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Facilitated methanogenesis involved in anaerobic digestion of dairy manure by soil

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ABSTRACT

Shaping the microbial community involved in anaerobic digestion (AD) systems to achieve highly efficient methane production is a major challenge. In order to improve the methane recovery from dairy manure, soil, which has the potential of enhancing methane production from biomass, was used in an AD system as an additive. The results showed that the AD process performance and methane production efficiency were significantly improved by soil; an improvement in daily methane production, reduced time to steady state, enhancement of the methane content, and reduction in carbon dioxide content were obtained. The maximum methane production was obtained at a feedstock: soil ratio of 2.5:1 with yellow soil addition, which was 147.7 L/kg volatile solids (VS) and 25.4% higher than that of the control. High-throughput sequencing of the 16S ribosomal RNA gene and synthetic solution with cations equivalent to those of yellow and black soil revealed that soil addition mainly stimulated the growth of bacterial genera *Ochrobactrum* and *Clostridium* and archaeal genera *Methanosaeta* and *Methanosarcina*. The method of ion extraction was used to extract ions from the soil, and the ions were verified to be a main contributor to methane production improvement. By mimicking the cation components of soil, a synthetic solution was prepared and used in the AD system. The results showed that the cations contained in the ion liquid played a key role in improving methane production. The contribution of VS in the soil to the AD system was studied and found to have no significant effect on the improvement in methane production. It was found that the cations in soil played a key role in enhancing AD efficiency. Therefore, the simplified, low cost, and efficient approach used in this study had good practicability and could be used for treating other various biowastes with high energy recovery, which has the potential of promoting the development of AD technology.

1. Background

Global concerns regarding the environment, climate change, and sustainability of fossil fuel production have inspired worldwide interest in the development of alternative energy from animal manure (Liew et al., 2011). The generation and inappropriate management of animal manure cause serious environmental problems. Animal manure is usually spread on land near confined feeding operations, which leads to a series of problems, such as the contamination of surface water and groundwater with pathogens, odor emission, loss of a potential green energy source, accumulation of excess phosphate (PO_4^{3+}) in soil, and deterioration of biological ecosystems (Ramos-Suarez et al., 2019; Zhang et al., 2018). Alternative approaches for treating animal manure are urgently

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needed. Anaerobic digestion (AD) has long been used for treating animal manure (Gaur and Suthar, 2017; Matheri et al., 2017), and has a history of reducing adverse impacts on the environment while providing a mechanism for energy generation and improving manure quality as a fertilizer (Abouelenien et al., 2010). However, the low efficiency of methane production of AD inhibits its further development and application. It is necessary to significantly enhance the efficiency of methane production to counteract the cost of digesters.

Numerous approaches have been taken in an effort to expand AD. The first approach to lower cost barriers is identifying rate limiting steps in the anaerobic digestion process. The kinetics of the slowest step will account for the overall kinetics of this multi-step process. For complex biomass, hydrolysis is often considered the limiting step, especially when the substrate is recalcitrance lignocellulosic material which does not easily break down to simple molecules. In the digestion of soluble organic matter, however, the rate-limiting step has been identified as methanogenesis (Tomei et al., 2009; Vavilin et al., 1996). The second approach to enhance and expand AD is enhancing the activity of microbes, including using higher temperature and immobilizing the biomass in biofilms or granules (Ward et al., 2008). The third approach to lower barriers is engineering improvements such as optimizing reactor types and enhancing mass transfer through adequate mixing. The fourth approach is removing process inhibition such as the ammonia inhibition occurred in AD of manures (Chen et al., 2008). Although great progress has been made through these various approaches, the cost of AD still remains the most significant factor which prevents this sustainable technology from being widely adopted.

Since AD is a relatively mature technology, significant improvements in efficiency will require a considerably novel approach. Another major opportunity that has not been adequately exploited is optimizing the microbial communities. This is primarily because of the complexity of the community that makes such an undertaking challenging. Without in-depth knowledge and tools, the microbial communities are often treated as a black box in the existing engineering and management tools for AD design and operation. Recent developments have started to reveal the potential for significant efficiency improvement by optimizing the syntrophic functions of the communities.

Soil has many properties that are beneficial for AD (Yao et al., 2015). The multiple cations contained in soil are beneficial for enhancing the balance of microbial communities, improving the efficiency of AD, and in turn improving methane production (Kato et al., 2012). Soil has buffering capacity, which means that it can maintain a constant pH (Makselon et al., 2018). Soil can be easily obtained owing to its convenient availability and abundance. Overall, the AD process stability can be enhanced; the AD technique can be simplified because there is no need to digest it with other materials for the purpose of process stability, and the cost can be reduced owing to the availability and abundance of soil. Therefore, the application of AD with soil addition is convenient, economical, and practical. However, there are various types of soil, the effects of which are different, so it is necessary to conduct comprehensive research to further develop AD technology with dairy manure in the future.

Based on the above, soil was first adopted in this study to enhance the performance and efficiency of AD with dairy manure as the substrate. The effect of soil addition on AD was investigated and the mechanism involved in the AD process was emphasized. Three tasks were proposed, namely (1) to study the effect of soil on AD process performance and methane production, (2) to investigate the mechanism of soil in enhancing AD efficiency, and (3) to optimize the conditions of AD.

2. Materials and methods

2.1. Feedstock

Dairy manure was obtained from the Washington State University (WSU) Dairy Center in Pullman, WA, USA, and was stored for 4 d at 4 °C prior to use. The inoculum was collected from an anaerobic digester at the Pullman Wastewater Treatment Facility. Soil samples were collected from two different sites, and were named Y soil (from WSU campus) and B soil (from wheat field). In order to exclude other interferences, such as microbes, the soil was dried under 105 °C for 48 h. Soil samples after drying were ground into powder and kept in a seal at 25 °C. The characteristics of the dry soil, inoculum, and dairy manure are shown in Table 1.

