in sewage sludge; (2) to improve the selective acetate production associated with CO_2 sequestration; and (3) to investigate the bacterial community in the sludge during anaerobic digestion.

2. Materials and methods

2.1. Sludge sample

Sewage sludge collected from the secondary sediment tank of Wuxi Lucun Wastewater Treatment Plant (WWTP), Wuxi, China, was used as seed sludge. It was concentrated by settling for 24 h, then heated at 120 °C for 2 h to kill the non-spore-forming methanogens [13,26]. Before used as seed sludge, the sludge was re-activated to recover the activity of acetogens as described by Nie et al. [27]. The main characteristics of the sewage sludge were: pH, 7.00 ± 0.08; Total Solid (TS), 1754.4 ±35 mg/L; Volatile Solid (VS), 1000.0 ± 30 mg/L; total protein, 616.8 ±7.5 mg/L; total carbohydrates, 206.6 ± 14 mg/L; total lipids, 16.1 ± 2 mg/L, and SO_4^{2-}, 6.7 mg/L.

2.2. Acetogen enrichment

To enrich the acetogens, the sewage sludge was inoculated in a 5 l up-flow anaerobic sludge blanket (UASB) reactor with concentration of 4 g VS/L. The 2-bromoethanesulfonate (BES) was added into the UASB to a final concentration at 50 mmol/L to inhibit methane generation [28]. The whole enrichment process was divided into five periods (Periods 1–5). The substrates were changed from glucose to sodium formate gradually and the volumetric loading rates (VLR) were controlled as shown in Table 1. The mineral salts in the influent were as follows: 500 mg/L NH_4Cl, 300 mg/L $MgCl_2$, 250 mg/L K_2HPO_4, 250 mg/L KH_2PO_4, 25 mg/L $FeCl_3$, 16 mg/L $NiSO_4$, 0.025 mg/L $CaCl_2$, 11.5 mg/L $ZnCl_2$, 10.5 mg/L CoCl, 0.5 mg/L $CuCl_2$, and 15 mg/L $MnCl_2$. The hydraulic retention time (HRT) was 24 h. During the whole process, gas and liquid samples were collected every day for chemical analysis. Sludge samples were taken on the first and last days of every stage for microbial community analysis.

2.3. Anaerobic acidogenic digestion test

Acetogen-enriched sludge was added into serum bottles as inoculum to test their influence on accumulation of VFAs and biogas. These bottles were sealed with rubber stoppers. There were a gas-vent on the rubber stopper for gas sampling and a port on the bottom for liquid sampling. The volume of the serum bottles was 500 ml and working volume was 350 ml. The sludge concentration was 1.0 g/L (as VS) and 27.8 mmol/L glucose was added as the organic substrate. BES (50 mmol/L) was added as methanogenesis inhibitor and the mineral salts were the same as the enrichment stage. Before the experiment commenced, oxygen was stripped by sparging nitrogen gas for 10 min. The temperature was controlled at 37 °C with a water bath shaker. The control experiment was the same as the acidogenic digestion mentioned above, except that there was no inoculation of acetogen-enriched sludge was added. Sampling of gas and liquid samples was conducted every 3 h.

2.4. Anaerobic acidogenic digestion sparged with CO_2

To explore CO_2 sequestration by acetogen-enriched sludge, CO_2 was sparged into the acidogenic reactors. In the control group, 1.62 mmol CO_2 was sparged into the headspace of the 500 ml serum bottle, 5.56 mmol/L glucose was used as the substrate and the seed sludge without acetogen was used as inoculum for the digestion. In the experimental group, in order to obtain a more significant change of the substrate and products, excess CO_2 was sparged into the headspace of the flask. After the sparging process was finished, the headspace gas was taken for measurement and found that 4.37 mmol CO_2 was sparged. The acetogen-enriched sludge was added with a concentration of 1.0 g/L (as VS). In the two groups, 50 mmol/L BES was added into the serum bottles to inhibit methanogenesis. The other digestion conditions were the same as the Section “Anaerobic acidogenic digestion test”.

