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Anaerobic digestion of spent mushroom substrate under thermophilic conditions: performance and microbial community analysis

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Abstract Spent mushroom substrate (SMS) is the residue of edible mushroom production occurring in huge amounts. The SMS residue can be digested for biogas production in the mesophilic anaerobic digestion. In the present study, performance of batch thermophilic anaerobic digestion (TAD) of SMS was investigated as well as the interconnected microbial population structure changes. The analyzed batch TAD process lasted for 12 days with the cumulative methane yields of 177.69 mL/g volatile solid (VS). Hydrolytic activities of soluble sugar, crude protein, and crude fat in SMS were conducted mainly in the initial phase, accompanied by the excessive accumulation of volatile fatty acids and low methane yield. Biogas production increased dramatically from days 4 to 6. The degradation rates of cellulose and hemicellulose were 47.53 and 55.08%, respectively. The high-throughput sequencing of 16S rRNA gene amplicons revealed that *Proteobacteria* (56.7%–62.8%) was the dominant phylum in different fermentative stages, which was highly specific compared with other anaerobic processes of lignocellulosic materials reported in the literature. *Crenarchaeota* was abundant in the archaea. The most dominant genera of archaea were retrieved as *Methanothermobacter* and *Methanobacterium*, but the latter decreased sharply with time. This study shows that TAD is a feasible method to handle the waste SMS.

Keywords Biogas · Lignocellulosic biomass · *Proteobacteria* · *Crenarchaeota* · *Methanothermobacter*

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Introduction

Spent mushroom substrate (SMS) is a by-product of edible mushroom production. In recent years, with the popularization of cultivation technologies, edible mushroom production increased significantly along with SMS. The annual output of SMS is more than 30 million tons, which not only gives challenges for the ecological environment but also brings great hidden danger to the edible mushroom cultivation industry (Finney et al. 2009). Biogas production from organic waste, especially agricultural residue, is considered one of the effective and attractive options to alleviate increasing concerns over rapid energy depletion and environmental problems triggered by fossil fuels (Nordell et al. 2016). As a by-product, the biogas residue is a good fertilizer for soil amendment (Pivato et al. 2016; Stefaniuk et al. 2015) that brings lots of benefits to develop an ecological and circulatory agriculture especially comprising mushroom production and crop cultivation. Previous studies have proved that SMS could be digested for biogas production in some mesophilic anaerobic digestion (AD) systems operated at 35~37 °C as sole substrate (Bisaria et al. 1983, 1990; Sharma et al. 1989) or co-digested with other organic wastes (Lin et al. 2014; Shi et al. 2014; Zhu et al. 2015). But, the methane yields of sole SMS, ranging from 50 to 100 mL/g volatile solid (VS), were

relatively low compared to other organic materials. Thermophilic anaerobic digestion (TAD), usually operated at 53~55 °C, has attracted more and more attentions due to many potential advantages, such as accelerating biochemical reactions; shortening the fermentation period; and increasing organic matter removal, solid–liquid separation rate, and pathogen deactivation (Guo et al. 2014; Jang et al. 2015; Lü et al. 2014). However, TAD performance of SMS for biogas production still lacks information.

AD of biomass comprises a series of metabolic steps, known as hydrolysis, fermentation, acetogenesis, and methanogenesis. These metabolic steps are executed by sophisticated microbial communities with tens to hundreds of operational taxonomy units, and many kinds of the microbes are uncultured (Stolze et al. 2015; Zakrzewski et al. 2012). The main raw materials for mushroom production are organic agricultural wastes and residues, such as sawdust, straw, beanstalk, corn cobs, cottonseed hulls, and so on (Zhu et al. 2012). As a solid fermentation by-product of fungus, SMS is also complex, containing lignin, cellulose, mycelium protein, minerals, and other nutrients (Phan and Sabaratnam 2012). Thus, microbial communities digesting SMS for biogas production may have some characteristics that have not been shown in other AD systems digesting lignocellulosic materials. The previous literature reported much work on AD performance of SMS but could not provide sufficient information about the microbial communities (Bisaria et al. 1983, 1990; Sharma et al. 1989; Zhu et al. 2015). Besides, the composition variation of solid residue in different AD stages may also impact the structure of hydrolytic bacteria, and then lead the whole microbial community to change. To better pilot the AD process of SMS, an integrated detection of the detailed structure of the microbial communities and its variation is required.

Therefore, the objectives of this study were (1) to evaluate the TAD performance of SMS as sole substrate, (2) to detect the composition variation of solid residue in different TAD stages, and (3) to investigate the microbial population structure and its changes.

