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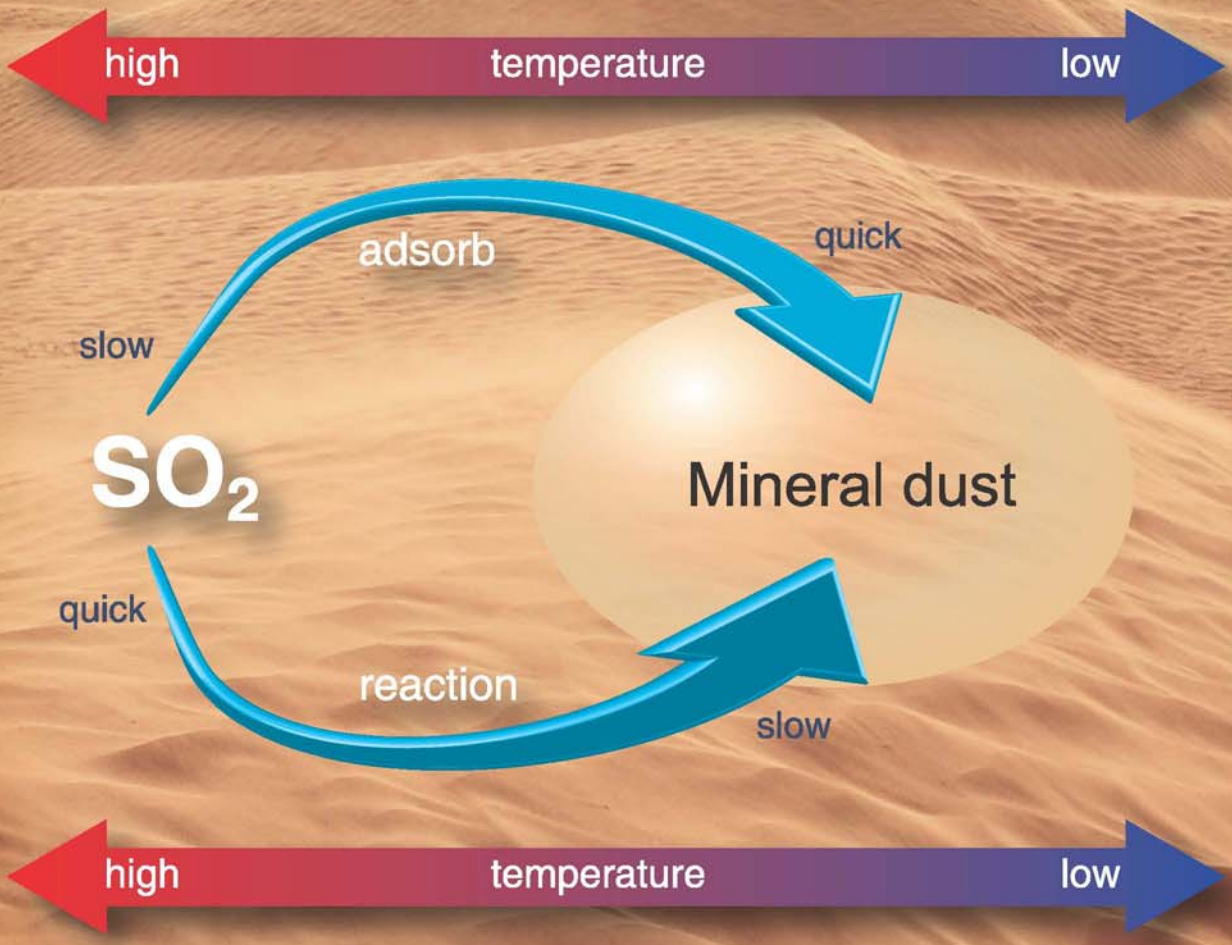

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CONTENTS

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Microbial community structures in an integrated two-phase anaerobic bioreactor fed by fruit vegetable wastes and wheat straw

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ABSTRACT

The microbial community structures in an integrated two-phase anaerobic reactor (ITPAR) were investigated by 16S rDNA clone library technology. The 75 L reactor was designed with a 25 L rotating acidogenic unit at the top and a 50 L conventional upflow methanogenic unit at the bottom, with a recirculation connected to the two units. The reactor had been operated for 21 stages to co-digest fruit/vegetable wastes and wheat straw, which showed a very good biogas production and decomposition of cellulosic materials. The results showed that many kinds of cellulose and glycan decomposition bacteria related with Bacteroidales, Clostridiales and Syntrophobacterales were dominated in the reactor, with more bacteria community diversities in the acidogenic unit. The methanogens were mostly related with *Methanosaeta*, *Methanosarcina*, *Methanoculleus*, *Methanospirillum* and *Methanobacterium*; the predominating genus *Methanosaeta*, accounting for 40.5%, 54.2%, 73.6% and 78.7% in four samples from top to bottom, indicated a major methanogenesis pathway by acetoclastic methanogenesis in the methanogenic unit. The beta diversity indexes illustrated a more similar distribution of bacterial communities than that of methanogens between acidogenic unit and methanogenic unit. The differentiation of methanogenic community composition in two phases, as well as pH values and volatile fatty acid (VFA) concentrations confirmed the phase separation of the ITPAR. Overall, the results of this study demonstrated that the special designing of ITPAR maintained a sufficient number of methanogens, more diverse communities and stronger syntrophic associations among microorganisms, which made two phase anaerobic digestion of cellulosic materials more efficient.