2.2. Anaerobic digestion set-up

For each digester, the required amounts of inoculum and dairy manure were 100 g and 400 g, respectively, based on wet weight. The total solids (TS) and volatile solids (VS) for each digester were 51.93 g and 42 g, respectively. The solids concentration for each digester was 10%. For both types of soil, powdered soil was added into the digesters to obtain 5:1, 2.5:1, and 1:1 feedstock: soil (F:S) ratios based on dry weight. For both types of soil, the amount of dry soil added for experiments with F:S ratios of 5:1, 2.5:1, and 1:1 was 10.4 g, 20.8 g, and 51.9 g, respectively. Experiments without soil were set as the control. The composition of the feedstock under each condition is shown in Table 2. At the laboratory scale, the experiments were conducted in batch mode. The headspace of each digester with 1 L of volume was flushed by nitrogen for 5 min to maintain anaerobic conditions; then, the digesters were tightly capped and shaken at 120 rpm (Lin et al., 2011). 35 °C were adopted. The experiments were ended after 22 d when there was little biogas production. They were repeated three times for each condition. This study was conducted according to the flow chart shown in Fig. 1.

For the purpose of verifying the importance of ions in soil to AD, a group of experiments with 200 g of dairy manure and 50 g of inoculum were conducted. Experiments with Y soil after ion extraction (soil slurry) and B soil slurry were conducted, as well as experiments with ion liquid after ion extraction from both types of soil to verify the impacts of ions contained in the soil on AD. Experiments with ion liquid and without soil slurry were set as the control. The extraction procedure is provided in Section 2.3. The composition of soil cations is shown in Table 3. The mass of soil slurry needed for each digester was based on the optimal F:S ratios related to methane production. The optimal F:S ratios were also used in all the following experiments.

A synthetic solution with a cationic equivalent to that of Y and B soil was created to further verify the effect of cations in enhancing AD efficiency. The composition for the solution was as follows: $Fe(OH)_2$, $CaCl_2$, Na_2SO_4, $MgSO_4$, K_3PO_4, $AlCl_3$, $FeCl_3$, and $MnCl_4$. The other conditions were consistent with those in the above paragraph, including the mass of the soil and inoculum, shaking

Table 1
Characteristics of cattle manure, inoculum, yellow soil and black soil.

Parameter	Cattle manure	Inoculum	Yellow soil	Black soil
TS (%)	12.6 ± 0.1	1.3 ± 0.1	91.2 ± 0.0	87.7 ± 0.1
VS (%)	83.2 ± 0.1	71.1 ± 0.0	4.6 ± 0.0	5.1 ± 0.0
TC (%)	40.8 ± 0.1	36.2 ± 0.2	-	-
TN (%)	5.6 ± 0.3	5.3 ± 0.0	-	-
H (%)	1.6 ± 0.0	5.4 ± 0.3	-	-
pH	8.4 ± 0.0	7.4 ± 0.0	7.2 ± 0.0	6.8 ± 0.1

Table 2
Total solids, volatile solids, total solids and volatile solids contents before anaerobic digestion.

Parameter	Control	Yellow soil			Black soil		
		5:1	2.5:1	1:1	5:1	2.5:1	1:1
TS (g)	43.9	51.9	59.9	83.9	51.9	59.9	83.9
TS (%)	7.1	8.3	9.5	12.8	8.3	9.5	12.8
VS (g)	36.1	36.4	36.8	37.9	36.5	36.9	38.1
VS (%)	82.1	70.2	61.5	45.1	70.2	61.6	45.4

condition, and temperature.

For determining the contribution of VS in soil to the enhancement of methane production, experiments with inoculum and Y and B soils were conducted. AD of inoculum alone was used as the control. The other conditions were consistent with those in the above paragraph, including the mass of the soil and inoculum, shaking condition, and temperature.

To confirm the chemical precipitation, experiments with 200 g of dairy manure and 50 g of inoculum were conducted. Based on the conditions of a 2.5:1 F:S ratio with Y soil addition and 5:1 F:S ratio with B soil addition, magnesium (Mg^{2+}) was added to the