2.5. Terminal restriction fragment length polymorphism analysis

The procedures for genomic DNA extraction have been described previously [11]. For terminal restriction fragment (T-RF) profiles, bacterial 16S rRNA genes were amplified with 5′ fluorescently labeled forward primer (27F labeled with 6-carboxyfluorescein, 5′-AGAGTTTGATCCTGGCTCAG-3′) and a universal reverse primer (1492R, 5′-GGTTACCTTGTTACGACTT-3′). Two independent 25 μl PCRs were performed for each sample and the products were combined and purified with the Gel Extraction Kit (TaKaRa Co., Japan). Aliquots of the purified amplicons were then digested with 10 U of Hae III, Hha I, and Msp I (TaKaRa Co., Japan) for 3 h at 37 °C. Each 200 μl tube was filled with 16 μl of amplicons, 2 μl of incubation buffer, and 2 μl of restriction enzyme, making up a total volume of 20 μl. The digested amplicons were mixed with GeneScan 1000 ROX size standards (Applied Biosystems Inc., USA) and analysed by capillary electrophoresis with GeneScan software (Applied Biosystems Inc., USA). Signals with a peak area that was less than 1000 relative fluorescence units were regarded as background noise and excluded from the analysis. The relative abundance of a detected T-RF within a given terminal restriction fragment length polymorphism (T-RFLP) pattern was calculated as the respective signal area of the peak divided by the peak area of all peaks of the T-RFLP pattern. The size of each bacterial T-RFLP species peak corresponded to the value for that species determined by in silico analysis of clone library with Lasergene (DNAStar Co., USA). Both the presence/absence and relative abundance of T-RFs were considered in data analysis.

Table 1
Operational parameters of acetogens enrichment in the UASB reactor.

Stage	Stage 1 (1–10d)	Stage 2 (11–25d)			Stage 3 (26–40d)	Stage 4 (41–55d)	Stage 5 (56–70d)
Substrates	Glucose	glucose/sodium formate			sodium formate	sodium formate	sodium formate
Concentration (g/L)	4	4/1.3	2.53/1.3	1.25/1.3	3.25	6.5	13
VLR(g COD/L•d)	4.37	4.67	3	1.8	0.76	1.52	3.04

2.6. Analytical methods

The quantification of acetogens was carried out by quantitative PCR described elsewhere [11]. Mixed liquor samples were regularly taken from the reactors and analysed for COD, pH and TS content according to Standard Methods [29]. Glucose was measured by the method described by Halhoul [30]. For VFA measurement, sludge samples were centrifuged at 7000 rpm for 10 min and the supernatants were filtrated through 0.45 μm filter membranes. A gas chromatograph (GC-2010, Shimadzu Co., Kyoto, Japan) equipped with an auto injector (AOC-20i, Shimadzu Co.), a flame ionization detector and a fused-silica capillary (PEG–20 M, 30 m × 0.32 mm, 0.5 μm, China) was used to measure VFA and alcohol. 4-Methylvaleric acid was added as internal standard and the samples were acidified with 3 mol/L phosphoric acid [28]. The GC column was initially held at 80 °C for 3 min, increased by 15 °C/min to a final temperature of 210 °C, and then held at this temperature for 2 min. The injection port and detector temperatures were both 250 °C. Total VFAs concentration was calculated as the sum of individual VFA concentrations. CO_2 and H_2 gas samples (0.05 ml) were taken with a gas-tight pressure lock syringe (Shimadzu, Japan), and quantified by gas chromatography (GC-2010, Shimadzu, Japan) equipped with a packed column Porapak Q (50/80 mesh) and a thermal conductivity detector (Shimadzu, Japan) [11]. All the measurements were repeated three times and the mean values were calculated.

3. Results

3.1. Quantification of acetogen and VFAs percentages

In order to investigate the acetogen enrichment in the sludge inoculums, the *fhs* gene encoding the formyltetrahydrofolate synthetase (FTHFS) was measured by quantitative PCR [11]. As shown in Fig. 1, the *fhs* gene copy numbers rose gradually from 1.3×10^7 copies/mL at stage 1 to 6.08×10^9 copies/mL at stage 5, an increase of 2 orders of magnitude. It was reported that the *fhs* gene quantity in different sources of natural environmental soil samples varied from 10^5 to 10^8 copies/g TS [11]. Compared to the natural background level, the *fhs* gene quantity in acclimated sludge inoculum in this study was clearly several orders of magnitude higher than the natural environmental samples. Consistent with the increase of acetogen, the percentage of acetate in total VFAs also increased from 21% at stage 1 to 100% at stage 5.

3.2. Profiles of chemicals during anaerobic acidogenesis

After the acetogen enrichment, the acclimated sludge was used as sludge inoculum for acidogenic digestion and the results were compared to that in control experiment (without acetogen inoculum). To study the impact of acetogen on chemicals, especially long term acetate accumulation in anaerobic digestion, three consecutive batches of anaerobic fermentation were conducted. Intermediates, such as VFAs, alcohol and CO_2, accumulated during the anaerobic acidogenesis process due to inhibition of methanogenesis by BES. Profiles of substrate utilization, accumulation of VFAs, ethanol and biogas were determined and shown in Fig. 2.