Introduction

The amount of agricultural wastes in China increased rapidly in recent years. According to China Statistical Yearbook 2011, a total amount of crop straw, which were mainly disposed by uncontrolled incineration in China, was about 423 million tons. Meanwhile, the fruit and vegetable wastes (FVWs), with a high organic content for more than 60% and the volatile

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solid (VS) content is 80%–90%, often cause heavy odor and plenty of leachate during collection and transportation (Lin et al., 2011). These residual biomasses rich in cellulose have constituted a major source of renewable energy for generating biogas through anaerobic digestion (AD) (Hobson and Feilden, 1982). In the AD process of residual biomass, the rate-limiting step was hydrolysis (Mata-Alvarez et al., 2000), and the materials containing cellulose often had troubles such as generating scum and foam in most of the developed technologies. These troubles usually resulted in low maximum loading rates and poor decomposition (Svensson et al., 2007).

For optimizing AD process efficiently, the single-phase process can be divided into two phases separately, with liquefaction and acidification in the first phase and methanogenesis in the second one (Mtz-viturtia et al., 1995; Weiland, 1993). Compared with the single-phase AD process, the two-phase technique is a feasible way to improve the biodegradation efficiency and the overall energy productivity by selecting and enriching special microbes in sequential reactors (Azbar and Speece, 2001). Meanwhile, the two-phase approach can ensure greater stability of the overall anaerobic process. A controlled acidogenic unit would provide a constant feed condition for methanogenic unit to avoid inhibition on methanogens (Koutrouli et al., 2009). However, the separation of fermentative and methanogenic environments may lead changes to biological pathways and intermediate metabolite formation. It is unclear whether all the biological pathways involved in AD can be optimized or not in a two-phase process when the acidogenic microbial consortia separated from the methanogenic ones. Though the different microbial groups differ in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions, they work altogether in the same environment of single-phase AD (Pohland and Ghosh, 1971; Demirel and Yenigun, 2002). The separation of acido/acetogenesis from methanogenesis may negatively affect the syntrophic associations, above all by preventing interspecies hydrogen transfer (Iannotti et al., 1973).

Designing a new reactor for anaerobic digestion of plant biomass should make the utilization of the agricultural biomass to a greater extent possible. The integrated two-phase anaerobic reactor (ITPAR) was a novel reactor better than single-phase reactor and two-phase reactor in conventional sense. It was suitable for anaerobic digestion of plant biomass that is rich in cellulose fiber, difficult to hydrolyze and easy to be separated from slurry. In the ITPAR, an integrated anaerobic digestion happened with a solid phase of hydrolytic acidification in the upper part and a liquid phase of methanogenesis in the lower part. The half-submerged perforated roller, used for containing cellulosic materials and enhancing mass retention and transfer, was installed in the upper part of reactor. The roller could not only avoid scum generation and separate the solids and liquids, but also segregate most of the methanogenic microorganisms (present in the lower part of reactor in the form of anaerobic granular sludge) and the feedings (agricultural biomass) in the same reactor. The dissolution and hydrolysis of submerged organic waste were enhanced by retained microorganism in the fermentation liquor. The hydrolysis products then got into liquid phase that can be well used by bacteria and methanogens in the middle and lower part of reactor. The methanogens in the lower part would not be washed out along with the feeding and discharging of reactor.

The objectives of this study are to evaluate both the methanogen and bacterium community structures involved in the different parts of ITPAR by 16S ribosomal ribonucleic acid (rRNA) gene clone library technology. The present study could provide a more precise view of the biological processes and the main microbial communities involved in co-digestion of FVWs and wheat straw (WS) in ITPAR and the mechanism of efficient anaerobic digestion.

1. Materials and methods

1.1. Reactor operating conditions and samples collection

The total volume of the newly designed ITPAR was 115 L, with the efficient reaction volume of 75 L and the roller volume of 25 L (Fig. 1). The half-submerged roller that was used to contain solid waste, rotated at 5 r/min in the upper part of reactor throughout the experiment. Inside the rotating roller, six parts were divided by three clapboards which could not only ensure complete mixing of raw materials but also avoid fiber twining, scum and incrustation while mixing materials with crude fiber. Many holes with 5 mm diameters were evenly distributed on the surface of the roller to allow sufficient transfer of hydrolysates and full contact of mass with fermentation liquor. Between the acidogenic unit and methanogenic unit installed, a recycling system used to circulate the hydrolysates from the acidogenic unit to the methanogenic unit, with an inner recycling flow of 0.45 m^3/hr during the experiment. It could provide sufficient substrates for the methanogenic unit and avoid the acid accumulation in the acidogenic unit. The biogas was collected from the top of the reactor. The operating temperature was kept at (35 ± 1)°C by a hot water jacket with hot water recycling.