Pleurotus eryngii is a typical edible and pharmaceutical fungus in the genus of *Pleurotus*, which has been cultivated for several countries with the annual production of more than 900,000 tons (Synytsya et al. 2009). In the present study, a laboratory-scale batch TAD experiment of SMS of *P. eryngii* (SMSPE) was carried out. The solid residues in different TAD stages were separated to analyze the chemical composition and to evaluate the degradation rate of different ingredients in the feedstock. For deep microbial analysis, high-throughput sequencing of 16S rRNA gene amplicons from both bacterial and archaeal communities in three stages was conducted on an Illumina Miseq sequencing platform.

Materials and methods

Characterization of feedstocks and inoculum

The SMSPE was obtained after three harvesting cycles in Gutian Edible Mushroom Base (Fujian, China). The original culture medium of *P. eryngii* (No. 50125, China Center of Industrial Culture Collection, Beijing, China) was composed of 48% sawdust, 24% straw, 20% wheat bran, 5% corn flour, 1% sugar, and 2% gypsum. The SMS was dried for 5 h in a drying oven at 60 °C, and then crumbed to 5–10 mm, and then stored in air-tight containers at 4 °C. Initial inoculum was provided from a 2000 L biogas digester fed with energy crops in Fujian Agriculture and Forestry University. This digester works well now, and part of the initial inoculum is still stored in a refrigerator at − 80 °C, which is accessible to provide the inoculum publicly. The initial inoculum was fed with SMSPE for 180 days to acclimate the microbes and to enlarge cultivation. After that, the biogas residue was employed as inoculum after filtering by double-deck, sterile gauzes to eliminate big particles. In the formal experiments, the filtered biogas slurry (inoculum) was mixed with SMSPE (feedstock) without addition of water. The characteristics of the SMSPE and inoculum are presented in Table 1.

Anaerobic digestion system

Aimed to analyze the performance and microbial community variation during the TAD process of SMSPE, simple lab-scale batch anaerobic digesters were employed. The digesters were 1.5-L plastic bottles closed with a thick rubber cap each. A thin needle could get through the cap for liquid slurry and biogas sampling. To measure daily biogas production, the outlet was connected to a water displacement system by a thin needle.

Experimental design

We have done a preliminary experiment (lasted for 30 days) to evaluate the biogas production capacity of this substrate with

Table 1 Characteristics of spent mushroom substrate of *Pleurotus eryngii* and inoculum

Contents	SMSPE[a]	Inoculum
Total solids (%)	46.35 ± 0.34[b]	5.12 ± 0.28
Volatile solids (%TS)	90.92 ± 0.44	92.78 ± 0.15
Total carbon (%TS)	41.65 ± 0.37	34.63 ± 0.19
Total nitrogen (%TS)	2.21 ± 0.03	3.02 ± 0.03
Carbon to nitrogen (C/N) ratio	18.85 ± 0.10	11.47 ± 0.11

[a] Spent mushroom substrate of *P. eryngii*

[b] Average ± S.D. of triplicates

the same inoculums and fermenting conditions. And, we found that about 85% of both biogas and methane were produced in the initial 12 days and selected 12 days as the biogas-producing period in the next formal experiments, which was enough to represent the biogas production capacity of spent mushroom substrate under thermophilic conditions. Eighteen replicated digesters were randomly divided into six groups with three bottles in each group. All the digesters were fed once at the same time with SMSPE to reach the total solid content of 2% and were kept at a thermostatic incubator at 55 °C. Four groups were taken out on days 3, 6, 9, and 12, respectively, for chemical component analysis. After being violently shaken for 3 min, the solid and liquid residue was separated by suction filtration with double-deck gauzes for solid residue composition investigation. The fifth group was used for biogas-producing detection and microbial investigation. The sixth group was set as control without feedstock. The biogas production, methane contents, slurry pH, soluble chemical oxygen demand (SCOD), and total volatile fatty acids (TVFAs) were detected daily and corrected by the control.

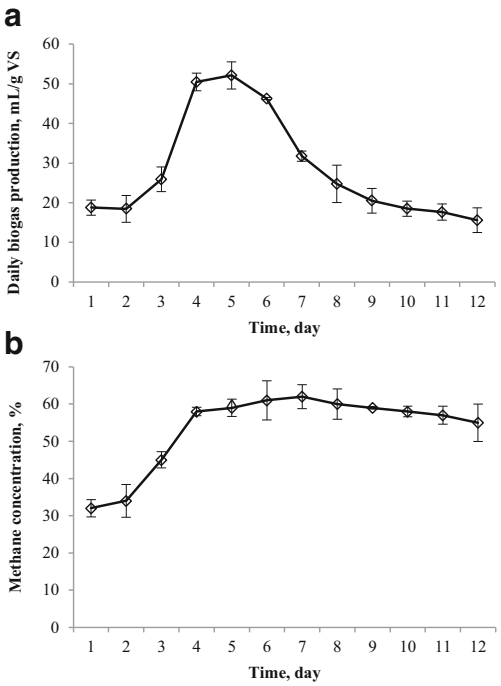

Fig. 1 Biogas production performance in the thermophilic anaerobic digestion of spent mushroom substrate of *Pleurotus eryngii*. **a** Daily biogas yield. **b** Methane concentration

