Enhanced Stepwise Spraying Effects on Gas Production by Anaerobic Solid-state Fermentation in a High-density Straw Bed

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Rice straw bales and fresh pig manure were used as feedstock, and added microbial fortification agent and stepwise spraying methods were used to investigate the effect on the gas yield characteristics and fermentation properties of garage-type anaerobic solid-state fermentation at ambient temperature. The test results showed that the added microbial fortification agent advanced the arrival time of the peak temperature by one day and increased the average fermentation temperature by 8.28%. The microbial fortification agent can increase gas production and the time of first yield peak and shorten the start-up time. The maximum gas yield of the enhanced groups was 13.01% higher than the ordinary groups, and the methane concentration increased 16.98%. After the second gas yield peak, the reduction of spraying frequency had almost no effect on the gas yield. The stepwise spraying method was helpful to improve the moisture distribution and the degradation rate of the middle layer. This study provides a basis for evaluating and improving the operating efficiency of the anaerobic solid-state fermentation systems.

Keywords: Rice straw; Manure; Anaerobic fermentation; High solid; Methane; Bioaugmentation; Spray frequency

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INTRODUCTION

Anaerobic solid-state fermentation technology (total solids concentration ≥ 20% [w/w]) has widespread applications because of its many benefits, which include good adaptability of feedstock, high organic load, low energy consumption, and lack of secondary emissions (Kothari *et al.* 2014; Li *et al.* 2014; Mazaheri *et al.* 2015). Therefore, it is an important approach for producing clean energy through the large-scale treatment of organic waste.

Spraying is an important way of improving heat and mass transfer during anaerobic solid-state fermentation (Yang *et al.* 2015). It improves the moisture content of feedstock and the effective distribution of bacteria, which provides an active transfer mechanism for microorganisms and accelerates transformation between substances (Yu *et al.* 2018). Moreover, gas yield with spraying is 29% higher than that without (Tadesse *et al.* 2014; Chao *et al.* 2016). The anaerobic fermentation processes of BEKON, GICON, and LOOCK all employ effective spraying to improve mass transfer, gas yield efficiency, and methane concentration (Zhang *et al.* 2014a). Different spraying processes are adopted according to the different digester structures, physical properties, ratios, and stacking characteristics of feedstock (Anil *et al.* 2015). Typically, optimization of the spraying frequency, ratio, and inoculum content are key factors to consider when choosing a spraying process. A previous study found that the addition of microorganisms and certain nutrients to the spraying process could improve methane yield and maintain stable fermentation (Nges *et al.* 2015; Degueurce *et al.* 2016). The enhanced microbial agent has a complex composition, and there is a synergistic relationship between the archaea in the process of decomposing the substrate, which can improve the stability of the anaerobic fermentation process and is not sensitive to environmental changes (Yuan *et al.* 2014; Hua *et al.* 2016). At present, the composite microbial agent is mainly used to improve the pretreatment efficiency of the hydrolysis acidification phase (Lei *et al.* 2012; Tuesorn *et al.* 2013). Moreover, a suitable spraying frequency could enhance mass transfer. However, different materials require different spraying frequencies (Sponza and Ağdağ 2004; Rico *et al.* 2015). At present, research into optimizing the spraying frequency and enhancing inoculum methods has predominantly been limited to the laboratory scale, and the use of a single functional microbial inoculum (Kusch *et al.* 2012; Mao *et al.* 2015; Pezzolla *et al.* 2017). Therefore, the influence of high efficiency spraying methods on process parameters, product properties, and biogas yield efficiency during large-scale anaerobic solid-state fermentation, particularly using high-density straw, has yet to be studied.

This study analyzed the effects of the enhanced spraying method on gas yield and fermentation properties in an applied scale experiment. Due to the improvement of the collection-storage-transport system of straw and application of bundling integrated equipment, the authors selected rice straw bundles as the main feedstock. Then, considering the rural planting and farming structure mode, dry swine manure from large-scale farms was added. The enhanced microbial agent was researched by the Biogas Institute of the Ministry of Agriculture (Sichuan Province, Chengdu, China), and its efficacy in the experiment had been studied in the authors' research group. On the basis of the above, the effects of different spray methods on the solid (feedstock), liquid (slurry), and gas (methane) in high-density solid anaerobic fermentation were compared, and the variation law between different fermentation stages under the action of enhanced spraying strategy was revealed. This research provides an experimental basis for the promotion and application of batch solid-state fermentation technology.

EXPERIMENTAL

Materials

Feedstock and inoculum characterization

The bales of rice straw were taken from Tianniang farm in Changshu city, Jiangsu Province, China. These were stored after collection and bailed by a rice-and-wheat combined harvester. The dimensions of the bales were approximately 700 × 460 × 360 mm^3. The average weight and density of a single bale was 10.1 kg and approximately 87.13 kg/m^3, respectively. Fresh swine manure was taken from a nearby pig farm and used as feedstock. The inoculated biogas slurry was collected from a continuously stirred tank of anaerobic digester to treat the pig waste. The physical and chemical properties of the raw materials are shown in Table 1, along with the volatile solid content (based on dry matter quality). A microbial fortification agent developed by the Biogas Institute of the Ministry of Agriculture (Sichuan Province, Chengdu, China) was employed, which contained 1.5×10^8/g methane (CH_4) Archeae, 4.5×10^8/g cellulose decomposition bacteria, and 5.5 ×

10^9/g fermentation bacteria. The microbial inoculants were supplied by the Biogas Institute of the Ministry of Agriculture, and they contained eight microorganisms. The total number of microbes was 6.1×10^9/g.