The anaerobic granular sludge with good methanogenesis activity was used as inoculum, which was taken from a full-scale Up-flow Anaerobic Sludge Bed (UASB) reactor (Qinhuangdao City, Hebei, China) treating starch-processing wastewater at 35°C. Raw FVWs were collected from a fruit/vegetable market in Beijing, China, which mainly contained residues of vegetables such as Chinese cabbage, carrot, lettuce, and different fruits, such as apple, banana, pear, and watermelon. Raw WS were collected from wheat fields in a suburban of Beijing, China. The FVWs were shredded to small pieces after the fruit cores were removed and the WS were sized to approximately 5 cm in length. The characteristics of FVWs and WS were shown in Table 1. Both the FVWs and WS were homogenized with a certain ratio (calculated by VS) before feeding. The digester was operated in a batch style in the acidogenic unit and a continuous style in the methanogenic unit. The residence time for the mixed waste in the acidogenic unit decreased from 24 to 17 days for the first four stages (considered as start-up periods) and then maintained at 10 days for the following seventeen stages. At the end of each stage, all the solid residues in the roller were removed and the additional water from raw materials (made up roughly 6%–14% of the total fermented liquid) was discharged from the liquid outlet respectively. The acclimated microorganisms retained in fermentation liquor would rapidly decomposed the new feedings by dissolution and hydrolysis.

For microbial structure analysis, sludge sample 1 to sample 4 were collected from S1 to S4 (different heights of the reactor) at the third day of stages 19, 20 and 21 (with the same operation condition) (Fig. 1). For each sample, three 50 mL mixed liquors were collected in three centrifuge tubes and then were centrifuged at 15,000 r/min for 20 min at 4°C (CR22G, HITACHI, Tokyo, Japan), the supernatants were removed and the sediments were stored at 4°C in refrigerator.

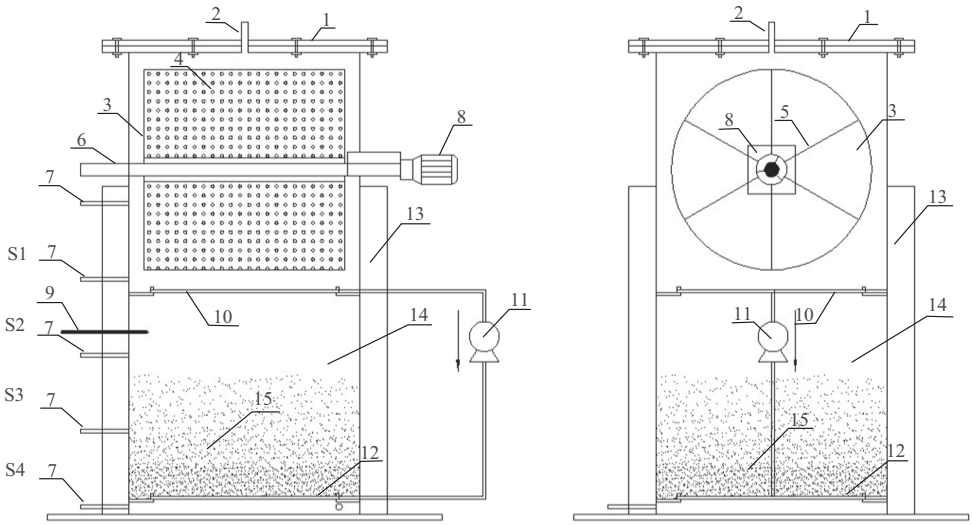

Fig. 1 – Schematic of the integrated two-phase anaerobic reactor (ITPAR). (1) Top cover, (2) biogas outlet, (3) acidogenic reaction roller, (4) holes on the surface of the roller, (5) inner clapboard, (6) rotation axis, (7) sampling orifice one to four, (8) rotation motor, (9) temperature probe, (10) recycling pipe, (11) recycling pump, (12) recycling distributor, (13) water jacket, (14) methanogenic unit, (15) anaerobic granular sludge.

1.2. Clone library analysis

The deoxyribonucleic acids (DNAs) of different sludge samples were extracted using the Fast DNA SPIN Kit for Soil (MP Biomedicals LLC., califonia, USA). The DNAs of triplicate samples were pooled together for further molecular analysis. Bacterial 16S rRNA genes were amplified using primers 63F (5′-CAGGCCTAACA CATGCAAGTC-3′) and 1387R (5′-GGG CGG WGT GTA CAA GGC-3′) (Marchesi et al., 1998). Archaea 16SrRNA genes were amplified with archaea primers 109F (5′-ACKGCTCAGTAACACGT-3′) and 915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Grosskopf et al., 1998). Polymerase chain reaction (PCR) conditions for bacterial 16S rRNA genes were as follows: 94°C for 5 min, 8 cycles of 94°C for 1 min, annealing at 65 to 58°C (reducing the temperature by 1°C per cycle) for 30 sec, and extension at 72°C for 2 min; additional 17 cycles of 94°C for 1 min, 58°C for 30 sec, and 72°C for 2 min; and final extension at 72°C for 10 min. PCR conditions for archaea 16S rRNA genes were as follows: 94°C for 5 min, 21 cycles of 94°C for 1 min, annealing at 62 to 52°C (reducing the temperature by 0.5°C per cycle) for 0.5 sec, and extension at 72°C for 1 min; additional 9 cycles of 94°C for 1 min, 52°C for 30 sec, and 72°C for 1 min; and final extension at 72°C for 10 min. The PCR products were purified with QIA quick PCR purification kit (Takara Biotechnology Co.,Ltd., Dalian, China) and then cloned into)pGEM-T easy vector (Promega,Wisconsin, USA). Clones were sequenced (completed by Shanghai Sangon Biotech, Co., Ltd., China) using a 3730XL DNA Analyzer (Applied Biosystems Co., USA).