Analytical method

The total solid (TS), VS, SCOD, and TVFA were determined according to standard procedures (APHA 1995). The NREL laboratory analytical protocol (Sluiter et al. 2008) was used to quantify cellulose, hemicellulose, and lignin in the feedstock and solid residues in different TAD stages. Soluble sugar, crude fat, and crude protein were determined according to Chinese Standard Procedures GB 6194 (CNBS 1986), GB/T 6433 (SAC 2006), and GB/T 6432 (SAC 1994), respectively. The biogas yields were determined by water replacement, and then the value was calibrated to that under standard conditions. The methane concentration in biogas was determined by a Biogas Analysis Meter (BX568, Henan Hanwei Electronics Co., Ltd., Zhengzhou, China). The pH values of slurry were measured with a pH meter (Delta 320, Mettler-Toledo Instrument Shanghai Co., Ltd., Shanghai, China) without any dilution.

Microbiological analysis

As a final product of metabolism, biogas production is an indicator of microbial structure and activity. In this study, according to the daily biogas production (Fig. 1a), the batch TAD process could be separated into three stages: initial (days 0 to 3, low biogas production increasing), middle (days 4 to 6, high biogas production), and final (days 7 to 12, low biogas production decreasing). In order to compare the microbial structures in different stages, samples from days 2, 5, and 9 were selected for microbiological analysis.

Biogas slurry of the fifth group was used for microbiological analysis on days 2, 5, and 9 after violent shaking with solid residue. The pellets were collected after centrifugation of 1-mL biogas slurry at 4 °C,12,000 rpm for 10 min. DNA was extracted from the pellets using the E.Z.N.A Soil DNA Kit (Catalog No. D5625–01, Omega Bio-tek, Inc., Norcross, USA) following the manufacturer's instructions.

The protocol to determine the diversity and composition of the microbial community was described previously (Caporaso et al. 2010). The V4 region of the 16S rRNA gene in bacteria was amplified by PCR with the primer set of 5′-GTG CCA GCM GCC GCG GTA A-3′ and 5′-GGA CTA CHV GGG TWT CTA AT-3′ following the protocol described previously. The primer set for archaeal 16S rRNA gene V4 region amplification were 5′-CAG YMG CCR CGG KAA HAC C-3′, and 5′-GGA CTA CNS GGG TMT CTA AT-3′. The PCR products were detected by 2% agarose gel electrophoresis, and the aimed strips were recovered by MinElute PCR Purification Kit (Catalog No. 28004, Tiangen Biotech (Beijing) Co., Ltd., Beijing, China). TruSeq® DNA PCR-Free Sample Preparation Kit (Catalog No. Kit FC-121-3001, Illumina, Inc., San Diego, USA) was used to construct the libraries, which were then sent for high-throughput sequencing on an Illumina MiSeq platform. All the sequence data were submitted to NCBI (Bioproject PRJNA384251).

Merging of pairs of reads from the original sequenced DNA fragments was conducted by FLASH (Magoč and Salzberg 2011). The QIIME software package was used to filter the

raw tag sequences and get clean tags (Caporaso et al. 2010). The UCHIME program was used to remove chimera sequences and get effective tags (Edgar et al. 2011). The operational taxonomic units (OTUs) were determined from effective tags by UPARSE at the threshold of 97% (Edgar 2013). RDP Classifier (Wang et al. 2007) and GreenGene database (Desantis et al. 2006) were used to annotate the OTU representative sequences (setting threshold of 0.8~1), and the community composition of each sample was statistically analyzed at various taxonomic levels. Homogenization of the sequences was performed according to the least amount of data in the samples. The alpha diversity analysis, including rarefaction curve, Chao1, and Shannon indices, was then conducted by QIIME alpha diversity analysis (Caporaso et al. 2010).