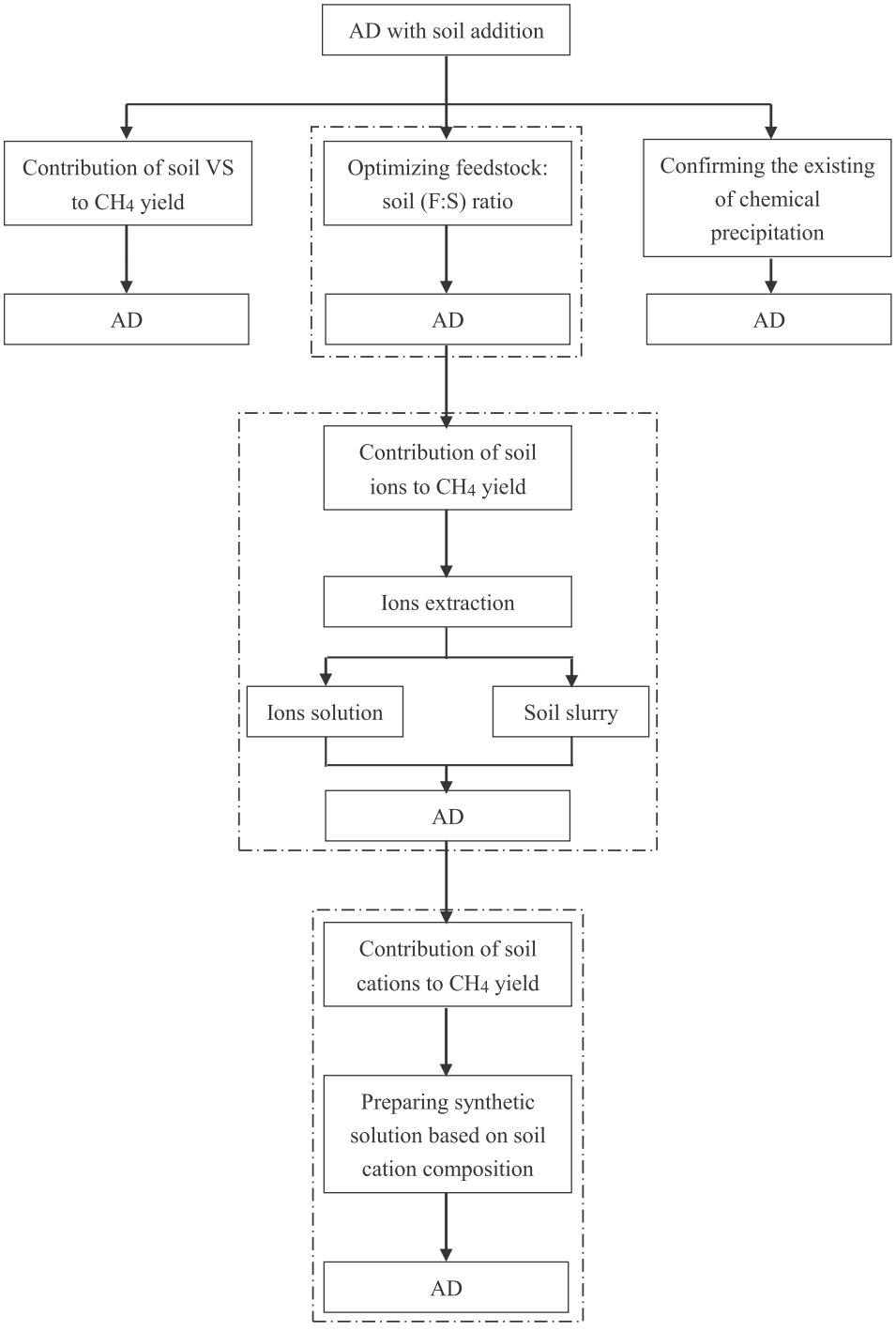

Fig. 1. The research work flow chart.

Table 3
Composition and content (ug/g) of soil cations.

Composition	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Al^{3+}	Fe^{2+}	Fe^{3+}	Mn^{4+}
Y soil	2110 ± 40.2	167.4 ± 16.8	401.7 ± 21	159.8 ± 4.7	0.4 ± 0.0	13.7 ± 3.2	22.8 ± 2.8	56.5 ± 5.0
B soil	736.4 ± 11.3	715.5 ± 10.8	799.7 ± 17.1	375.9 ± 3.4	3.2 ± 0.6	3.7 ± 0.2	6.6 ± 1.1	33.1 ± 1.5

experiments with Y soil and B soil slurry. The other conditions were the same as those in the above paragraph.

2.3. Analytical methods

2.3.1. Analysis of the microbial community

Samples were taken from digesters at stable status and stored at −20 °C for later analysis of the microbial community by high-throughput sequencing of the 16S ribosomal RNA (16S rRNA) gene. Each sample was divided into aliquots of appropriate masses for commercially available DNA extraction kits. Genomic DNA was extracted using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's protocol. Briefly, bead beating and spin filter technology were used to extract DNA from the samples (500 mg). Glass beads and SLX-Mlus buffer were added to samples and vortexed to mix thoroughly. The samples were lysed and centrifuged for 3–5 min using 100% isopropanol to precipitate the DNA at −20 °C for 1 h. Then, an elution buffer was added and the DNA pellet was dissolved. Using a cHTR reagent and centrifuging at maximum speed for 2 min, the cleared supernatant was then transferred to a HiBind DNA column. After centrifuging and washing, DNA was eluted from the column and stored at −20 °C. The genomic DNA concentrations were quantified using the Nanodrop. The V3-4 hypervariable regions of bacterial 16S rRNA (341F/785R) (F- CCTACGGGNBGCASCAG, R- GACTACNVGGGTATCTAATCC) and V4-5 hypervariable regions of archaeal 16S rRNA (519F/915R) (F- CAGCCGCCGCGGTAA, R-GTGCTCCCCCGCCAATTCCT) were amplified by PCR using the barcoded fusion primers (Hugoni et al., 2015; Takahashi et al., 2014). Each 25 μL reaction included 5 μL of KAPA2G GC Buffer A, 5 μL of 5X KAPA Enhancer 1, 0.5 μL of dNTP Mix, 1 μL of each primer, 0.5 μL of KAPA2G Robust Standard Polymerase, and template DNA. The purification of PCR products was conducted using a QIAGEN Gel Extraction Kit according to the manufacturer's protocol. Then, KAPA Hyper Prep Kits for Illumina were used to construct the sequencing library, and PCR amplicons were further sequenced on the Illumina Hiseq2500 platform. Sequence reads and 16S rRNA data analysis were accomplished at the Microread Genetics Company in Beijing, China.

2.3.2. Chemical analyses

TS and VS were measured according to previous methods (Sluiter et al., 2008). A pH meter was used to measure pH. According to the Standard Methods, the TAN content was determined using a Tecator 2300 Kjeltec Analyzer (Eden Prairie, MN, USA; 4500-NorgB; 4500NH3BC) (Association, 1994). Total carbon, total nitrogen, and total hydrogen were measured via an elemental analyzer.

2.3.3. Ion extraction and analysis

For ion extraction and analysis, 0.25–0.30 g of soil was placed in 50 mL centrifuge tubes, and then 25 mL of 0.1 M $BaCl_2$ was added. Then, a reciprocal shaker was used to shake the tubes for 4 h. The samples were centrifuged at 4000 rpm for 30 min in a Sorval RC-5B Superspeed Centrifuge, which was conducted at room temperature. The ion liquid was then used for Na, Ca, Fe, Mg, K, Mn, and Al measurement using an Agilent 4200 MP-AES and the verification experiment. The remaining solid/soil slurry after separation was used for the verification experiment.

2.3.4. Biogas composition analyses

The volume of produced biogas and the total volume of biogas were measured based on previous methods. The methane content of biogas was analyzed via a gas chromatograph (CP-3800, Varian, Inc., Palo Alto, CA, USA) (Ma et al., 2013b).