Table 1. Characterization of the Raw Materials Used in the Experiments

Parameters	Rice Straw (RS)	Swine Manure (SM)	Inoculum Sludge (IS)
Total solid content (%)	86.17 ± 0.05	20.85 ± 0.13	2.06 ± 0.02
Volatile solid content (%)	74.55 ± 0.09	72.23 ± 0.18	2.55 ± 0.04
Total organic carbon content (%)	50.12 ± 0.16	39.68 ± 0.25	0.18 ± 0.09
Total nitrogen content (%)	0.91 ± 0.04	3.14 ± 0.18	0.05 ± 0.04
chemical oxygen demand (mg/L)	-	13390 ± 2.56	2360 ± 1.38
pH	-	7.24 ± 0.13	7.09 ± 0.06

Table 2. Characteristics of the Microbial Inoculants

Bacterial Strain	DSMZ Preservation Number[1]	Percent (%)
Pseudomonas alcaligenes	DSM 19550	15
Pseudomonas nitroreducens	DSM 14399	15
Smithella propionica	DSM 16934	15
Enterococcus aquimarinus	DSM 17487	15
Clostridium celerecrescens	DSM 5628	25
Methanosaeta concilii	DSM 2139	5
Methanosarcina mazei	DSM 2053	5
Methanocorpusculum sp.	DSM 4274	5

1. Deutsche Sammlung von Mikroorganismen und Zellkulturen [the German Collection of Microorganisms and Cell Cultures], Leibniz Institute, Berlin, Germany

Reactor Design: Garage-type Anaerobic Solid-state Fermentation System with a Flexible Roof Membrane

The garage-type anaerobic solid-state fermentation system with a flexible roof membrane (GASFS-FFM), which is a type of sequencing batch fermentation system, was independently researched and developed. It consists of a fermentation reactor, a circulation spraying system, and a monitoring system (Fig. 1a). Four fermentation reactors were utilized, with internal sizes of 8200 × 3200 × 3000 mm^3. The single effective volume was 78.72 m^3. A pool was built under the ground to store the leachate. The walls and ground of the reactors were installed using sandwich-type heat preservation, and the roofs were installed using heat preservation and a greenhouse.

A spraying system was designed, which mainly consisted of a nozzle, filter, circulation pump, controller, and a heating device (Fig. 1b). At the top of the four reactors, u-shaped polypropylene-random (PPR) spraying pipes were adopted with a diameter of $\Phi = 32$ mm (Fig. 1c). The spraying system used a modified wide-angle fan nozzle (1/2K-SS60; Feizhuo, Shanghai, China), with a spraying angle of 120°. The spacing between the two nozzles was 500 mm, and they were installed using a combination of multi point and multi angle methods (Fig. 1c). Nozzle angles could be adjusted to 0°, 90°, 180°, or 270°. The system could additionally improve the uniformity and coverage area of spraying. All four reactors used the above spraying system.