As shown in Fig. 2(a), glucose was completely consumed within about 25 h and the degradation curves were similar among two of the three digestion batches. A small difference can be seen in the third one, which showed a slight lag for glucose consumption. In the three digestion batches, the glucose utilization rate with acetogen-enriched sludge inoculums was a bit faster than that of the control, indicating that acetogen improved substrate utilization in the anaerobic digestion. However, there was no difference in the glucose utilization extent between the digestions with and without acetogen-enriched sludge inoculums.

Fig. 2(b, c and d) illustrates the changes of acetate, butyrate and ethanol concentrations in the three digestion batches. In the digestion with enriched acetogen, the acetate and butyrate concentrations reached 30.46 mmol/L and 17.2 mmol/L at the stable stages of the three digestion batches, respectively. It is obvious that the digestion with enriched acetogen had much higher acetate and butyrate concentrations than the control. The ethanol concentration of acetogen-enriched digestion increased to about 14 mmol/L at the early stage and then decreased quickly at the subsequent digestion. In contrast to what was observed for acetate and butyrate, the ethanol concentration of the acetogen-enriched digestion was much lower than the control. It could be the result from transformation of ethanol to acetate due to enriched acetogen [31]. On the other hand, acetate and ethanol concentrations showed no significant difference in the first two digestion batches, but in the third one they were lower, probably due to the decrease of acetogen quantity after two fermentation batches (section "Shift of microbial community"). Therefore, a selective pressure is important to keep the dominance of acetogens in the sludge inoculum for the long term operation.

The CO_2 and H_2 concentrations are shown in Fig. 2(e and f). In the first batch of digestion, CO_2 concentration in control experiment increased to 10.66 mmol/L at the early stage and then gradually decreased to 6.97 mmol/L at the end stage. For the H_2, it rose to 26.65 mmol/L and decreased to 21.32 mmol/L at the end stage. The other two batches also showed similar trends. However, in digestion with acetogen-enriched sludge inoculums, CO_2 and H_2 concentrations decreased sharply at the end stage in each batch because of the acetogenic reaction to generate acetate, showing a significant difference to the control experiment.

3.3. Profiles of chemicals in acidogenic digestion filled with CO_2

In order to investigate CO_2 sequestration by acetogen, CO_2 was sparged into the headspace of acidogenic digesters with or without acetogen-enriched sludge inoculum. The pH, glucose, CO_2 utilization rate and acetate yield changes in anaerobic acidogenic digesters were measured and shown in Fig. 3.

As shown in Fig. 3, without the inoculum of enriched acetogen, pH values decreased quickly to 4.77 within 16 h and increased gradually to 5.95 after 68 h (Fig. 3a). Glucose was quickly consumed completely at 20 h, while the CO_2 decreased gradually from 1.62 to 0.71 mmol/L at 78 h. As for the acetate yield, it increased drastically at 20 h to 5.72 mmol/L and then maintained stably at 5.53 mmol/L. On the other hand, in the acetogenic digester with the inoculum of enriched acetogen, the pH change trend was similar to the change in Fig. 3a. However, the CO_2 consumption showed a faster decrease rate than that in the acidogenic digester without

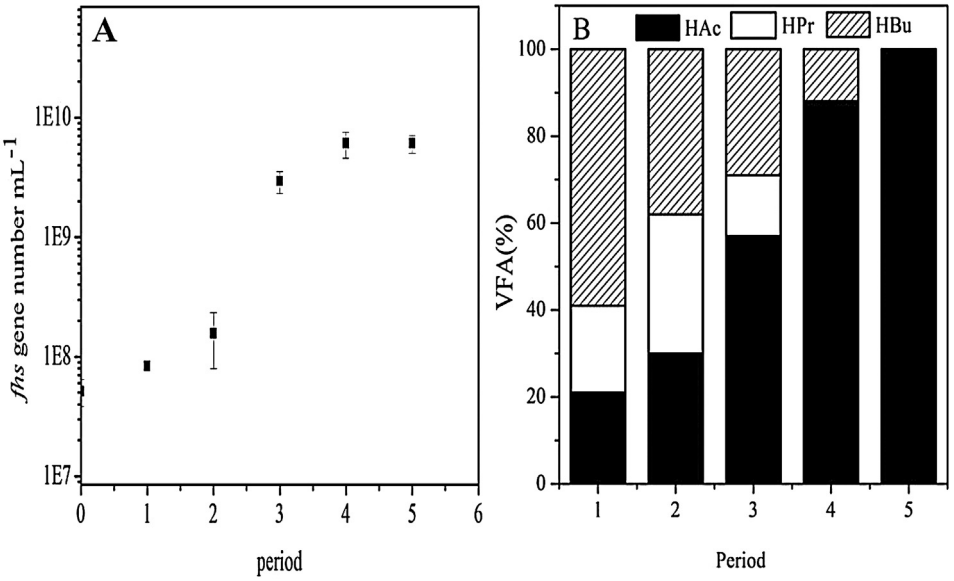

Fig 1. The *fhs* gene quantification (a) and the VFA percentages (b) in enrichment process.