2.3.5. Statistical analysis

SPSS 19.0 software was used to determine the standard deviations and whether observed differences between two or more groups of experimental results were significant. Differences were compared using a p value of 0.05.

3. Results and discussion

3.1. Methane production

For all experiments, the trends of daily methane production were similar (Fig. 2A). Daily methane production increased rapidly, and then a temporary rapid decrease was observed followed by a gradual increase. For the control, the daily methane production was lower than those of the experiments with soil. For all experiments, peaks appeared on the same day. For the trends of daily methane production, as shown in Fig. 2B, the TAN contents for all experiments were lower than the AD ammonia inhibition range of 1.5–3.0 g/L (Yao et al., 2017), thereby indicating that no ammonia inhibition occurred in this study. It is possible that acidification occurred owing to the accumulation of acidic products, such as acetate (Abouelenien et al., 2010). For all experiments, pH experienced an initial decrease followed by a gradual increase (Fig. 2C). In the AD system, the microbial consortium consisted of fermentative bacteria, acetogenic bacteria, and methanogens (Gong et al., 2011). The growth rate of fermentative and acetogenic bacteria is faster than that of methanogens, thereby making methanogens more sensitive to the changes in the AD environment (Gong et al., 2011). The accumulation of acidic products, such as acetate, usually occurs in the initial AD period, which leads to a decrease in pH (Yao et al., 2014). A period of adaptation and cultivation of methanogens occurs later, the acidic products are used for methane formation, and the pH increases accordingly (Yao et al., 2014). This result indicated that both Y soil and B soil addition had a positive effect on the increase in daily methane production.

The trends of methane content for all experiments were similar, except for that of the control (Fig. 2D). The methane content for the control was 48.8–56.0%; those for experiments with Y soil addition at F:S ratios of 5:1, 2.5:1, and 1:1 were 53.1–59.8%, 53.3–59.8%, and 53.0–59.5%, respectively; and those for experiments with B soil addition at F:S ratios of 5:1, 2.5:1, and 1:1 were 52.9–60.0%, 52.4–60.1%, and 51.4–59.1%, respectively. The range in methane content was close to that in the AD of organic waste (Bouallagui et al., 2009). However, the time used for achieving steady state for the control was twice as long (4 d) as that for the experiments with soil addition (2 d to steady state). Methane content greater than 50% (≥50%) indicates the achievement of stable status (Brown and Li, 2013). In view of the relatively low standard deviation, the

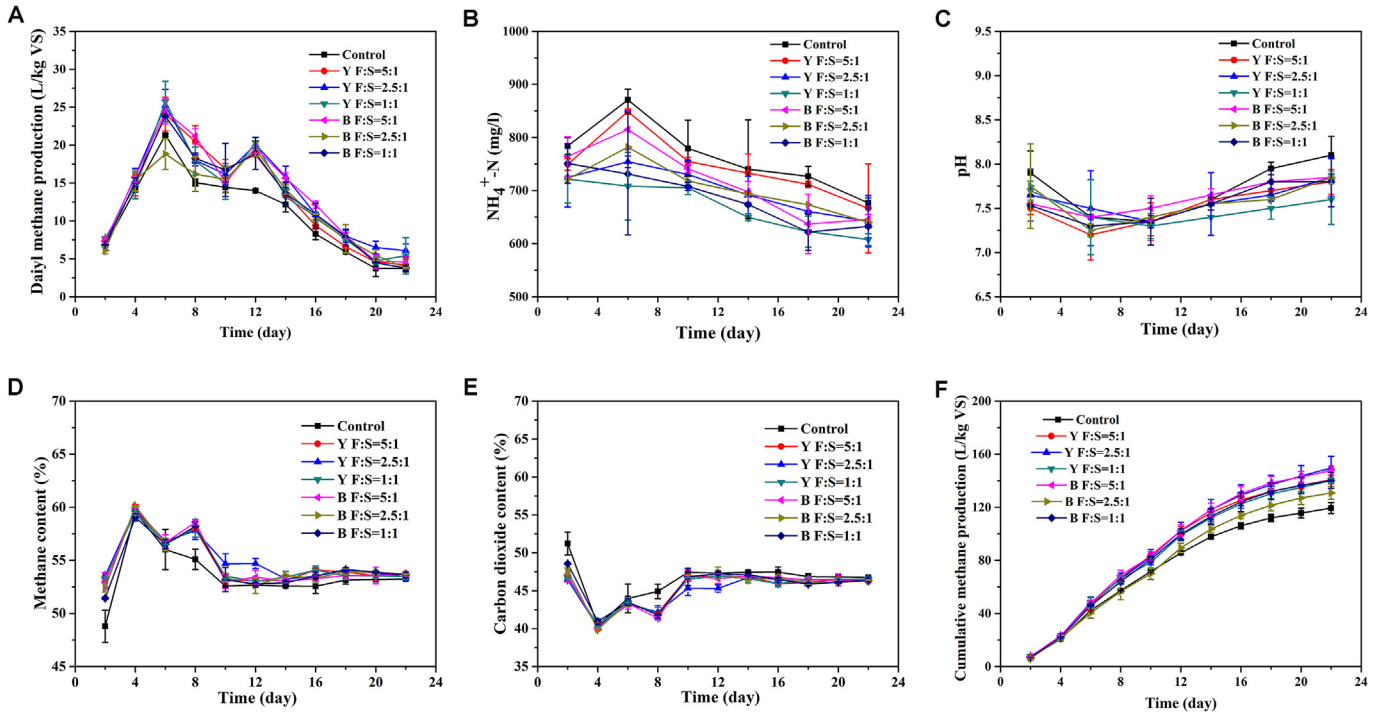

Fig. 2. Process performance and methane production of anaerobic digestion. A: daily methane production of the control and experiments with different feedstock:soil (F:S) ratios; B: TAN contents of the control and experiments with different F:S ratios; C: pH levels of the control and experiments with different F:S ratios; D: methane content of the control and experiments with different F:S ratios; E: carbon dioxide content of the control and experiments with different F:S ratios; F: cumulative methane production of the control and experiments with different F:S ratios..