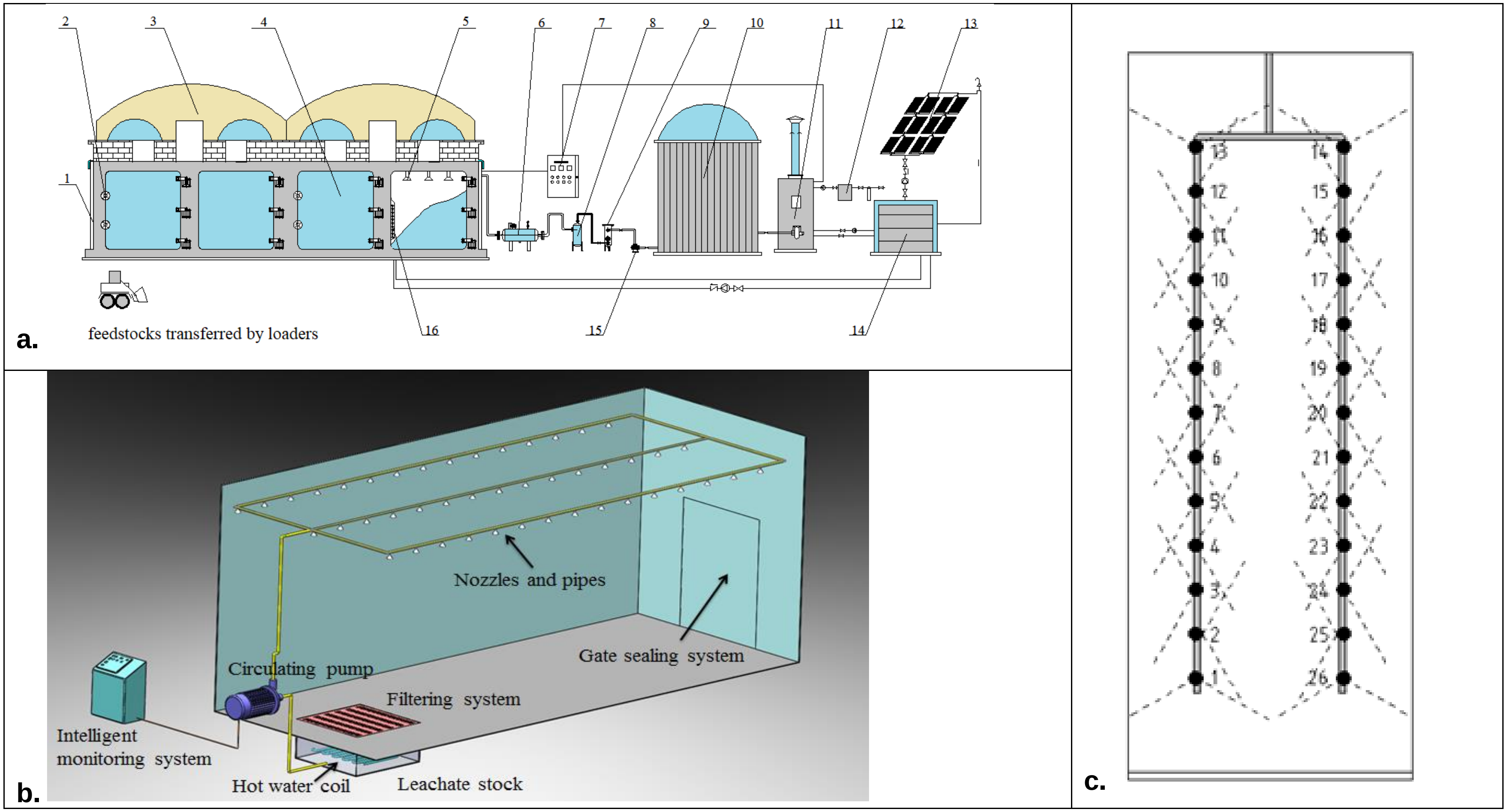

Fig. 1. Garage-type anaerobic solid-state fermentation system with flexible roof membrane: (a) Schematic diagram of the system: 1- Thermal insulation, 2- Retaining mechanism, 3- Heat preservation and greenhouse, 4- Gate sealing system, 5- Circulation spraying system, 6- Pressure stabilizing tank, 7- Monitoring system, 8- Biogas dehydrating tank, 9- Biogas desulfurization tank, 10- Biogas storage tank, 11- Biogas boiler, 12- Water softening device, 13- Solar heating system, 14- Heat water storage tank, 15- Booster fan, 16- Hot water coil; (b) Structure diagram of combined spraying system; (c) Installation diagram of position and angle of nozzle

Experimental Design

The experimental period spanned 40 days. The feedstocks were transferred from the gate and roof by loaders and conveyor devices. First, the straw bales were placed horizontally at the bottom of the reactor in one layer. The stirred fresh pig manure and microbial fortification agent were then spread evenly on the straw bales. This process was repeated until the height of the stacking material was approximately 2.4 m. The ratio of straw to pig manure was 1:2. The carbon–nitrogen ratio was adjusted to 30:1.

Four treatments were performed in the experiment: ordinary continuous spraying (OCS), ordinary stepwise spraying (OSS), enhanced continuous spraying (ECS), and enhanced stepwise spraying (ESS). The spray volume of four treatments was 60% of the total leachate volume. Reactor A, using the OCS method, adopted continuous spraying every 8 h, and the biogas slurry was not treated. Reactor B, using the OSS method, adopted the stepwise spraying at 8 h intervals before the second gas yield peak, every 24 h after the peak, and the same biogas slurry as that in the OCS method. Reactor C, using the ECS method, adopted the same spray frequency as that in the OCS method, but a microbial strengthening agent was added to the biogas slurry. Reactor D, using the ESS method, adopted the same spray frequency as that in the OSS method, and the same biogas slurry as that of the ECS method. The experimental design for four treatments of anaerobic solid-state fermentation are shown in Table 3.

Table 3. Experimental Design for Four Treatments of Anaerobic Solid-state Fermentation

Reactor No.	Treatments	Spraying frequency		Type of biogas slurry
Reactor A	Ordinary continuous spraying (OCS)	Day 1 to Day 40	8 h	No treated
Reactor B	Ordinary stepwise spraying (OSS)	Day 1 to Day 2nd peak*	8 h	No treated
		Day 2nd peak to Day 40	24 h	
Reactor C	Enhanced continuous spraying (ECS)	Day 1 to Day 40	8 h	Added a microbial strengthening agent
Reactor D	Enhanced stepwise spraying (ESS)	Day 1 to Day 2nd peak	8 h	Added a microbial strengthening agent
		Day 2nd peak to Day 40	24 h	

*. Day 2nd peak: the second yield peak day

Three temperature measuring layers were selected in the vertical direction of the material, at heights from the ground of 600 mm, 1200 mm, and 1800 mm. A total of 13 temperature test points were set up for each temperature layer. Gas samples were collected every day, and biogas slurry samples were collected after completion of the spraying operation. The biogas residue samples were collected promptly after the fermentation, which had nine sampling points in three layers (Fig. 2).