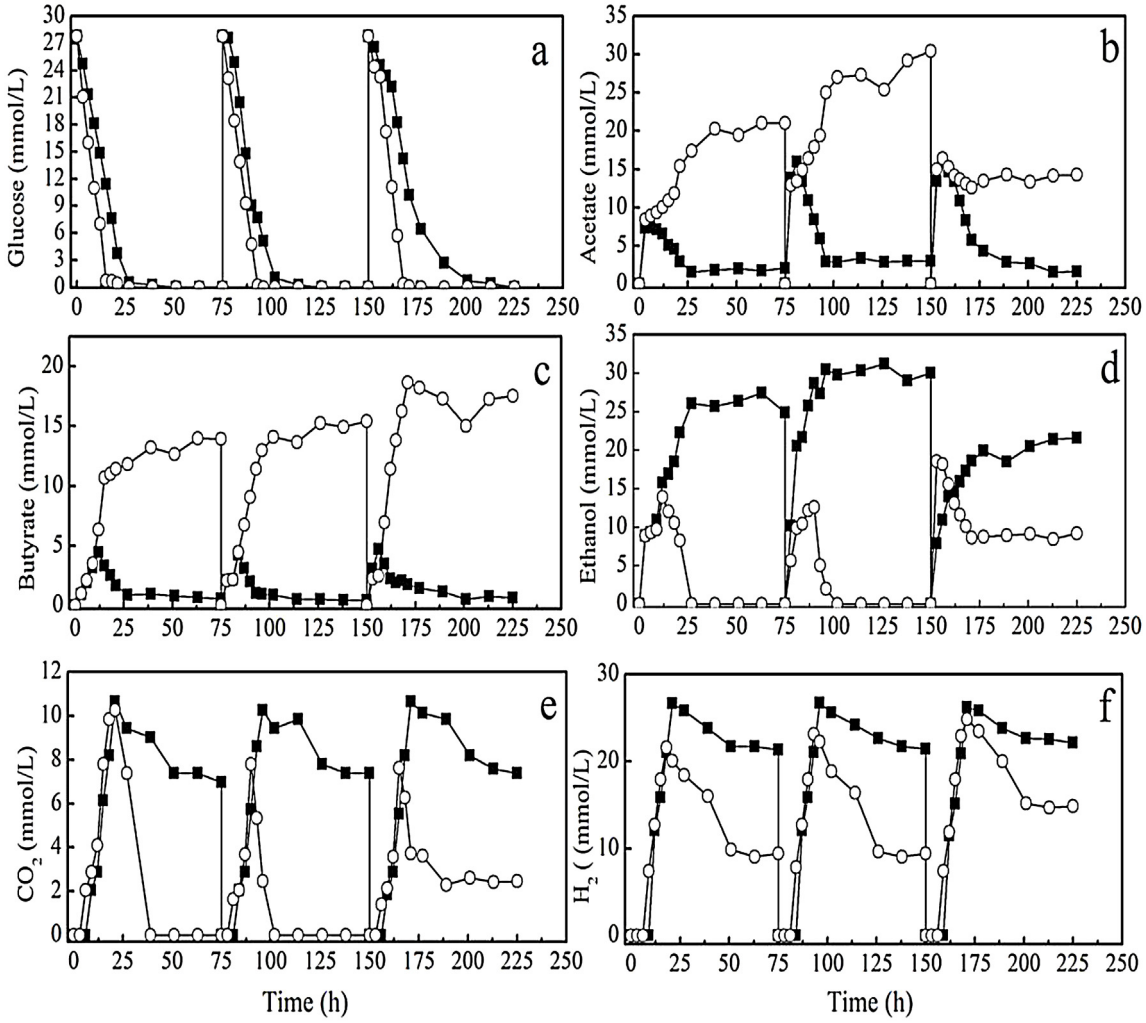

Fig. 2. The glucose utilization (a), acetate (b), butyrate (c), ethanol (d), CO_2 (e), H_2 (f) changes in anaerobic acidogenesis digesters. The open cycle: with acetogen-enriched innoculmn; the solid square: without acetogen-enriched inoculum.