Results

Biogas production

The lab-scale batch TAD of SMSPE lasted for 12 days, and the daily biogas production is shown in Fig. 1a. The daily biogas production abruptly increased from day 2 to day 4, and the high biogas-producing status lasted for 3 days. Then, the daily biogas production gradually decreased with the increasing digestion time. After that, a respectively low but stable biogas-producing process (from the day 9 to day 12) appeared. In the initial process, the methane content was relatively low but increased very sharply, from 31.8 to 59.1% within 4 days, followed by a steady-state phase with high methane content (Fig. 1b). The maximum concentration of 62.0% was achieved on day 7. After that, the methane content began to decrease slightly but was still higher than 52%. The total biogas yield from SMSPE by TAD was 328.65 mL/g VS within 12 days. The cumulative methane yield of 12 days was 177.69 mL/g VS. The average methane concentration was 53.8%, indicating that the TAD of SMSPE performed well. The peak biogas-producing period was from days 4 to 6, accounting for 39.31% of the total biogas and 42.4% of methane.

Property variation of biogas slurry

The SCOD in the slurry increased dramatically in the initial stage from 2.93 to 16.2 g/L on day 3 (Fig. 2a), decreased to 4.64 g/L on day 6, and finally stabilized at about 4 g/L from day 7 onwards. The evolution of TVFA in the slurry is shown in Fig. 2b, which demonstrates that the TVFA concentration experienced a drastic change. The concentration of TVFA on day 3 was the highest as a result of the fast degradation of soluble and easily digestible portions of SMSPE. Then, it decreased sharply from 6.44 g/L (day 3) to 1.10 g/L (day 6), followed by a gradually decreasing process. The rapid

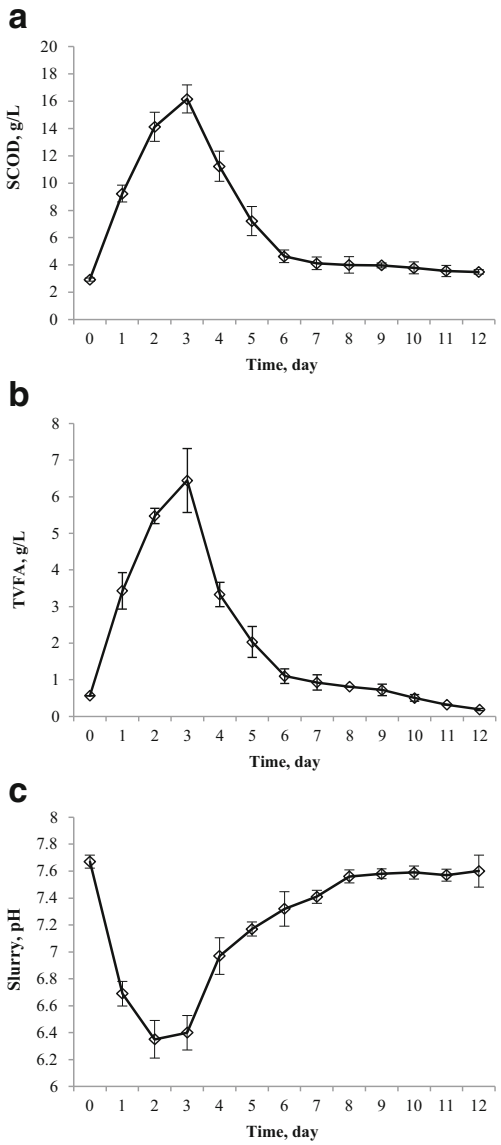

Fig. 2 Biogas slurry in the thermophilic anaerobic digestion of spent mushroom substrate of *Pleurotus eryngii*. **a** Soluble chemical oxygen demand (SCOD). **b** Total volatile fatty acids (TVFA). **c** Slurry pH

increase in TVFA in the initial 3 days was attributed to the conversion of some original degradable substance to TVFA by hydrolysis microbes in biogas liquid. Meanwhile, as shown in Fig. 2c, the slurry pH was linearly decreased from 7.67 (day 0) to 6.35 (day 2), which was the lowest pH during the fermentation process. And then, it sharply increased from 6.40 (day 3) to 6.97 (day 4), followed by a slightly increasing period from day 4 to day 8 and stable stage (7.45~7.59) from day 8 to day 12.