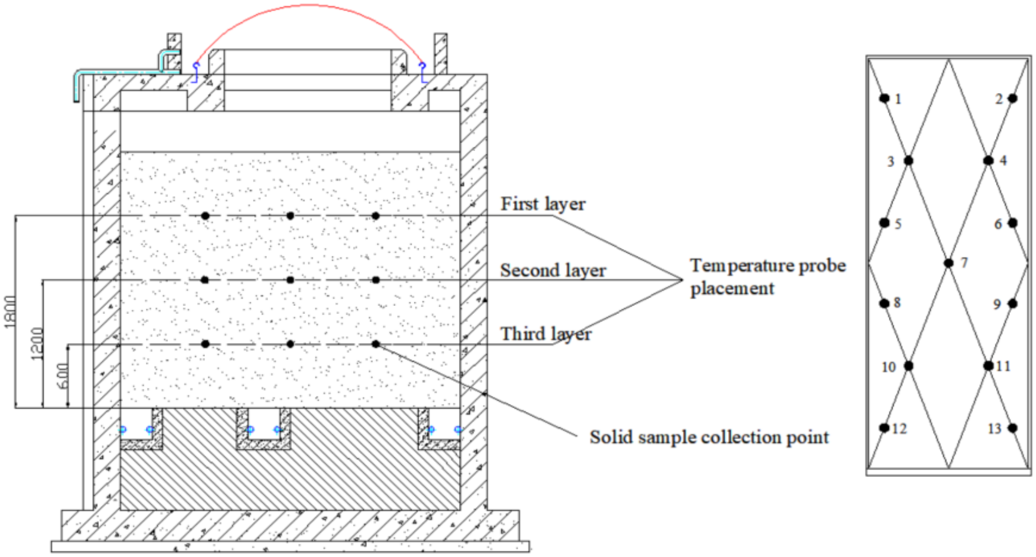

Fig. 2. Schematic diagram of solid sample collection point and temperature probe placement

Chemical Analysis

The TS (total solid) and VS (volatile solid) were analyzed by the standard methods of the American Public Health Association (2005). Temperature was monitored and recorded using a temperature sensor (JWB/33; ColliHigh, Beijing, China), with the accuracy of ± 0.2% F•S, and a data-acquisition module (KL-M4542B; ColliHigh, Beijing, China). The temperature sensor was calibrated with a standard mercury thermometer before the experiment. Biogas yield was measured with an ultrasonic gas flow meter (BF-30008-160; Sifang Company, Wuhan, China). Methane and carbon dioxide were analyzed by daily sampling using a gas chromatograph (GC9890A; Renhua Instrumentation Factory, Shanghai, China). All resulting values are the mean of at least three replicate analyses. The pH and oxidation-reduction potential were measured by a PHS-25 meter (Leichi Instrumentation Factory, Shanghai, China). Cellulose, hemicellulose, and lignin content were determined by a cellulose analyzer (220i; ANKOM, Macedon, NY, USA) based on Fan's washing method.

RESULTS AND DISCUSSION

Temperature Changes of Feedstock in Different Spraying Modes

The influence of different spraying methods on the temperature distribution of feedstock in the GASFS-FFM is shown in Fig. 3 (a, b, c, and d). During the early stage, no stratification of the temperature field was observed in the four reactors. However, temperature stratification was clear after the temperature peak. In the OCS and OSS groups (Fig. 3a and 3b, respectively), the feedstock of each layer reached the highest temperature on Day 14. The order of the temperature stability of each layer was, from highest to lowest, third layer, first layer, and second layer for OCS and then third layer, second layer, and first layer for OSS. In the ECS and ESS groups (Fig. 3c and 3d, respectively), each layer reached the highest temperature on Day 13, one day earlier than the OCS and OSS groups. The average temperatures of the first and third layer in the ECS and ESS group were higher than those in the OCS and OSS groups, whereas the average temperature of the second layer was approximately the same. The temperature stability of each layer was better in the ECS and ESS groups. After the second gas yield peak in the four reactors, the reduction of spraying frequency had a greater effect on the material temperature of the first layer. However, it had no obvious effect on the temperature of the middle and lower layers. In the ECS and ESS groups, this effect was more obvious.