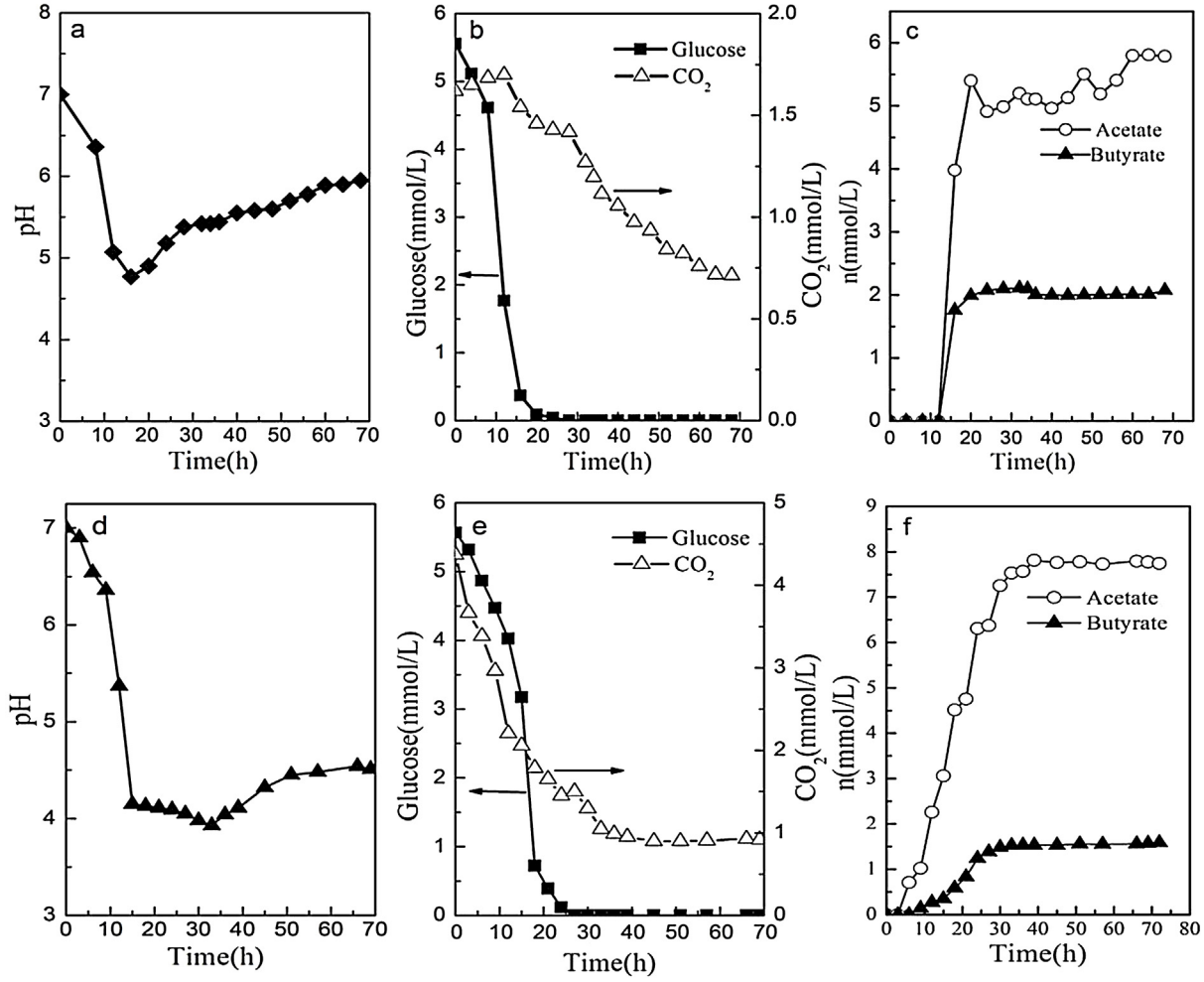

Fig. 3. The pH, glucose and CO_2 sequestration, and acetate yield changes in anaerobic acidogenic digesters filled with CO_2. The figures a–c and d–f represent the fermentation results without and with acetogen-enriched sludge inoculum, respectively.

the enriched acetogen. Furthermore, there was also an almost complete CO_2 consumption with the final concentration at 0.92 mmol/L. It should be noted that the CO_2 conversion efficiency in the acidogenic digester without the inoculum of enriched acetogen is 56.17% while in the digester with the enriched acetogen is 78.95%, indicating that the acetogen improved the CO_2 conversion. Consequently, in accordance with the glucose and CO_2 utilization, the acetate yield increased significantly and reached 7.81 mmol/L. It was 1.36 times higher than that in the acidogenic digester without the enriched acetogen.