Solid residue degradation and main component variation

The temporal evolution of solid residue degradation is presented in Fig. 3a. After feeding the feedstocks, the weight of

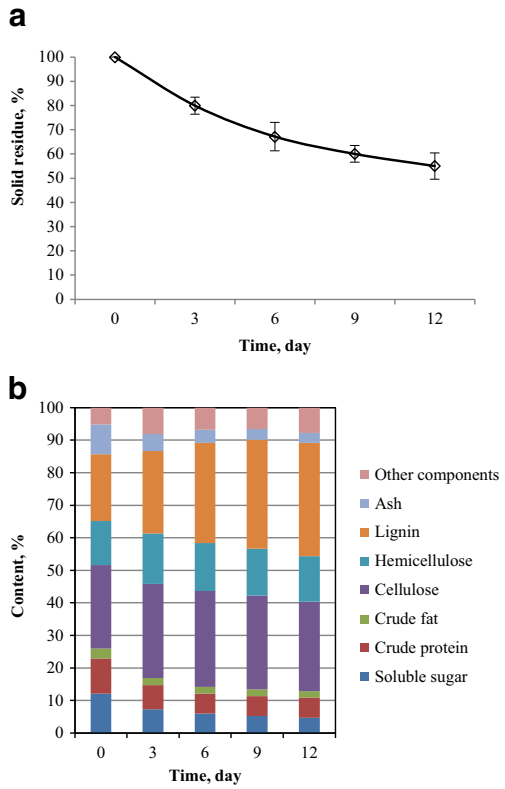

Fig. 3 Solid residues in the thermophilic anaerobic digestion of spent mushroom substrate of *Pleurotus eryngii*. **a** Degradation rate of solid residues. **b** Contents in solid residues

daily solid residue decreased, and the final solid residue weight (day 12) was 55.01% of the initial. About 20.03% of the raw material was degraded in the initial stage, which was the fastest period of degradation. When the fermentation time went to day 6, about 32.84% of the raw material was digested. In the following days, the degradation process in the further degradation appeared quite slow.

The variations of main components in the solid residues are shown in Fig. 3b. The contents of soluble sugar, crude protein, and crude fat decreased very fast in the initial 3 days and became stable in the following period. The lignin content kept increasing during the whole process and reached the highest value of 34.79%. The content changes of cellulose and hemicelluloses were different from above. They were stable around 27 and 14%, respectively.

Microbial community analysis

To better understand the bacterial and archaeal diversity, samples of days 2, 5, and 9 were selected to represent the typical stages in the batch fermentation. The total DNA from these samples was extracted, and the V4 regions of 16S rRNA were sequenced to identify the species and their abundance in the bacterial and archaeal community, respectively.

Analysis of the bacterial and archaeal communities by Illumina Miseq sequencing resulted in 288,566 sequences after quality trim and chimera check, with a range from 61,896 to 100,632 sequences for bacteria and 15,020~22,450 sequences for archaea. The number of OTUs for bacteria ranged from 569 to 654, and a comparatively lower value of 317~394 for archaeal communities was shown (Table 2). Based on the observed species and the Chao1 index, the sequencing covered 62.10~91.48% of the bacterial community, which is lower than that of archaea. The observed species, Chao1, and Shannon index revealed the same general trend that both bacterial and archaeal communities had lower diversity and evenness in the biogas-producing peak stage (day 5). The low diversity of day 5 was also confirmed by rarefaction curve (Fig. 4).

In general, *Proteobacteria* (56.7–62.8%) was identified as the predominant phylum in different fermentation stages studied here, followed by *Firmicutes* (18.5–27.6%), *Thermotogae*, *Actinobacteria*, *Fusobacteria*, and *Chloroflexi* (Fig. 5a). The majority sequences classified to the *Proteobacteria* phylum were assigned to the genera *Ochrobactrum*, *Achromobacter*, and *Erwinia*(Fig. 5b). The phylum *Firmicute* was detected in all different TAD stages of SMSPE, but at different relative abundance, from 18.46 to 27.59% and 23.04% (Fig.5a). The genera of *Clostridium*, *Ruminococcus*, and *Sedimentibacter*, all belonging to order *Clostridiales*, were relatively abundant in the phylum *Firmicute*.

The bacterial community was relatively stable at phylum level but changed obviously at the genus level (Fig. 5). In the initial stage, *Ochrobactrum*, *Achromobacter*, and *Clostridium* were more abundant than other genera. The abundance of *Clostridium*, and *Ruminococcus* increased in the middle stage. In the final stage, *Cetobacterium*'s proportion increased.