repeatability and reproducibility of biogas quality with soil addition were high. This result indicated that soil benefited the achievement of steady state, which enhanced the operability and practicality of the AD system. The carbon dioxide (CO_2) content for the control was the highest throughout the AD process (Fig. 2E). A reasonable hypothesis for this was that the activity of methanogens was enhanced in the digesters with soil addition, which enabled the conversion of much more CO_2 to methane compared to that of the control. Chuang et al. (2011) and Zhen et al. (2016) analyzed the efficiency of CO_2 emission reduction in detail and stated that the produced energy from biomass could mitigate the CO_2 emitted from non-renewable sources; the further reduction in CO_2 emissions from AD in this study would be significant. Therefore, soil addition helped to reduce the CO_2 content of biogas.

As shown in Fig. 2F, the maximum total methane production obtained at the F:S ratio of 2.5:1 with Y soil addition (149.7 L/kg VS) was 25.4% higher than that of the control (119.4 L/kg VS) ($p < 0.05$). The difference in total methane production between the experiment with Y soil addition at a F:S ratio of 5:1 (the maximum total methane production) and the experiment with B soil addition at a F:S ratio of 5:1 was not significant ($p>0.05$), which meant that the Y soil and B soil had equal effects on the improvement in methane production. Greater reductions in VS were associated with higher total methane production (Table 4). The maximum VS reduction, which was 44.7% higher than that of the control, was obtained at a F:S ratio of 2.5:1 with Y soil addition. Cellulose and hemicellulose degradability were associated with methane production as that of VS (Yao et al., 2013), so soil addition also enhanced the breakdown of cellulose and hemicellulose. TS reduction did not correspond with total methane production because of the low VS content in the soil, as discussed in previous studies (Yao and Chen, 2016). The greater feedstock reduction could have been attributed to soil addition.

3.2. Microbes driven by soil and their relationship with intermediates

3.2.1. Microbial community changes

Based on the efficiency of total methane production, the experiment with Y soil addition at a F:S ratio of 2.5:1 and the experiment with B soil addition at a F:S ratio of 5:1 were selected for the microbial community analysis; the control was included as a comparison. We analyzed the microbial community of AD to gain further insight into microbial metabolism. Genomic DNA was extracted from the enrichment culture and sampled from stable digesters, and was terminated; 16SrRNA gene fragments were amplified by PCR using universal primers, and randomly chosen clones were sequenced on an ABI 3730 genetic analyzer at the Microread Genetics Company in Beijing, China. Notable differences in the microbial communities in the control and experiments with soil addition were observed (Fig. 3).

For the purpose of excluding microorganism interferences, the soil was dried under 105 °C for 48 h. The genera *Ochrobactrum* and *Escherichia* were observed for experiments with soil addition; their relative abundance was 14.3% and 3.0% for the experiment with Y soil addition and 13.5% and 1.9% for the experiment with B soil addition, respectively, thereby indicating that soil addition tended to simulate the growth of *Ochrobactrum* and *Escherichia*. The genus *Clostridium* was observed for the control and experiments with soil addition. The relative abundance of *Clostridium* in experiments with soil addition was higher than that in the control. For the archaeal group, the relative abundance of genera *Methanosaeta* and *Methanosarcina* for experiments with soil addition was higher than that of the control. *Methanosaeta* produced more methane than other methanogens owing to its ubiquitous distribution and high affinity for acetate. Acetate is the precursor of more than half of the methane in most methanogenic environments (Smith and Ingram-

Table 4
Total solids (TS) and volatile solids (VS) degradations after anaerobic digestion.

Parameter	Control	Y soil			B soil		
		5:1	2.5:1	1:1	5:1	2.5:1	1:1
TS (%)	21.3 ± 2.2	18.1 ± 2.2	16.7 ± 3.1	13.1 ± 2.2	18.4 ± 1.5	15.4 ± 1.7	12.0 ± 3.2
VS (%)	25.9 ± 1.4	30.6 ± 1.6	37.5 ± 0.5	30.5 ± 1.1	33.1 ± 0.9	28.4 ± 2.3	30.5 ± 1.3

Smith, 2007). The ability of *Methanosaeta* species to produce methane with electrons derived from direct interspecies electron transfer may add to their competitive advantage. In this study, it was found that soil addition facilitated methanogenesis.

3.2.2. Microbial mechanism involved in the variation of TAN, CO_2, and pH

The relative abundance of the bacterial genus *Clostridium* for the experiments with soil addition was higher than that of the control. *Clostridium* produce a complex of multi-cellulolytic enzymes called cellulosome, which conducts the breakdown of cellulose and hemicellulose (Lynd et al., 2002). This indicates that an increase in *Clostridium* leads to the increase in biomass breakdown, which is beneficial for the improvement in methane production and the enhancement of VS reduction, as shown in Table 4. Dairy manure is a recalcitrant substrate with a high lignocellulose content. For biomethanation of substrates with a high lignocellulosic content, hydrolysis is the rate-limiting step (Ma et al., 2013a); in turn, the rate of methane production is dependent on the degree of hydrolysis (Buffiere et al., 2018). Members of the genus *Clostridium* also participate in acetogenesis by producing a variety of extracellular enzymes that can degrade biopolymers for methane production (Alam et al., 2005). Therefore, the changes in methane production in the experiments were in line with the variation in the relative