3.4. Acetate yields, percentages and glucose utilization efficiencies

To quantitatively reflect the role of acetogen on acetate production in acidogenic digestion, acetate yields, percentages and glucose utilization rates were calculated and the results are shown in Table 2.

As shown in Table 2, the average acetate digestion yield with acetogen-enriched inoculum was 4.14 mmol/g COD, the average acetate percentage and substrate utilization rate were 50.9% and 0.046 g/L/h, respectively. However, acetate yield, acetate percentage and glucose utilization rate were 2.33 mmol/g COD, 43.2% and 0.068 g/L/h, respectively, in digestion without enriched acetogen. Evidently, digestion with enriched acetogen was more effective in the production of acetate in comparison with digestion without acetogen-enriched sludge. With CO_2 sparged into the headspace of the digesters, the acetogenesis conversion was further significantly enhanced with the acetate yields at 7.37 mmol/g COD and percentage at 83%, which was 1.78 times higher than that in the acetogen-enriched digestion. In addition, the substrate degradation rate was also improved with the rate at 1.340 g/L/h. The above results indicated that the acetogen improved the acetate yield and increased the acetate percentage in the digestion significantly. With the substrate (CO_2) addition, the acetogenesis was enhanced and led to a higher acetate yield and contribution, suggesting that the acetogen played an important role in converting the CO_2 into acetate in the anaerobic fermentation.

3.5. Shift of microbial community

A combination of T-RFLP fingerprinting with sequencing was used to characterize the microbial community in digestion with acetogen-enriched sludge and control digestion. Microbial community structures in the two kinds of sludge were summarized in Fig. 4a and b.

In the initial acetogen-enriched inoculum (Fig. 4a), TRF 519 bp was dominated in microbial community with the percentage of 71.81%. The remained TRFs were 496 bp (8.11%), 488 bp (7.14%), 88 bp (4.94%), 545 bp (4.17%), 92 bp (3.84%). However, after three fermentation batches (Fig. 4b), the number of microbial species increased and TRF 483 bp (21.2%), 12 bp (15.37%) and 486 bp (13.63%) were the major fragments. Interestingly, the T-RF 519 bp,

Table 2
The acetate yields, percentages and glucose utilization efficiencies of the digestion in different situations.

	Acetate yields (mmol/g COD)	Acetate percentages (%)	Glucose utilization rate (g/L/h)
Digestion without enriched acetogens	2.33 ± 0.16	43.2 ± 3.18	0.068
Digestion with enriched acetogens	4.14 ± 0.46	50.9 ± 2.72	0.046
Digestion with enriched acetogens and sparged CO_2	7.37 ± 0.89	83.0 ± 0.75	1.340

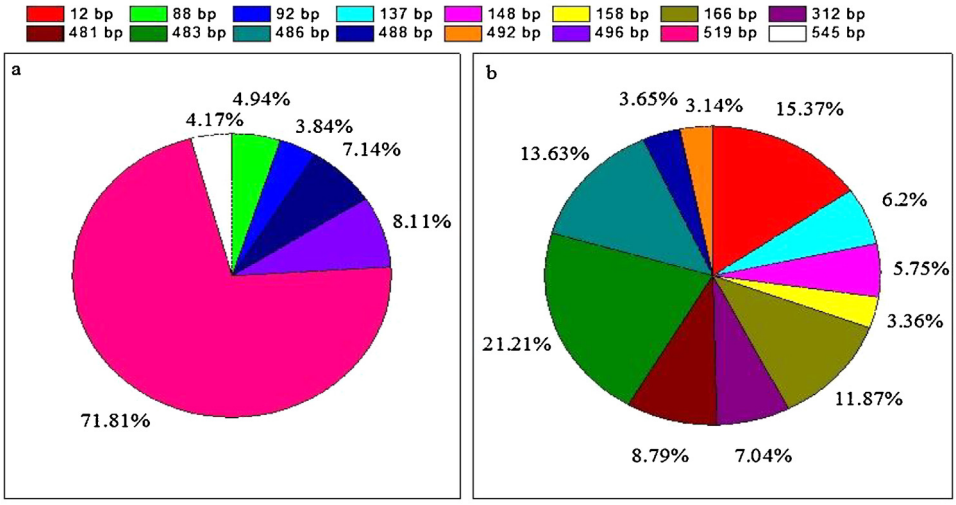

Fig. 4. The microbial community structures of acetogen-enriched sludge (a) and glucose-fed fermented sludge (b).