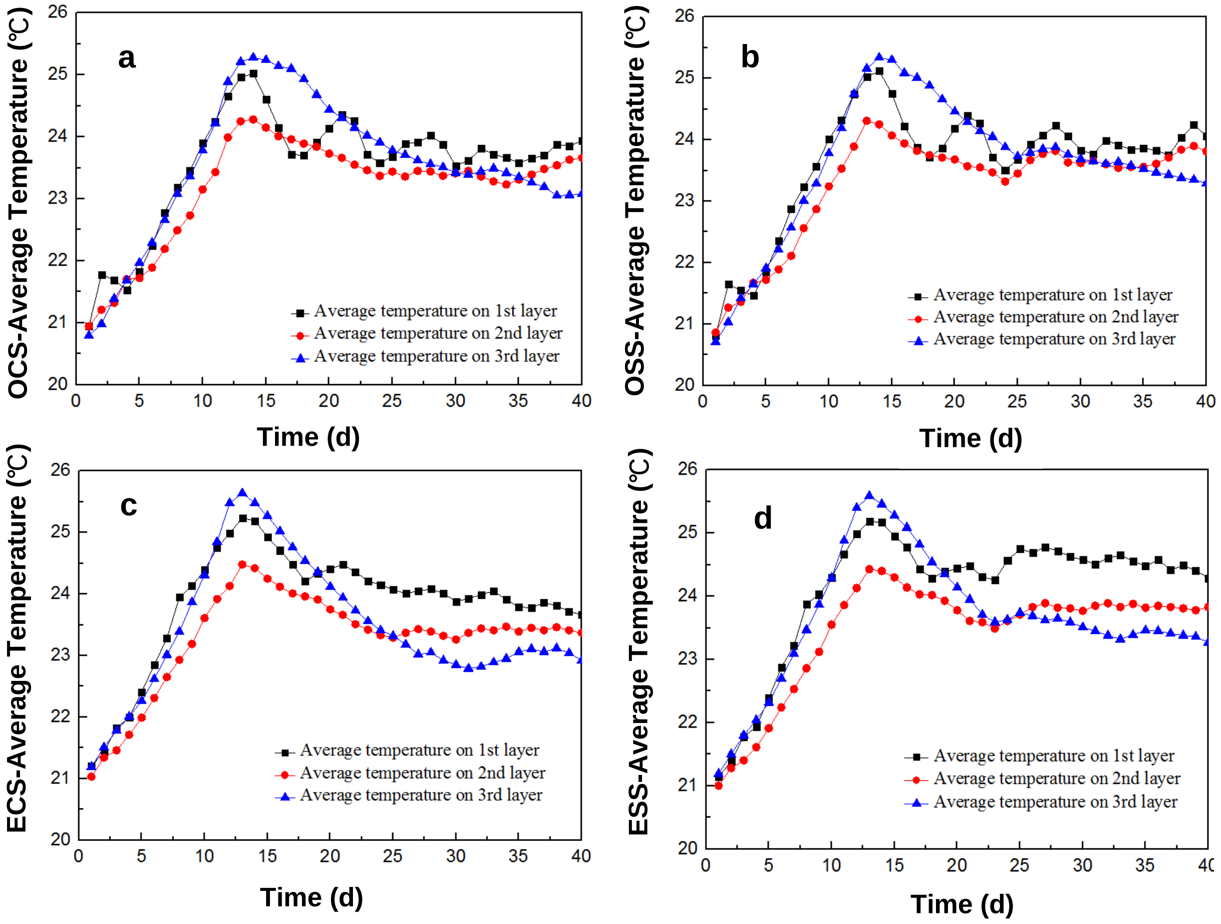

Fig. 3. (a) Temperature changes of feedstock in OCS group; (b) Temperature changes of feedstock in OSS group; (c) Temperature changes of feedstock in ECS group; (d) Temperature changes of feedstock in ESS group.

Due to a lack of sufficient core microorganisms, the metabolic capacity of the microorganisms decreased, and the early temperature slowly increased in the OCS and OSS groups. However, the temperature in the ECS and ESS groups increased rapidly, which could be due to the fact that the addition of the microbial fortification agent ensured the relative abundance of methanogens, and generated substantially more biological heat with the residual oxygen in the reactor, which is consistent with the findings of Lindorfer *et al.* (2006) and Jian *et al.* (2013). After the second gas yield peak, temperature fluctuations of the material were greater in the OCS and ECS groups than in OSS and ESS groups, especially in the top layer (≥ 0.3 °C per day). This was mainly due to the frequent spraying of leachate with lower temperature, removing heat from the feedstock. Further, the lower spraying frequency reduced the scouring of microorganisms and maintained a relatively stable environment. Hence, in the middle and later stages of the fermentation, the appropriate reduction of spraying frequency was beneficial for maintaining a higher fermentation temperature and stable environment. Moreover, the change of spraying frequency had little effect on the temperature of the bottom material in the later period. This may be because the accumulation of macromolecular substances that had not been fully degraded by long-term spraying increased the amount of suspended and colloidal substances in the biogas slurry.

The accumulation led to a gradual increase in the viscosity of biogas slurry and affected the heat transfer in the feedstock (Wu *et al.* 2015). Therefore, the added microbial fortification agent can advance the arrival time of the peak temperature by one day and increase the average fermentation temperature 8.28%. The stepwise spraying method can maintain the stability of the temperature environment (≤ 0.3 °C per day), and a higher fermentation temperature in the later stages of fermentation.

Characteristics of Biogas Yield in Different Spraying Modes

The influence of different spraying methods on the daily gas yield is shown in Fig. 4(a).