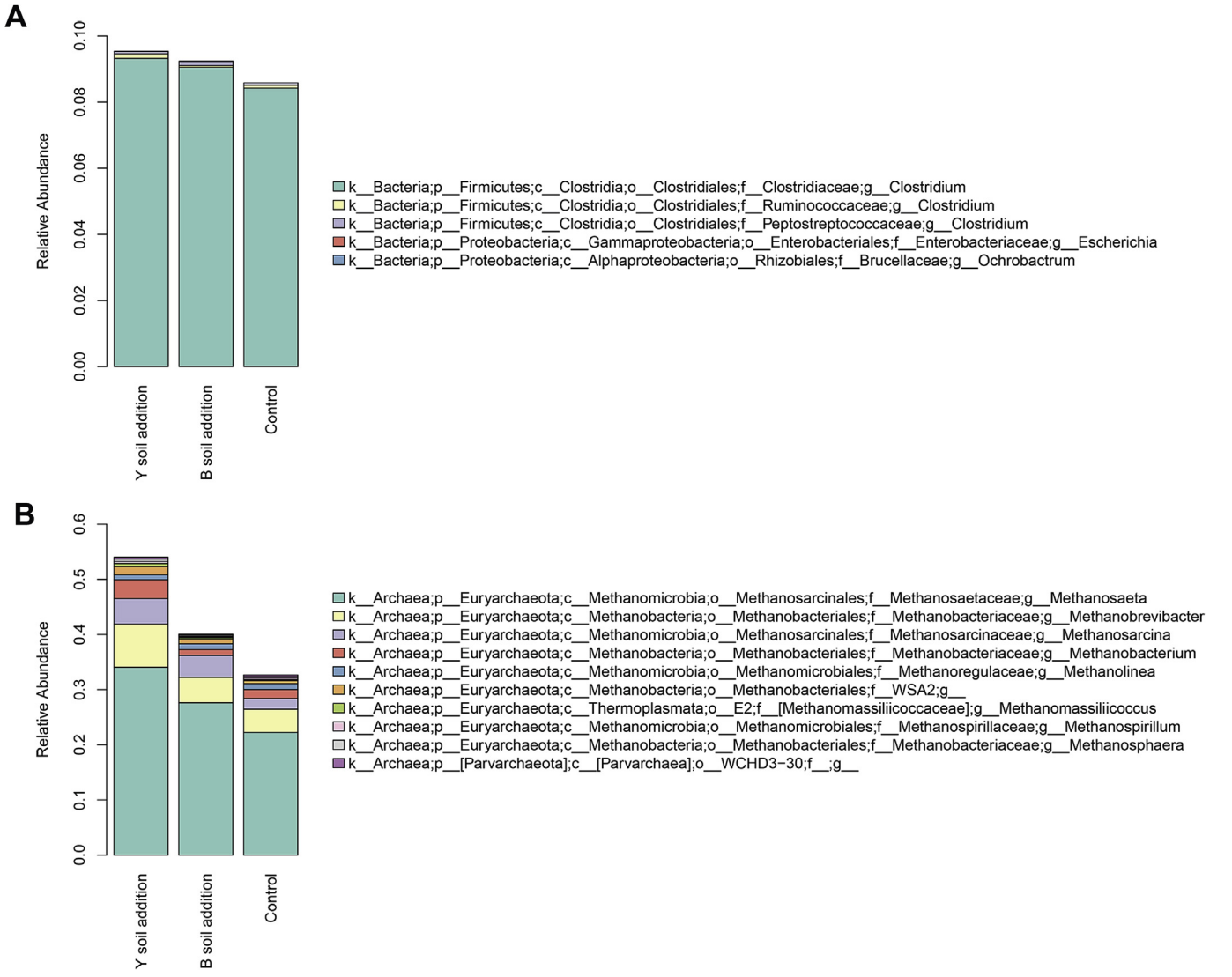

Fig. 3. Taxonomic composition of microbial communities from the control and experiments with Y soil at the feedstock:soil (F:S) ratio of 2.5:1 and B soil at the F:S ratio of 5:1. A: the relative abundance of the taxonomic composition of bacterial communities; B: the relative abundance of the taxonomic composition of archaea communities.

abundance of this genus. *Ochrobactrum* was only detected for experiments with soil addition (Anderson et al., 2003). *Ochrobactrum*, as acetogenic bacteria, were able to either decompose complex macromolecules or reduce nitrate and aerobically utilize other small molecules, such as gluconate, glucose, galactose, D-fructose, acetate, propionate, butyrate, ethanol, lactate, cellobiose, glycerol, and sucrose (Zuo et al., 2008). The presence of this genus in digesters is mainly due to soil addition. The shift in the balance to *Clostridium* as ammonia concentrations increased in the control indicated that *Ochrobactrum* was more sensitive to ammonia. Therefore, the low TAN content and the relatively neutral pH permitted the increase in *Clostridium,* thereby resulting in the high efficiency of cellulose and hemicellulose breakdown, which contributed to the improvement in methane production. It has been estimated that methanogens that utilize acetate account for about two-thirds of the produced methane in nature (Horn et al., 2003). *Methanosaeta* spp. and *Methanosarcina* spp., as acetoclastic methanogens, are most sensitive to ammonia (Rajagopal et al., 2013) and function optimally at a neutral pH (Horn et al., 2003). *Methanosaeta* spp. is dominant in digesters that are inoculated with wastewater treatment plant sludge, and can work well under low ammonia concentrations (De Vrieze et al., 2012), which is consistent with the results of this study. This means that experiments with relatively neutral pH levels in the presence of soil permit the increase in the relative abundance of *Methanosaeta* and *Methanosarcina.* Genus *Methanosarcina* increased more owing to its ability to switch between metabolic pathways for methanogenesis (methyl, hydrogen, and acetate), thereby making it a preferable candidate for methanogenesis compared to other methanogens (St-Pierre and Wright, 2015). For the reduction of CO_2 to methane, *Methanosaeta* species can utilize acetate for methane formation, while they cannot utilize H_2 or formate as electron donors (Smith and Ingram-Smith, 2007).