Table 3
Possible microbial genuses in the sludge.

TRFs	Possible genus
80 bp	*Bacillus, Methanobrevibacter, Nitrosomonas, Streptococcus*
147 bp	*Methanobrevibacter, Telluria., Capnocytophaga, Thiobacillus*
184 bp	*Rathayibacter, Microbacterium, Paenibacillus,*
196 bp	*Acinetobacter calcoaceticu, Clostridium,*
312 bp	*Butyrivibrio, Pasteurella,*
439 bp	*Acetobacter, Clostridium, Methyloarcula, Ancylobacter, Rhodobacter*
479 bp	*Methylophilus, Microbacterium, Francisellas, Haliscomenobacter*
483 bp	*Clostridium, Eubacterium, Methylophilus, Nannocystis*
485 bp	*Achromatium, Acidovorax, Clostridium, Methylococcus*
519 bp	*Granulicatella*
570 bp	*Clostridium, Haloanaerobium, Lactobacillus*
578 bp	*Lactobacillus, Pediococcus acidilactici, Weissella*
588 bp	*Pediococcus pentosaceus.*

which dominated in acetogen-enriched sludge, disappeared in the end of the third fermentation batch. Combining the results in Fig. 2 and Fig. 4, it can be seen that although VFAs accumulation profiles showed similar pattern between the three fermentation batches, the structure of acetogen changed significantly.

The possible genuses of microorganisms represented by the T-RFs are listed in Table 3. In the acetogen-enriched sludge, the dominant TRF 519 bp was affiliated to *Granulicatella* genus, and other TRFs were probably belonged to *Bacillus*-generating anaerobic microorganisms.

4. Discussion

The results provide various evidences to support that acetate production was selectively improved through acetogen-enriched sludge inoculums in anaerobic acidogenic digestion and the process was simultaneously associated with CO_2 sequestration.

Firstly, the results of quantification of acetogen and VFAs percentages in Fig. 1 clearly indicate that acetogen were enriched in the acclimated sludge inoculum, which verified that sodium formate can be used as an effective selective substrate for the acetogen enrichment in sludge. Ryan also demonstrated that sodium formate could be used to enrich acetogenic bacteria in high rate anaerobic reactors under mesophilic and thermophilic conditions [32]. In addition to sodium formate, acetogenic communities were enriched with syngas substrate from thermophilic anaerobic sludge. Parameswaran et al. reported that homo-acetogens made effective positive syntrophic interaction in MXC anodes for efficient consumption of H_2 to produce electrical current [33].

According to the chemical profiles during digestion (Fig. 2), acetogen-enriched digestion showed a higher acetate concentration but a lower H_2 and CO_2 partial pressure. As described previously, acetogenic bacteria can grow autotrophically on H_2 and CO_2 and/or heterotrophically on numerous organic compounds [34]. Under the condition of autotrophically growth, H_2 and CO_2 are transformed into acetate according to the following reaction equation: $4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$ ($\triangle G^{0'} = -95$ KJ/mol). Therefore, based on the concentration differences of H_2, CO_2 and acetate between digestions with and without acetogen-enriched sludge inoculums, it can be concluded that enriched acetogen significantly improved the conversion of H_2 and CO_2 to acetate. According to the chemical profiles in acidogenic digesters, it was obvious that acetogen-enriched inoculum improved CO_2 sequestration and acetate yield due to acetogenic reaction based on the Wood–Ljungdah pathway, indicating the acetogen inoculum would be beneficial to CO_2 emission mitigation in anaerobic digestion [35].

It is obvious that the microbial community structure shifted significantly after three fermentation batches with the acetogen-enriched inoculum. The change of the microbial community was in accordance with the profiles of VFAs pattern. This further proved the role of acetogens for acetate accumulation in fermentation. On the other hand, it also demonstrated the loss of dominance of acetogens in the system without the selective pressure (sodium formate substrate), suggesting the microbial community is unstable. A number of studies have investigated the microbial community shift in closed anaerobic digester and explained the different disturbance of operational parameters and how to control the stability of the

Conventional digestion process	Novel digestion process
Acetate yield: 2.33 mmol/g COD	Acetate yield: 7.37 mmol/g COD
Acetate output: 198.52 kg/d	Acetate output: 627.92 kg/d