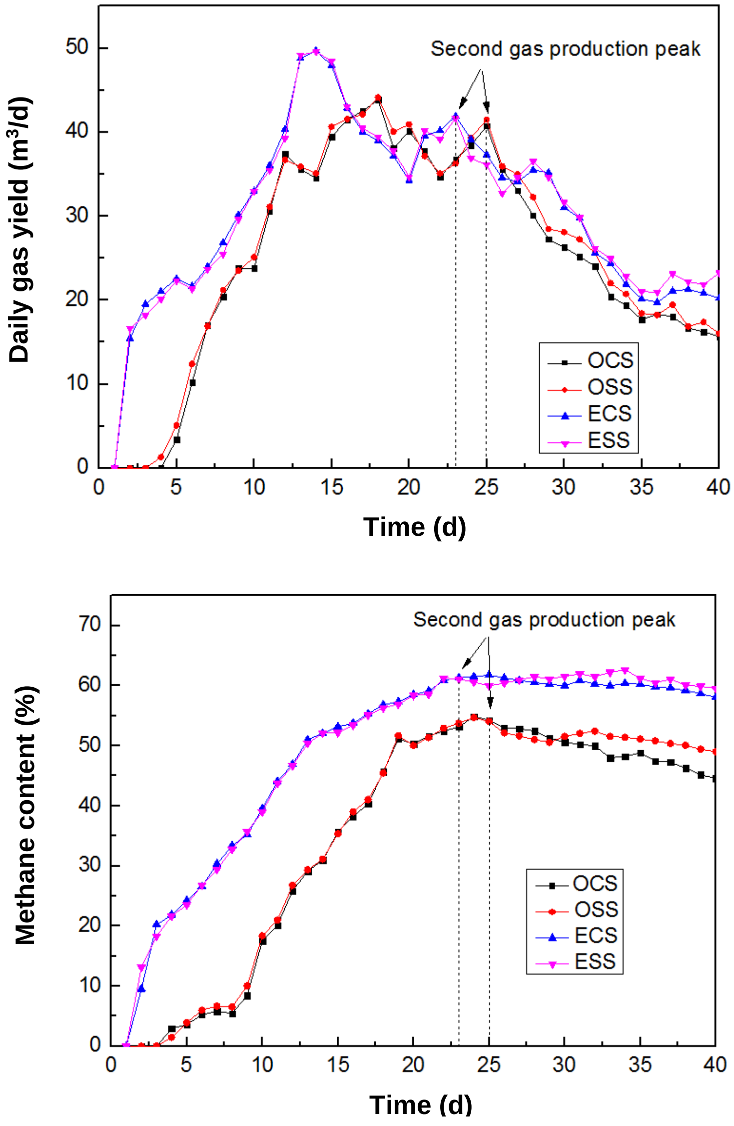

Fig. 4. (a) Influence of different spraying modes on the daily gas yield; (b) Changes of methane content for different spraying modes

The OCS and OSS groups generated gas on the fifth day and exhibited a longer start-up time than the ECS and ESS groups, which started to generate gas on the second day. For the OCS and OSS groups, the first daily biogas yield peak was 43.86 m^3 and 44.13 m^3, respectively, on Day 18, with the volume of gas yield being approximately 0.58 $m^3/(m^3 \cdot d)$. However, the second yield peak was reached on Day 25. For the ECS and ESS groups, the first biogas yield peak was 49.72 m^3 and 49.56 m^3, respectively, on Day 14, with the volume of gas yield being approximately 0.66 $m^3/ (m^3 \cdot d)$, and the second yield peak was reached on Day 23. This confirmed that the microbial fortification agent can effectively increase gas yield and shorten the start-up time by 3 days. In the enhanced group, the first yield peak was 4 days earlier than the ordinary group, and the second peak gas yield was extended by 2 days. The maximum gas yield was 13.01% higher. Experimental data showed that the spraying strategy was changed to obtain similar methane flow generation for the ECS and ESS group, after the second gas yield peak (Day 23). Following the second gas yield peak, the gas yield of the OSS group increased slightly by 2.24% compared with that of the OCS group, and the difference between the two was similar.

Therefore, it is necessary to determine the more suitable spraying method to result in higher gas yield and lower energy consumption following the second yield peak. The data showed that the addition of fortified bacteria greatly increased the gas yield and shortened the start-up time, as well as that following the second gas yield peak. Reducing the number of sprays had no effect on the gas yield. Many studies have indicated that the quantity, quality, and frequency of inoculum had a substantial influence on gas yield efficiency and quality. The spray frequencies suitable for different kinds of material were narrow, with too many or too few limiting methane yield (Haleh *et al.* 2012; Pezzolla *et al.* 2017; Riggio *et al.* 2017). André *et al.* (2015) showed that, due to structural changes in the feedstock, no recirculation is required after the second yield peak.

The changes in methane (CH_4) content (volume fraction) for different spraying methods are shown in Fig. 4(b). The CH_4 content in both groups gradually increased, then stabilized. At the yield peak, the volume fraction of methane stabilized at 53% in the OCS and OSS groups and 62% in the ECS and ESS group. In the start-up phase, the ECS and ESS group not only exhibited a more rapid start but additionally a more rapid rise than the OCS and OSS groups. The CH_4 content in the ordinary and enhanced groups reached 50% on Day 19 and Day 13, respectively. The enhanced group therefore reached the 50% CH_4 content 6 days earlier than the ordinary group and increased the average CH_4 content 16.98%. After the second yield peak, excessive spraying frequency was not conducive to the increase of methane content in the system, especially in the ordinary biogas slurry system with lower inoculation concentration (Degueurce *et al.* 2016). In the ordinary groups, the reduction of the spraying times increased the methane content 2.59%. Degueurce *et al.* (2016) showed that a low inoculum concentration of the system and high spraying time is not conducive to increasing the methane content. Thus, the system lacks sufficient methanogens to transfer intermediate products (organic acids) for methane yield. During the gas yield peak, the methanogens were vigorously metabolized in the enhanced group, and the appropriate amount of organic acid provided raw materials for methanogenesis. The methane concentration not only continued to rise but remained high.

Changes in pH Value for Different Spraying Modes

The pH values of the four groups first decreased, then increased, and finally stabilized (Fig. 5). The pH value of the anaerobic digestion process was normally in the range of 6.5 to 8.5 (Jian *et al.* 2013). The pH of the OCS and OSS groups decreased to the lowest point (6.41 and 6.38) on Day 7. This may be due to the limited amount of inoculum in the previous biogas slurry, resulting in accumulation of organic acids (Laura *et al.* 2017). The pH value returned to 7.00 until Day 25. From Day 25, the OSS group adopted the spraying every 24 h, which increased the pH. After Day 34, the pH value of the two groups was almost the same. The pH value of the ECS and ESS groups decreased to the lowest point (6.88 and 6.78) on Day 8 and then became neutral on Day 15. The change of spray frequency had little impact on the pH value in the enhanced groups with the adequate amounts of inoculum. Further, during the period of decreasing pH the temperature decreased. This may reflect the higher propionic acid and butyric acid content, causing an oxidation reaction with energy absorption to acetic acid, hydrogen, and carbon dioxide. This indicates that a suitable spray method could promote the conversion of organic acid and ammonia nitrogen and maintain a neutral pH value. Thus, the fermentation system not only needs to maintain adequate amounts of inoculum, but additionally maintain the structural stability of the microbial community and reduce the scour effect of leachate on microflora (André *et al.* 2015).