The genome sequences of *Methanosaeta* spp. contain genes encoding the enzymes required for CO_2 reduction, as well as *Methanosarcina* (Rotaru et al., 2014). *Methanobrevibacter,* as hydrogenotrophic methanogens that utilize H_2 and CO_2 as substrate for methanogenesis, was the third most abundant archaeal genera in all experiments (Rittmann et al., 2015). This result indicated that more CO_2 was reduced for methane formation in the experiments with soil addition compared to that of the control. Therefore, the CO_2 content of biogas for experiments with soil addition was reduced (Fig. 2C).

3.3. Verification of the role of soil cations in anaerobic digestion

3.3.1. Contribution of ions in soil to the improvement in methane production

Experiments with ion liquid or soil slurry were set up to study the impacts of ions in soil on methane production, and the results are shown in Fig. 4. After ion extraction from soil, the ion liquid and soil slurry were obtained. The total methane production for experiments with ion liquid addition obtained from Y and B soils was 20.3% ($p < 0.05$) and 18.3% ($p < 0.05$) higher than that of the control, respectively. On the other hand, soil slurry was used to verify the importance of ions to AD performance. The total methane production for experiments with soil slurry addition obtained from Y and B soil was only 5.7% ($p > 0.05$) and 0.9% ($p > 0.05$) higher than that of the control, respectively (Qiao et al., 2015). This demonstrated that the difference in total methane production between the experiments with soil slurries and the control was not significant. It could also be concluded that the methane production of the experiment with soil addition was higher than that of the experiment with ion liquid because the soil was separated into two sections of ion liquid and soil slurry. In addition to the significant effect of the ion liquid on total methane production, soil slurry also had an effect on the improvement in total methane production, for which the mechanism is discussed later.

As a result, the enhanced AD efficiency could have been due to the ions in the soil; soil slurry had no significant impact on the improvement in methane production.

3.3.2. Improvement in methane production by cations contained in the ion liquid

In this batch of experiments, synthetic solution, which mimicked the cation composition of the ion liquid, was prepared to directly verify the role of cations in improving methane production because the ion liquid may have contained other components that could affect AD performance. The increases in methane production for experiments with Y and B synthetic solutions were 18.5% ($p < 0.05$) and 15.3% ($p < 0.05$), respectively, compared with that of the control (Fig. 5). The effect of the synthetic solution in enhancing methane production was close to that of the actual ion liquid extracted from soil samples. This result further verified that cations contained in the ion liquid played a primary role in enhancing AD efficiency.

3.3.3. Contribution of available matter contained in soil to the improvement in methane production

Experiments with inoculum and Y and B soil and experiments with just the inoculum (control) were conducted to study the effect of VS in soil on the improvement in methane production. The content of the available matter for microbes (VS) contained in Y and B soils was 4.6% and 5.1%, respectively. It has been shown that no more than 50% of VS contained in the substrate of AD can be used for methane production (Yao et al., 2013); therefore, the actual amount of VS in soil used for methane production was much less than the absolute amount of VS, which was calculated based on the 4.6% and 5.1% values. Compared with the control, the increases in methane production for the experiments with the inoculum and Y and B soil were only 2.5% and 4.4%, respectively (Fig. 6), so the contribution of VS in soil to the increase in total methane production was not significant ($p > 0.05$).

3.3.4. Chemical precipitation from soil ions

Experiments with Y and B soil slurry and experiments without soil slurry were conducted. As shown in Fig. 7, the TAN contents for

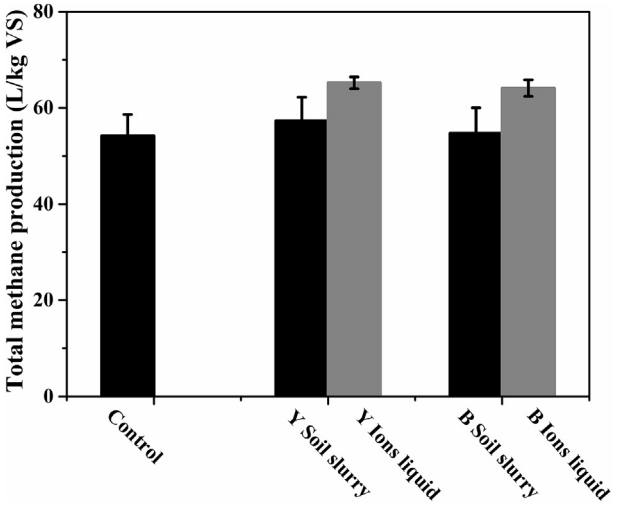

Fig. 4. Total methane production of the control, Y soil slurries, Y ion liquid, B soil slurry, and B ion liquid.

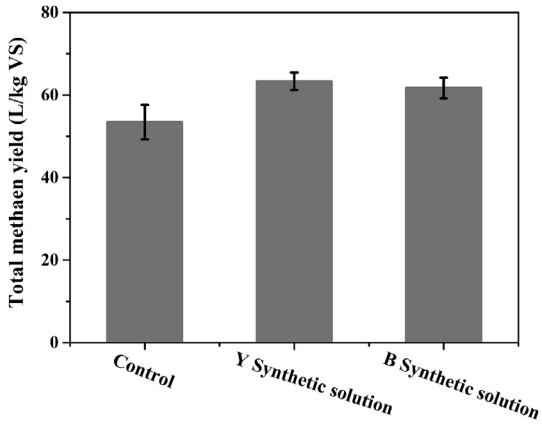

Fig. 5. Total methane production of the control, Y synthetic solution, and B synthetic solution.

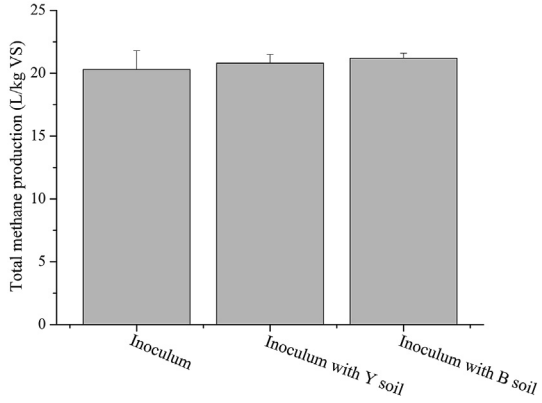

Fig. 6. Total methane production of the inoculum with and without soil.

all experiments were similar, namely no chemical precipitation occurred. Mg^{2+} was added equally to the soil in the experiment with soil slurry to confirm this conclusion. With Mg^{2+} addition, the TAN contents for the two experiments with Y and B soil slurry were lower than that of the control.