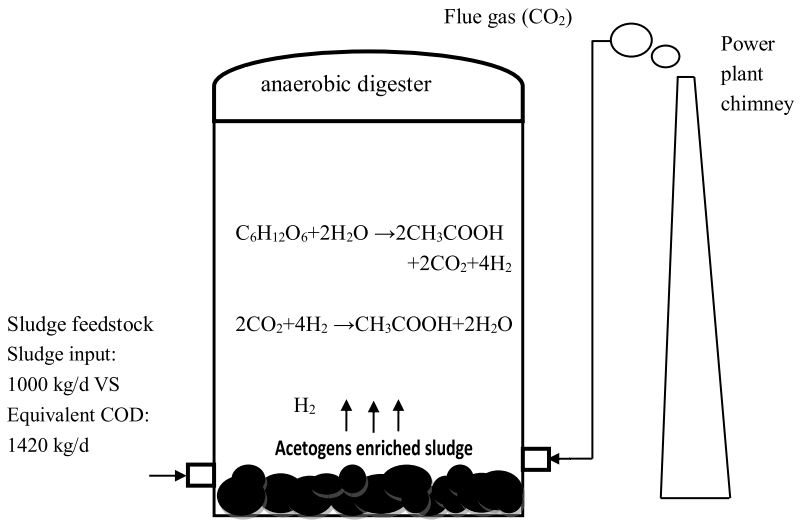

Fig. 5. The schematic process of the enhanced acidogenic fermentation process.

microbial community [36,37]. In that case, acetogen enriched seed sludge might be reseeded into the reactor occasionally to keep the high content of the acetogens. In future, some methods could be used to diminish the washout of the acetogens in the anaerobic fermentor, such as longer sludge retention time, recirculation of the effluent, etc.

It can be concluded that the acetate yield will be selectively improved and CO_2 in the headspace will also be converted into acetate by acetogen-enriched inoculum for glucose anaerobic fermentation. Therefore, on the basis of the above experiments and relative literature reports, we came up with the potential application by enriched acetogens sludge inoculums. A potential enhanced process (Fig. 5) for the selective acetate production and reduction of CO_2 emission is proposed when organic wastes like sludge are anaerobically fermented for VFAs production. As shown in Fig. 5, the acetogen-enriched sludge is inoculated into the fermentor as inoculum, sludge feedstock is used as the raw material and the flue gas containing CO_2 from the electricity power plant is also sparged into the fermentor. In that way, acetate will be selectively enhanced and the CO_2 will be sequestrated and transformed into acetate.

The calculation of acetate production and CO_2 emission mitigation was conducted to show the effectiveness of this potential enhanced process based on the results in this study. In conventional acidogenic fermentation process without acetogen-enriched inoculums, 1000 kg/d sludge feedstock (calculated as VS weight) which was input into the acidogenic fermentor will produce 198.52 kg/d acetate and 331.30 m^3 CO_2 based on the acetate yield in Table 2 and equations in Fig. 5, while in the potential enhanced process with the enriched acetogen as inoculum and additional CO_2 in flue gas is sparged into the fermentor, the acetate output would be 627.92 kg/d and nearly no CO_2 emission, which means 320.77 m^3 of CO_2 will be sequestrated and converted into acetate.

This process has a significant economic and environmental advantages over conventional acidogenic fermentation processes by selectively increasing the acetate yield and mitigating CO_2 emission. The flue gas from electric power plants is a major CO_2 emission source and it can provide CO_2 to the acetogens in this enhanced system. Meantime, it is well known that the hydrogen can be produced and accumulated during the hydrolytic and fermentative stages in the anaerobic digestion, so the hydrogen can be used as another feasible supply for the acetate production by the potential enhanced process.

5. Conclusions

In this study, the acetogens were successfully enriched in raw sludge by using sodium formate as substrate in a UASB reactor. When acetogens-enriched sludge was used as inoculum in anaerobic digestion for selectively acetate production, acetate percentage in VFAs were significantly improved and the yield of acetate 4.14 mmol/g COD, which was 1.77 times higher than the control. With CO_2 sparging in the headspace of the reactors, the acetate yield was 7.37 mmol/g COD which was 3.16 times higher than the control due to the conversion of CO_2 into acetate by acetogenesis reaction. The microbial community structure in the acetogens-enriched inoculums shifted significantly after in the anaerobic fermentation. A potential enhanced process was proposed for selective acetate production and reduction of CO_2 emission with sludge as feedstock. Based on the calculated results, the enhanced process showed significant economic and environmental advantages over the conventional acidogenic fermentation processes.

Acknowledgments

This work was financially supported by Fundamental Research Funds for the Central Universities (JUSRP51633B), National Natural Science Foundation (No. 51678280), Natural Science Foundation of Jiangsu Province of China (No. BK2012121).

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