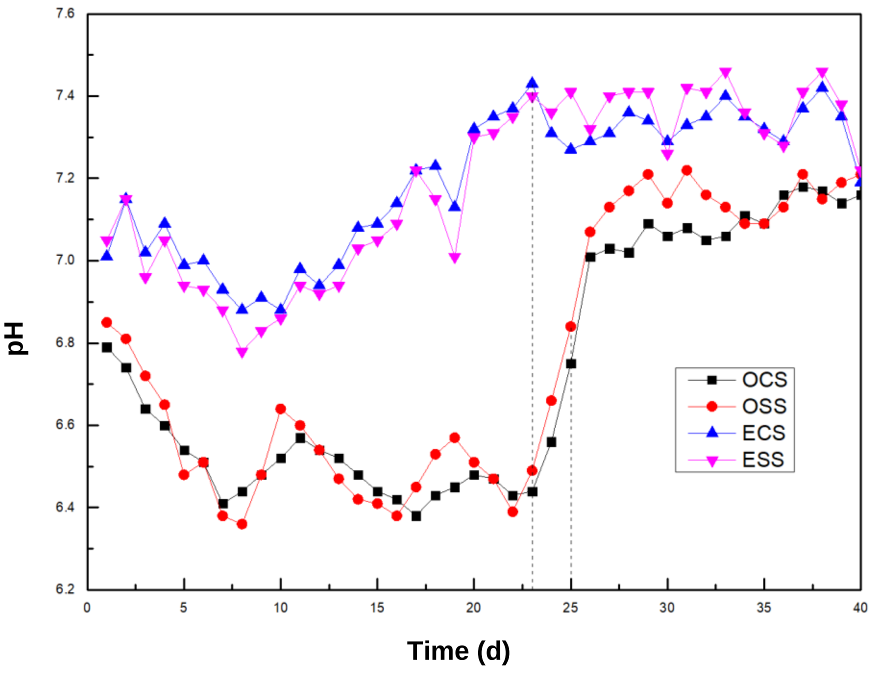

Fig. 5. Changes in pH value of different spraying modes

Changes in Moisture Content for Different Spraying Modes

The influence of different spraying methods on the distribution of moisture content in the vertical section is shown in Fig. 6. The moisture content in four groups increased with a decrease in feedstock height. In the materials of the first and third layers, the water content of the OCS the ECS groups was higher than that of the OSS and the ESS groups. In the middle layer materials, the water content of the four groups was relatively close.

As fermentation progressed, the porosity of the feedstock in the middle and bottom layers became low, and the pore distribution became more uniform and compact. Meanwhile, the moisture content of the feedstock was typically saturated, and the viscosity of the biogas slurry was large. Therefore, the permeability of the gravity-oriented leachate decreased, and the flow path began to immobilize. André (2015) showed that different spraying methods did not affect the flow path in the feedstock after the second gas yield peak. The continuous spraying method improved the water content of each layer, especially in the first- and third-layer materials. The increased spraying frequency and fixed flow paths resulted in the moisture content of the bottom layer exceeding the saturation level by 74.29% and reaching a complete water seal state, which increased the viscosity of the substrate and restricted gas transmission. The stepwise spraying method allowed the top liquid buffer time to infiltrate evenly. This resulted in the bottom layer not becoming saturated and maintaining good air permeability. Furthermore, the addition of the fortified bacteria agent increased the degradation of the material and the settlement deformation. This caused the water content of each layer of the ECS and ESS groups to be higher than that of the OCS and OSS groups.