The archaeal 16S rDNA gene sequences were distributed to two phyla, *Crenarchaeota* and *Euryarchaeota* (Fig. 6a). The proportion of *Euryarchaeota*, phylum of methanogens, was stable around 5% during the TAD of SMSPE. However, the *Crenarchaeota* increased from 1.54 to 8.36%. The most dominant genera of archaea were consistently retrieved

Table 2 Summary of observed OTUs, Chao1, and Shannon index

Sample	OTUs	Chao 1	Shannon
BacD2[a]	654	898	5.58
BacD5	569	622	5.57
BacD9	644	1037	5.25
ArcD2[b]	393	395	5.64
ArcD5	317	330	4.58
ArcD9	318	371	4.78

[a] Bacterial community on day 2

[b] Archaeal community on day 2

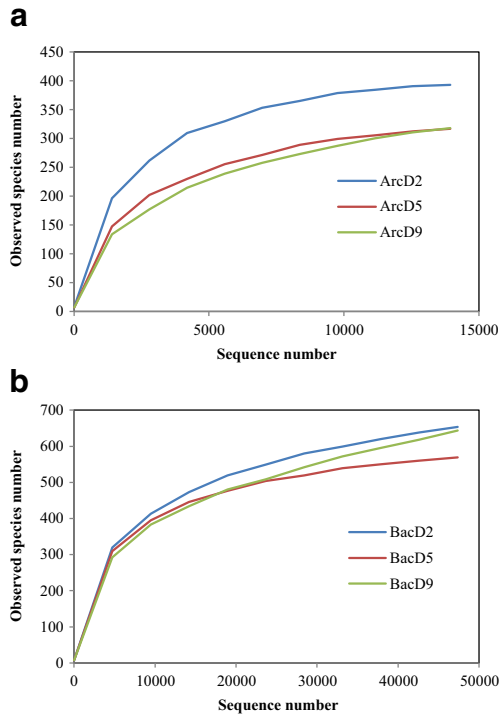

Fig. 4 Rarefaction analysis of bacterial (**a**) and archaeal (**b**) communities in the thermophilic anaerobic digestion of spent mushroom substrate of *Pleurotus eryngii*

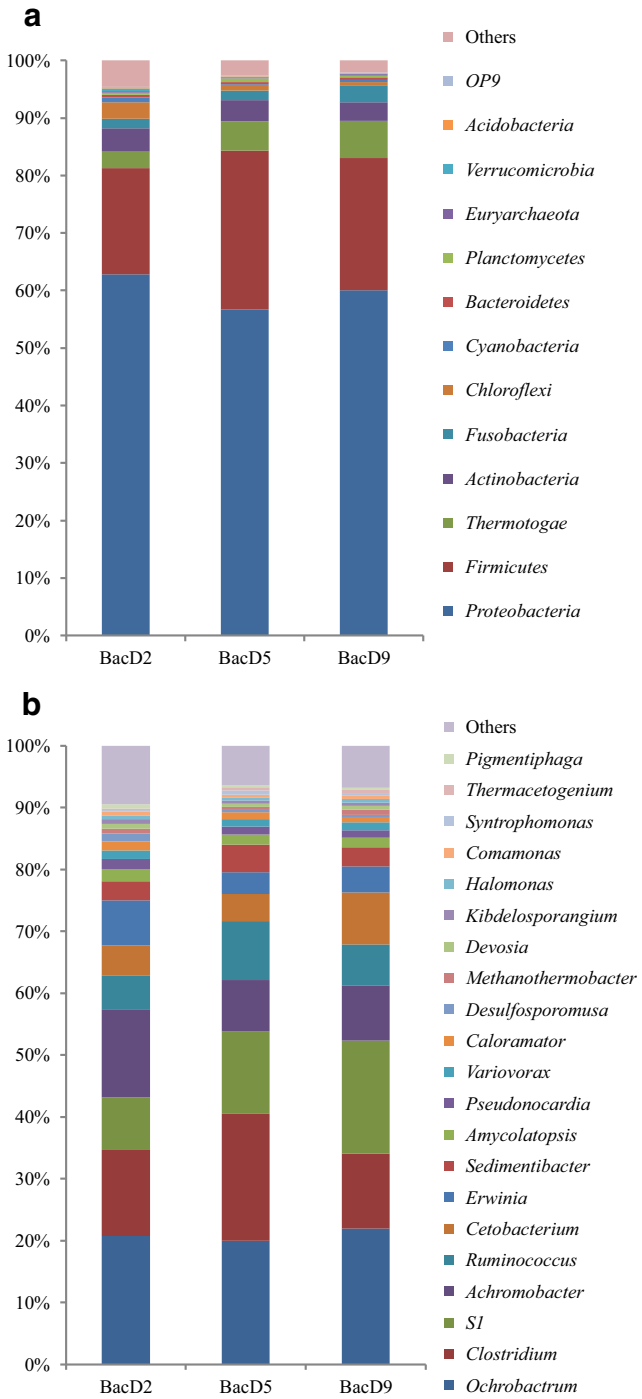

Fig. 5 Relative abundance of bacteria based on V4 region sequencing of 16S rRNA gene at phylum level (**a**) and genus level (**b**). BacD2: Bacterial community on day 2

as *Methanothermobacter* and *Methanobacterium* (Fig. 6b), both of which belong to the family of *Methanobacteriaceae*. *Nitrosopumilus*, *Candidatus Nitrososphaera*, and *Haloarcula* were identified in quite small ratios. In addition, a low proportion of sequences were attributed to *Methanobacterium* on day 5 and day 9, and *Methanothermobacter* took the absolutely dominant position.