Chimeras were excluded from further analyses using Mallard 1.02 (Cardiff School of Biosciences, United Kingdom) (Ashelford et al., 2006). Sequences sharing identity greater than 97% identity was grouped into one operational taxonomic unit (OTU) using the Distance-Based OTU and Richness (DOTUR) program. The DOTUR program was also used to calculate the diversity indexes (Schloss and Handelsman, 2005). One representative clone was chosen from each selected OTU, and then submitted to the BLASTN (NCBI, USA) to obtain the closest relatives. Phylogenetic trees were constructed using MEGA 5.10 (Center for Evolutionary Medicine and Informatics, USA) (Tamura et al., 2011). Taxonomic identities of the clones in each OTU were assigned using the Ribosomal Database Project (RDP) II analysis tool "classifier" (Wang et al., 2007). The beta-diversity index was calculated to determine the difference between two communities (Legendre et al., 2005).

2. Results and discussion

2.1. Bioreactor performance

The ITPAR has been operated to anaerobically co-digest FVWs and WS for 21 stages at different organic loading rates and

Table 1 – Characteristics of fruit/vegetable wastes (FVWs) and wheat straw (WS).

Substrate	Total solid (%)	Volatile solid (wt.% TS)	Elemental compositions (wt.% TS)				C/N ratio	Particle size (mm)
			C	H	O	N		
FVWs	7.37	88.06	43.26	5.18	33.01	2.77	15.60	<5
WS	94.47	88.63	42.22	5.36	39.68	0.85	49.67	<50

TS: total solids.

WS/FVWs ratios. During the experimental period, the organic loading rate reached 1.37 kg VS/(m^3·day), with the maximum daily biogas generation rate of 328 L/day on the first day of the stage. The methane contents in the biogas generated ranged from 64.9% to 76.7% in each stage and the VS removal efficiency was higher than 85%. The maximum biogas production was approximately 760 L/kg VS (removed). These results were comparable with those obtained from grass silage digestion in certain two-phase anaerobic reactors (Nizami et al., 2010; Nizami and Murphy, 2011).

The final three stages (stage 19 to stage 21) were operated with the same operation condition. The feeding amount and the reactor efficiency from stages 19 to 21 were summarized in Table 2. The average value and the standard deviation of VS removal efficiency and total biogas production indicated that the reactor had a stable performance under similar operation condition.

Considering that the daily biogas production, pH and VFA concentration from all stages showed similar trends, we only presented the data in stage 19 to illustrate the operation state of ITPAR. As shown in Fig. 2, the daily biogas production peaked in the first day and kept higher in the first three days. It was possible that the retention of acclimated microorganisms in ITPAR was critical to the quickly startup of each stage. The pH values were more volatile in the acidogenic unit than those in the methanogenic unit. Methanogens that were subject to the environmental fluctuations kept stable and active in the optimized conditions of methanogenic unit to ensure the efficient conversion of VFAs. The variant VFA concentrations in the two units and the less VFA accumulation indicated the efficient mass transfer of the reactor. The acidogenic and methanogenic units can be separated in a combined reactor but can also be combined *via* mass transfer to achieve efficient anaerobic digestion.

2.2. Evaluation of 16S rDNA clone library

Based on ARDRA and sequence analysis, OTUs and diversity indexes were both determined at the 3% sequence difference level using the DOTUR program (Table 3). The 55, 31, 23 and 22 OTUs were recovered from sample 1 to sample 4 of bacterial community, and 23, 23, 15 and 18 OTUs were recovered from sample 1 to sample 4 of archaea community. The coverage and Shannon–Wiener index of these libraries were showed in Table 3. Shannon index presented a reducing trend from sample 1 to sample 4 in both bacterial and archaea clone libraries, indicating the richness and diversity of microbial communities were decreasing from top to bottom of the reactor, *i.e.*, the microbial (both bacteria and archaea) diversity was richer in acidogenic unit than that in methanogenic unit. Coverage C proved that these libraries were large enough to yield unbiased estimates (Kemp and Aller, 2004).

Table 2 – Feeding amount and efficiency of the reactor in sampling stages.

Stage [a]	Removal efficiency of total VS	Average	Total biogas production (m^3)	Average
19	87.4%	88.8% ±	0.641	0.689 m^3 ±
20	89.1%	1.3%	0.677	0.055%
21	90.0%		0.749	

[a] Stage 19 to stage 21 were operated in the same operation condition. The loading rate was 1.37 kg VS/(m^3·day), the VS_{WS}: VS_{FVWs} in feeding was 0.22:1 with 0.19 kg VS_{WS} and 0.84 kg VS_{FVWs}. VS: volatile solids.