Chemical precipitation is useful for reducing ammonium (NH_4^+). PO_4^{3+} and Mg^{2+} contained in the soil can combine with NH_4^+ and form insoluble $MgNH_4PO_4 \cdot 6H_2O$ (Yetilmezsoy and Sapci-Zengin, 2009). The solubility of stable white orthorhombic crystals is low (0.023 g/100 mL H_2O at 0 °C); the reaction is expressed as follows (Li and Zhao, 2001):

$$Mg^{2+} + NH_4^+ + PO_4^{3+} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \downarrow$$

The result confirmed that chemical precipitation occurred in the AD system with soil addition.

3.4. Prospective of this strategy

In order to overcome the challenges in the development of AD technology, it is necessary to develop a novel and applicable approach that creates immediate and long-lasting impacts. Numerous approaches have been used in an effort to expand AD. The first approach to lower cost barriers is to identify the rate-limiting steps in the AD process. For complex biomass, hydrolysis is often considered the limiting step, especially when the substrate is recalcitrant lignocellulosic material that does not easily break down to simple molecules. However, for soluble organic matter, methanogenesis has been identified as the rate-limiting step in AD systems (Adekunle and Okolie, 2015). The second approach to enhance and expand AD is to enhance the activity of microbes, including using higher temperature and immobilizing the biomass in biofilms or granules (Ward et al., 2008). The third approach to lower barriers is to make engineering improvements, such as optimizing reactor types and enhancing mass transfer through adequate mixing. The fourth approach is removing process inhibition (Chen et al., 2008). Although great progress has been made through these various approaches, the cost of AD still remains the most significant factor that prevents this sustainable technology from being widely adopted. In this study, soil was easily collected and was abundant and available, so the cost of collecting soil was relatively low. The soil could be directly added to the AD system without any complex treatment, which made this AD technology simple and further reduced the cost of engineering. AD was operated under mesophilic rather than thermophilic temperatures; in addition, low-cost heat produced as waste heat by gas engines could be used as the energy source for maintaining the operating temperature of AD, and this is being conducted at some full-scale biogas plants (Yao et al., 2017a). For the purpose of avoiding ammonia inhibition, diluting nitrogen-rich materials using water resources before AD is usually used in practical applications, which leads to the increase in effluent and exacerbates environmental pollution. The ammonia content was reduced via chemical precipitation in this study; thus, the method has the potential of alleviating ammonia inhibition in thermophilic AD and had no negative effects in terms of environmental benefits.

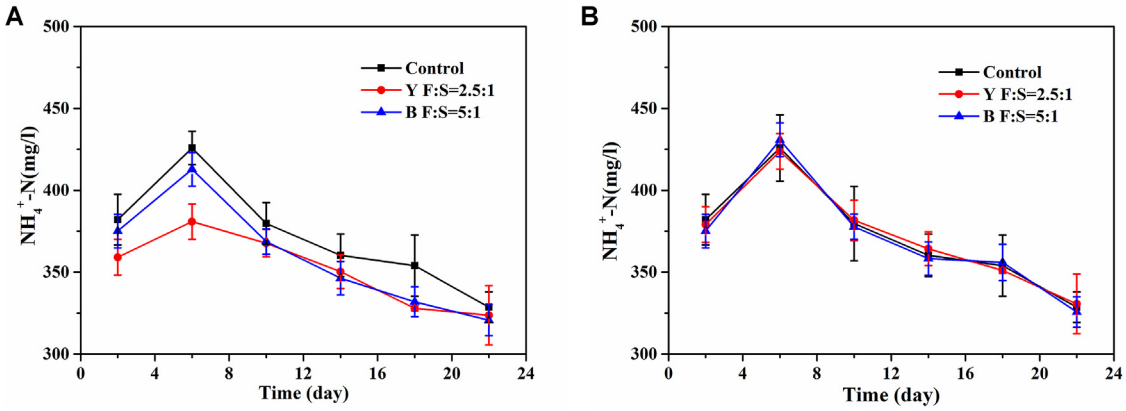

Fig. 7. TAN contents of the control, yellow, and black soil slurries. A: with Mg2+; B: without Mg2+.

Therefore, the method raised in this study had positive economic and environmental benefits and has relatively good potential for use in large-scale applications. However, in this study, we just selected two types of soil around campus and verified that the soil has the potential of enhancing the efficiency of AD, while the type of soil is various, it is not an absolute conclusion that other types of soil have the similar effects as shown herein. In the future, it is necessary to carry a systematic research to ascertain the characteristics of soil regarding to AD.

4. Conclusions

The AD of dairy manure with soil addition was tested in this study. It was found that soil had a positive effect on AD process performance and methane production; the daily methane production and content was improved, digester start-up time was shortened, TAN content was reduced, CO_2 content was reduced, and total methane production was improved. After a series of verifications, the improved AD efficiency was attributed to soil cations. This study proposed an alternative approach for AD development that will broaden researchers' horizons for treating biowastes with high bioenergy recovery. It is a cost-effective and environmentally friendly technique, and has the potential of realizing maximal resource recovery and methane production in the field of bioenergy recovery from nitrogen-rich materials. However, there are some research gaps and scientific questions that need to be addressed in the future. There are various types of soil, and the effects of different soil types on the AD of dairy manure remain unknown, which need to be investigated. In addition, the detailed mechanism of improvement in AD efficiency by soil still needs to be investigated, including the possibility of soil cations enhancing the interspecies electron transfer between symbiotic microorganisms.

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