Discussion

The mesophilic batch AD of SMS usually lasts for 30 to 60 days, and the methane yields range from 50 to 100 mL/g VS (Lin et al. 2014; Sharma et al. 1989; Zhu et al. 2015). In this study, TAD of SMSPE generated methane yields of 177.69 mL/g VS, which was about 1.5 to 3 times higher than the yields obtained in previous studies with SMS. Although SMS is produced from different substrates, this study indicates that TAD technology would be a better candidate for SMS treatment.

TVFA content variation was in overall good agreement with slurry SCOD and pH changes. The fast accumulation of TVFA during the start-up phase indicated that the hydrolytic and fermentative bacteria were at a high metabolic rate and converted much organic components of SMSPE to VFA beyond its consuming rate. The biogas-producing microbial community functions optimally within a narrow pH range around 7. The excessive accumulation of VFAs led to pH drop and subsequently inhibited activities of methanogens (Xu and Li 2012). This explains the low biogas yields and methane content during the initial phase (Fig. 1a, b). On day 3, the VFAs were abundant and the pH began to increase,

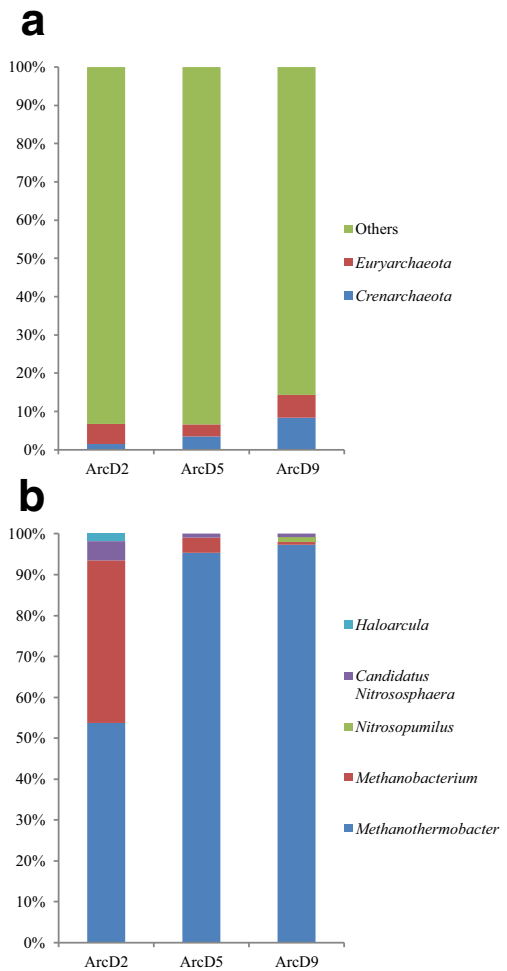

Fig. 6 Relative abundance of archaea based on V4 region sequencing of 16S rRNA gene at phylum level (**a**) and genus level (**b**). ArcD2: archaeal community on day 2

providing enough food and suitable environment for methanogens. The substantial decrease of SCOD indicated that the consumption of soluble materials was very fast in the stage from days 3 to 6. Then, the consuming rate of VFAs began faster and the biogas yields and methane content increased sharply, leading peak methane production from day 4 to day 6. After day 6, the TVFA content in slurry was quite low, and the final biogas yields dropped subsequently.

Intrinsic qualities of the biomass source are key factors determining its conversion efficiency, and the component condition is important to explain the fermentation process. The soluble sugar was easy to be dissolved in water and utilized by microbes, so its degradation rate was much higher than others. Proteins, fat, and soluble sugars are easily biodegradable and can be converted to nitrogenous substance or carbonaceous energy matter, which are accessible for microbes to grow and multiply. After the nutritional and easily degradable substrates were consumed, the metabolism of microbes was limited, affecting fermentation of cellulose and hemicellulose in the final period. For lignocellulosic biomass, the overall digestibility of the substrates is tightly related to lignocellulose characteristics, such as amount of lignin, access to cellulose, and cellulose crystallinity. Lignocellulose is difficult to degrade for its unique structure and composition (Zheng et al. 2014). In a previous study, the degradation rates of cellulose and hemicellulose of SMS by mesophilic AD were below 40 and 20% (Lin et al. 2014). TAD of SMSPE has achieved the degradation rates of 47.53%, 55.08% (calculated by solid residue degradation rate and composition) for cellulose and hemicellulose, respectively, which demonstrated the relatively high degrading capability of TAD.