2.3. Identification of microbial community structures

The compositions of bacterial family in four samples were illustrated in Fig. 3a. The total sequenced clones could fall into seven families. Bacteroidales formed the most frequent order in the clone library, which accounted the 62.1%, 66.6%, 77.9% and 81.3% of the total clones in four samples. Four strictly anaerobic families making up the Bacteroidales were Bacteroidaceae, Rikenellaceae, Porphyromonadaceae and Prevotellaceae (Suzuki et al., 1999). The mesophilic anaerobe Bacteroidaceae possessing the ability to degrade cellulose (Khan et al., 1980), accounted for a vast majority of the total number of Bacteroidales. The prevalence of them reflected the ability to metabolize a variety of organic compounds including protein, lignin, cellulose, sugars, lipids and amino acids. Rikenellaceae was a type shown to ferment carbohydrates such as glucose to produce propionic acid and succinic acid (Svensson et al., 2007), suggesting that they were involved in acidogenesis. The groups Porphyromonadaceae and Prevotellaceae were much less than the two above groups. Previous studies showed that protein hydrolysate could significantly stimulate the growth of Porphyromonadaceae and Prevotellaceae, and they could break down some sugars in anaerobic conditions (Tao et al., 2007). Other dominant families were Clostridiaceae and Syntrophomonadaceae that belonged to the order Clostridiales, while Syntrophaceae belonged to the order Syntrophobacterales. The genus *Clostridium* was known for degradation of complex organic materials such as cellulose, starches and lipids (Rintala and Puhakka, 1994) and often predominated in high concentrations of acetic acid. The genus *Syntrophomonas* was reported as those which were able to produce acetic acid from butyrate (Schmidt et al., 2013). Syntrophaceae was able to degrade propionic acid to acetic acid (Tang et al., 2007). The even distribution of Clostridiaceae and Syntrophomonadaceae explained a syntrophic relationship of hydrolysis/acidogenesis and acetogenesis between the two groups of microorganisms throughout the reactor. On the whole, the acidogenic unit had more diverse communities and greater metabolic diversity. The different trophic groups that recovered from acidogenic unit suggested the co-digestion FVWs and WS mainly supported acetate, propionic and butyrate-producing bacteria.

The compositions of archaea genus in four samples were illustrated in Fig. 3b. The *Methanosaeta* constituted the dominant genus (accounted for 40.5%, 54.2%, 73.6% and 78.7% of the total clones in four samples) in the clone library, followed by *Methanosarcina* (accounted for 19%, 14.4%, 8.26% and 5.6% of the total clones in four samples). The lower part of the reactor had a relatively higher abundance of *Methanosaeta* known to use acetate as the only substrate for methanogenesis. Species from the *Methanosarcina* preferred methylated compounds such as methanol and methylamines

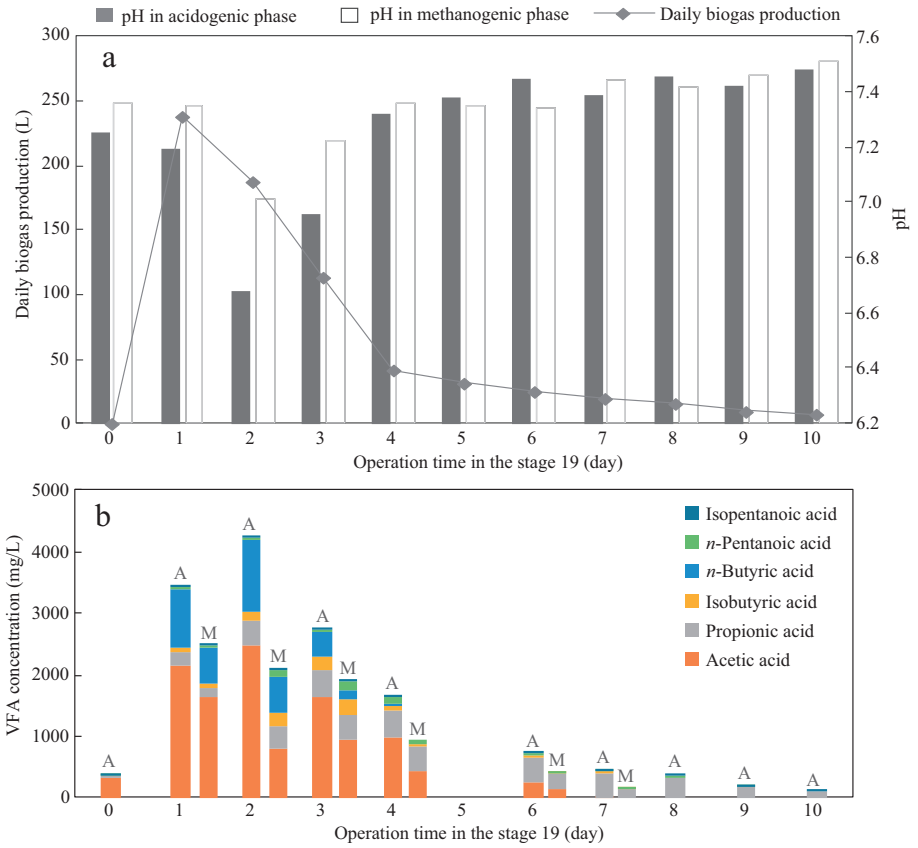

Fig. 2 – (a) Daily biogas production rate and pH values in acidogenic unit and methanogenic unit of ITPAR in the stage 19 and (b) VFA concentrations in acidogenic unit and methanogenic unit of ITPAR in the stage 19. A: acidogenic unit; M: methanogenic unit.