The dominant phylum *Proteobacteria* has previously been found in various biogas reactors processing sewage sludge (Sundberg et al. 2013; Yang et al. 2016). *Ochrobactrum* can degrade chemical compounds efficiently (Bhattacharya et al. 2015; Chaturvedi and Verma 2015; Subba Reddy et al. 2016). Members in *Achromobacter* were reported having powerful capacity of biodegradation (Kowalczyk et al. 2016; Singh and Singh 2011) and synergism with cellulolytic microbes by producing β-glucosidase (Chen et al. 2015). *Erwinia* comprises lots of plant pathogenic bacteria (Mikiciński et al. 2016; Zhang and Nan 2014). The decrease of *Erwinia* illustrated the pathogen deactivation functions of TAD.

The phylum of *Firmicute* comprises lots of hydrolytic and acidogenic bacteria, taking very important roles in the transformation of biopolymers to organic acids, which has also been found in other studies (Schlüter et al. 2008; Stolze et al. 2015). *Clostridium* comprises lots of members that can hydrolyze cellulose, starch, lipid, and protein, such as *C. thermocellum*, *C. sporogenes*, and *C. clariflavum* (Niu et al. 2013). The *Firmicute* phylum was not the most dominant phylum during this process, but it is stable and relatively high proportion supports its active role.

The reason might be that members in these genera could degrade protein and soluble sugar with higher efficiency. The abundance of *Clostridium* and *Ruminococcus* increased in the middle stage of the TAD, suggesting importance of these bacterial groups to digest cellulose and hemicellulose. In the final stage, both the hydrolysis of feedstock and formation of methane were very weak, indicating that the population of microbes decreased and many dead cells need to be degraded.

It is generally accepted that most of the bacterial population in the phylum *Firmicute* has cellulolytic, hemi-cellulolytic, and proteolytic properties which carry out the initial degradation of organic substrates into soluble products. *Firmicute* usually dominate TAD of lignocellulosic materials (Lü et al. 2014; Niu et al. 2015; Tian et al. 2013). *Proteobacteria* has previously been found in various biogas reactors processing sewage sludge (Sundberg et al. 2013; Yang et al. 2016). It has also been found in anaerobic digestion of lignocellulosic materials (Wan et al. 2013). But, the proportions of *Proteobacteria* in previous studies were usually below 10%.

In comparison, it was not frequent to find in this study *Proteobacteria* dominating the AD process of lignocellulosic materials at a high proportion of 56.7–62.8%. It is possible that *Proteobacteria* comprises some hydrolytic species and can play an important role in the TAD process of lignocellulosic materials as the dominating bacteria.

Methanogens are considered the dominating archaea in anaerobic digesters (Coats et al. 2012). Our findings give more possibilities that the phylum *Crenarchaeota* may also play an important role in the TAD process. Populations of *Methanosaeta* and *Methanobacteriaceae* in mesophilic AD of SMS were measured using fluorescent in situ hybridization in a previous work (Zhu et al. 2015). However, both of the communities were not identified in this study. *Methanothermobacter*, a genus of thermophilic and hydrogenotrophic methanogens, has previously been reported in many TAD processes (Guo et al. 2014), and it was the dominant archaeal genus in some TAD digesters (Chen et al. 2008; Lü et al. 2014). Both *Methanothermobacter* and *Methanobacterium* are hydrogenophilic types that mainly produce methane using H_2 and CO_2 (Jang et al. 2015; Lü et al. 2014). This agrees with previous studies, which have recorded the dominant position of hydrogenotrophic methanogens in TAD digesters. The sharp decrease of *Methanobacterium* (Fig. 6b, 39.81% on day 2 to 3.72% on day 5) indicated a distinct variation happened when the bath TAD system changed from the VFA-accumulating stage to the fast biogas-producing stage.

As far as we know, this study was the first to evaluate the TAD process of SMS systematically. The property variation of biogas, slurry, and solid residues were detected as well as microbial structures. The results indicated that the TAD technology can be a potential and powerful candidate for SMS treatment that can enhance methane yields, shorten fermentation periods, and improve the cellulose and hemicellulose degradation rates. The microbial community, characterized by high proportion of *Proteobacteria* and *Crenarchaeota*, was quite different from other biogas-producing communities degrading lignocellulosic materials. The detailed functions of the two phyla in TAD process still need further research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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