as substrate for producing methane (Shin et al., 2010). Because of the high co-digestion efficiency in ITPAR for the first three days of stage, there was a low acetic acid concentration during the last few days of stage in the reactor (especially in the methanogenic unit). Over time, the low acetic acid concentration made the *Methanosaeta* finally outcompeted *Methanosarcina* to be the dominant population for its higher half-saturation constant (K_s, 0.44 mmol/L) and lower maximum growth rate (U_{max}, 0.11 per day) (Harper and Pohland, 1986). This hypothesis may explain the predominance of *Methanosaeta* in the ITPAR especially in the methanogenic unit. The relative abundance of hydrogenotrophic methanogens decreased in four samples. The *Methanobacterium* and *Methanoculleus* accounted for 15.5%, 10.4%, 5.0%, 5.6% and 12.1%, 6.8%, 6.6%, 3.2% in four samples respectively. The *Methanospirillum* appeared primarily in the upper part of reactor (sample 1 and sample 2). All these results indicated that methanogenesis was mainly carried out by acetoclastic methanogens throughout the reactor. Meanwhile, hydrogenotrophs were also involved in the biogas production especially in the acidogenic unit. This was different from the results in other studies that the hydrogenotrophs methanogens were the major microbials of acidogenic unit in the conventional two-phase anaerobic digester. (Shimada et al., 2011).

The co-digestion of ITPAR gave rise to diverse microbial communities and metabolic pathways in the reactor. The transient accumulation of VFAs and the hydrogen partial pressure in acidogenic unit after feeding led to an increase of

Table 3 – Operational Taxonomic Units (OTUs) and diversity indexes (calculated at 0.03 difference level) of archaea and bacterial clone libraries.

Library	Number of clones	Number of OTUs	Shannon–Wiener index	Coverage (%)
Bacteria 1	132	55	1.11	74.2
Bacteria 2	135	31	0.93	80.7
Bacteria 3	131	23	0.71	87.0
Bacteria 4	139	22	0.66	89.2
Archaea 1	116	23	0.86	85.3
Archaea 2	118	23	0.82	87.3
Archaea 3	121	15	0.66	92.6
Archaea 4	126	18	0.57	91.3

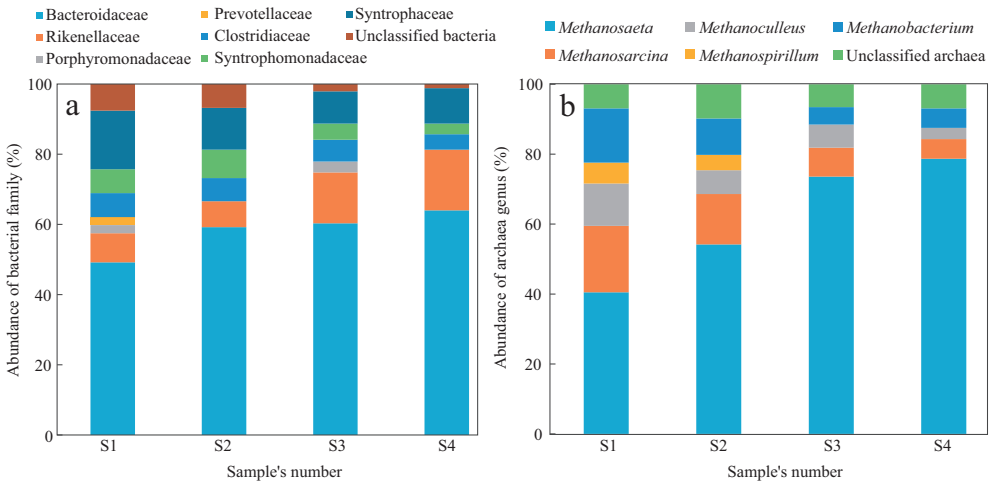

Fig. 3 – Abundance of bacterial family (a) and archaea genus (b) in four samples.

relative abundance of *Methanosarcina* and hydrogenotrophic methanogens and a corresponding decrease of *Methanosaeta* in the upper part of the reactor. Meanwhile, the *Methanosaeta*, proved to be the major microbes for methanogenesis in the reactor, outcompeted to be dominant by the neutral conditions (pH 6.8–7.5) and the low concentration of acetic acid in most time of stage. Also, the ITPAR maintaining syntrophic associations among microorganisms in two units and guaranteeing sufficient number of methanogens in methanogenic unit ensured the efficiency in AD process.

Beta diversity index was calculated to determine the community difference between each two samples. The beta-diversity characterizations between each pair samples (S1–S2, S2–S3, S3–S4, S1–S3, S2–S4, S1–S4) were shown in Fig. 4. Both the archaea and bacterial diversity indexes were augmented with sampling distance increase and the overall archaea indexes were higher than that of bacterial. Among the adjacent samples, S1–S2 reached the maximum value in bacterial clone while S2–S3 was in maximum index in archaea clone, illustrating that the bacterial composition in sample one was much different from other samples, and the composition difference of methanogens were more similar in both acidogenic unit (sample 1 and sample 2) and methanogenic unit (sample 3 and sample 4) than between them (sample 2 and sample 3). On the whole, there existed a more even bacterial distribution but an obvious methanogens variation between acidogenic unit and methanogenic unit in the reactor. The differentiation of methanogenic community composition in acidogenic unit and methanogenic unit, as well as pH values and VFA concentrations indicated the successful phase separation in the integrated reactor. Meanwhile, the co-existence of the anaerobes as well as many intermediate metabolites in one reactor will be good for syntrophic associations among microorganisms.

2.4. Phylogenetic analysis of the major OTUs

The phylogenetic relationship of the presentative sequences of each major OTU (with at least three clones) was submitted to Genbank database for phylogenetic analysis. In Fig. 5a, the phylogenetic tree of archaea 16S rRNA gene sequences showed that the methanogens were grouped mainly with the genus *Methanosaeta*, *Methanosarcina*, *Methanoculleus*, *Methanospirillum* and *Methanobacterium*. The sequence belonging to *Methanosaeta* showed a 100% identity with related to *Methanosaeta concilii*, a kind of acetoclastic methanogens that can directly use acetate. The sequence belonging to *Methanosarcina* shared a 100% similarity to *Methanosarcina mazei*, the substrates of which were variety such as H_2/CO_2, acetate, all methylamines and methanol (Osumi et al., 2008). Among the hydrogen-utilizing

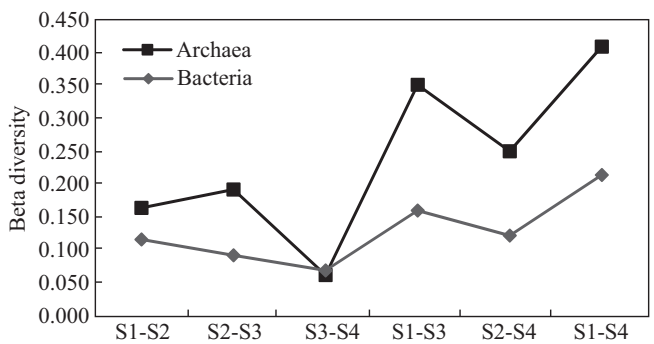

Fig. 4 – Beta diversity of archaea and bacteria between each pair samples.

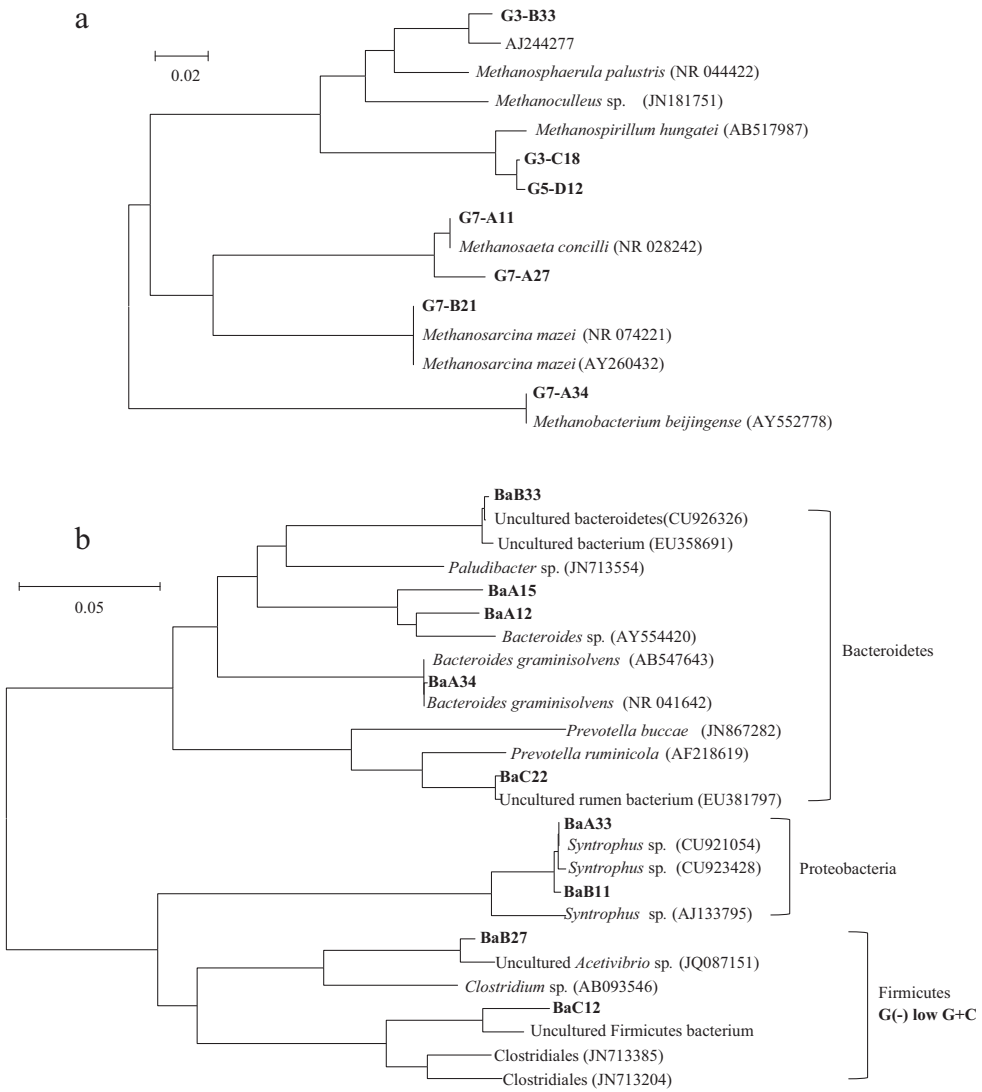

Fig. 5 – Phylogenetic tree of archaea (a) and bacterial (b) 16S rRNA gene sequences.

methanogens, *Methanobacterium* was with 95% identity related to *Methanobacterium beijingense* that uses H_2/CO_2 and formates for its growth and methane production (Ma et al., 2005). *Methanospirillum* was with 99% identity related to *Methanospirillum hungatei* (Iino et al., 2010). The sequences affiliated with *Methanoculleus* had high identity to methanogenic archaea in stable anaerobic cellulose-degrading reactor (Chin et al., 1999).

Phylogenetic tree of bacterial 16S rRNA gene sequences was shown in Fig. 5b. Members of the phyla Bacteroidetes, Firmicutes and Proteobacteria were involved in the co-digestion of FVWs and WS. Sequences BaA12, A15, B33, C22, B27, C12 and A33 were particularly abundant through the entire reactor suggesting that they were key components of the community. BaA12 belonging to the family Bacteroidaceae was related with 96% identity to several cultured sequences such as the novel anaerobic cellulolytic *Bacteroides* sp. (AY554420.1) isolated from a landfill leachate bioreactor. Another *Bacteroides* sp. (NR_041642.1), a xylanolytic anaerobe isolated from a methanogenic reactor treating cattle waste (Nishiyama et al., 2009), also shared 96% similarity with sequence BaA12. This strictly anaerobic bacterial strain produced acetate, propionate and succinate by utilizing xylan and sugars including arabinose, xylose, glucose, mannose, cellobiose, raffinose and pectin. All of these were in accordance with the major components of ITPAR feedstock (FVWs and WS), which contain high concentrations of cellulose and saccharides. The sequence BaA15 belonging to the family Rikenellaceae showed to be 99% identical with *Petrimonas sulfuriphila*, which is a kind of H_2-producing acetogens that have to grow fairly close to the hydrogen-utilizing Methanobacteriales to produce acetate, hydrogen and CO_2 during glucose fermentation (Grabowski et al., 2005). The sequence BaB33 belonging to the family Porphyromonadaceae showed to be 99% identical with uncultured bacterial CU926326 and EU358691, but only shared 89% identical with *Paludibacter propionicigenes*, a strictly anaerobic, propionate-producing bacteria utilized various sugars and produced propionate and acetate as major fermentation products with a small amount of succinate (Ueki et al., 2006).

The sequence BaC22 belonging to the family Prevotellaceae had 99% similarity with uncultured bacterial GQ327017 and EU381797, it showed to be 94% identical with *Prevotella* sp. which was identified as FOS- and GOS-degrading bacteria. Firmicutes (*e.g.*, *Clostridium*) known to produce cellulases, lipases, proteases and other extracellular enzymes (Leven et al., 2007) suggested that they were involved in hydrolysis and acidogenesis. The sequence BaB27 belonging to the family Clostridiaceae was 98% identical with uncultured bacterial JQ087151 and 92% identical with *Clostridium clariflavum*, formatting of either ethanol acetate and formate as principal fermentation products as well as lactate and glycerol as minor products by utilizing cellulose and xylan (Sizova et al., 2011). The sequence BaC12 belonging to the family Syntrophomonadaceae could be co-cultured with the hydrogenotrophic methanogen *M. hungatei* to oxidize straight-chain saturated fatty acids with carbon chain lengths of C4–C18 (Hatamoto et al., 2007). The sequence BaA33 belonging to the family Syntrophaceae was 95% identical with *Syntrophus* sp. (AJ133795) and 100% identical with uncultured bacterial CU921054.

3. Conclusions

In the present study, microorganisms were acclimated in an innovative ITPAR. Based on the 16S ribosomal DNA clone library analysis, the archaea and bacterial compositions were identified in different parts of the reactor. The co-digestion of FVWs and WS mainly supported lignocellulose and sugar degradable bacteria. The upper part of the reactor had more diverse communities and metabolic pathways. Methanogenesis was mainly carried out by acetoclastic methanogens in the reactor, though hydrogenotrophs also involved in the biogas production especially in the acidogenic unit. The differentiation of methanogen community composition in acidogenic unit and methanogenic unit, as well as pH values and VFA concentrations indicated the successful separation of the acidogenic unit from the methanogenic unit in the ITPAR. The diverse acclimated microbial communities, strong syntrophic associations among microorganisms along with the stable and active methanogens resulted in the efficient anaerobic co-digestion of cellulosic wastes in the ITPAR.

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