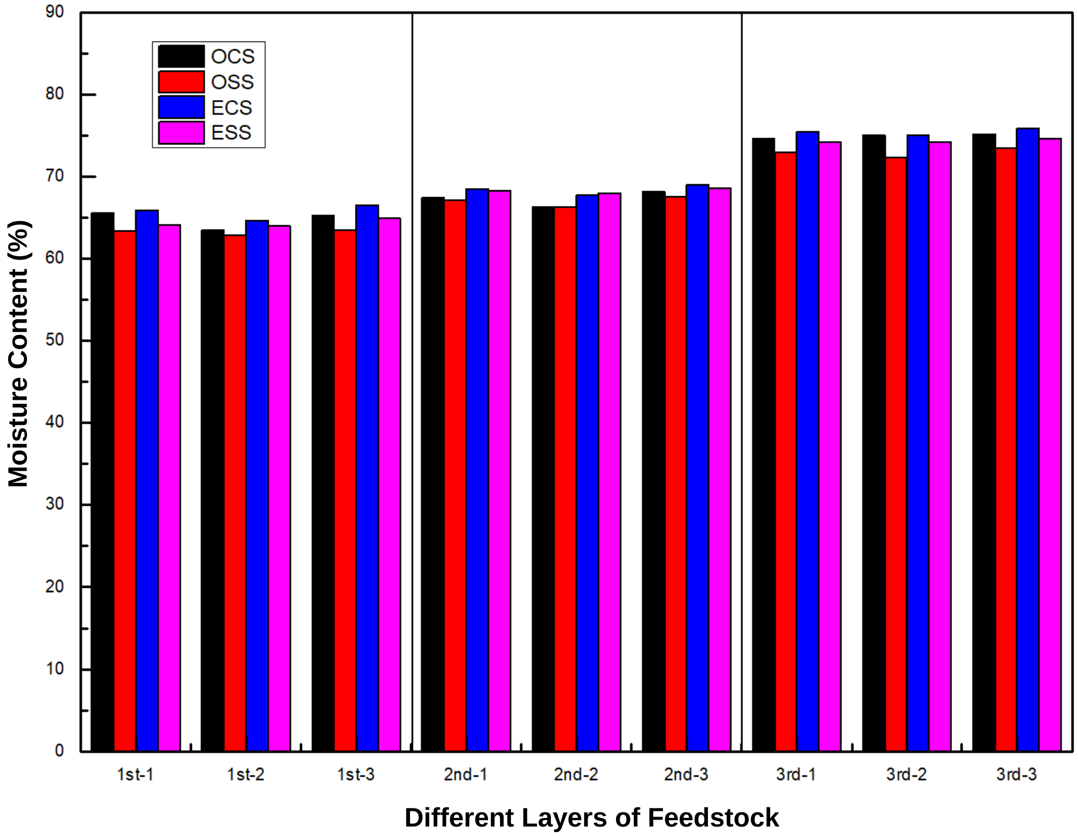

Fig. 6. Influence of different spraying modes on the distribution of moisture content in the vertical section

The moisture content in the first and second layers, in the four groups, presented the characteristics of high moisture content on both sides and low in the middle layer. The flow distribution of leachate in the feedstock was not uniform. This may be because the non-uniform fast macroporous flow of leachate decreased gradually with fermentation. Leachate collected in the channel flow at the top layer then flowed down the reactor boundary, resulting in a moisture depression zone in the high-density feedstock. These results were consistent with the findings of Kunlun *et al.* (2017).

Changes in Degradation Rate for Different Spraying Modes

Rice straw is mainly composed of cellulose, hemicellulose, and lignin.

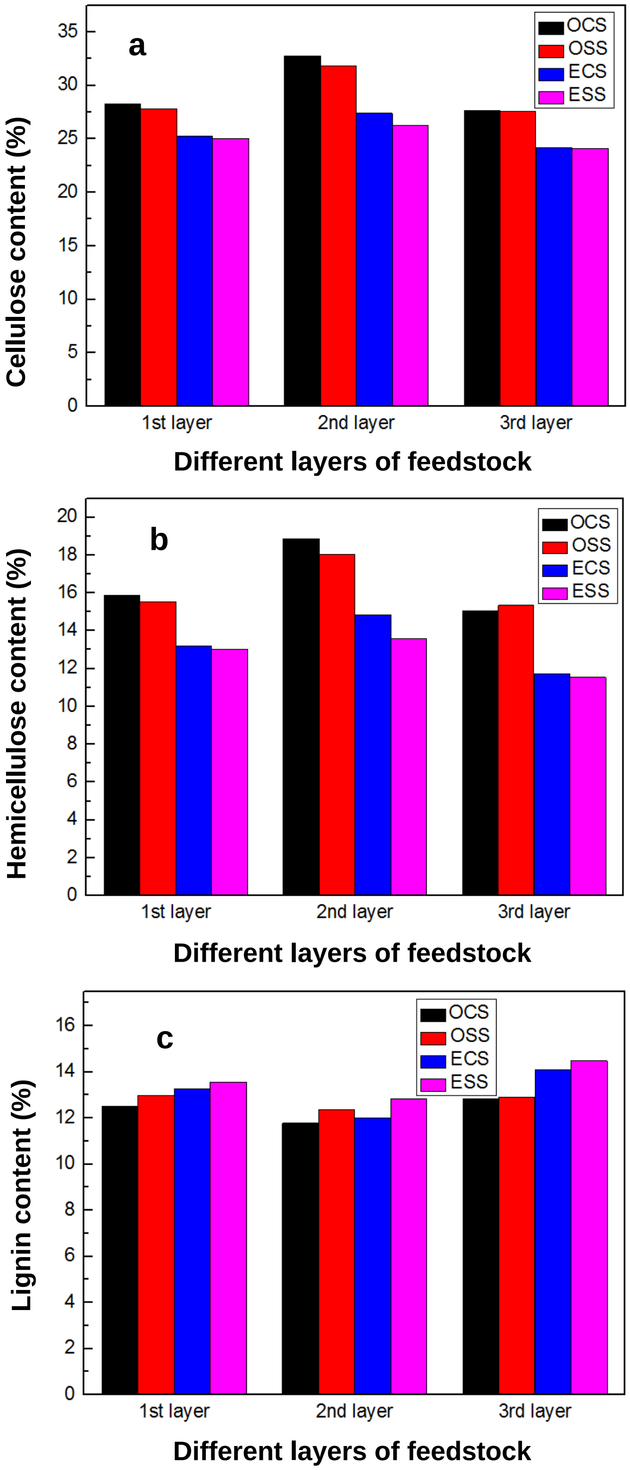

Fig. 7. (a) Cellulose content of the different layers; (b) Hemicellulose content of the different layers; (c) Lignin content of the different layers

It is believed that methane yield is mainly achieved by anaerobic digestion of the first two components, while lignin has little effect (Mussoline *et al.* 2013; Yu *et al.* 2014). The mass fractions of cellulose, hemicellulose, and lignin in the rice straw were 36.32%, 26.16%, and 11.08%, respectively. Following the end of the fermentation cycle, changes in the three elements in the vertical profile of the feedstock due to different spraying methods are shown in Fig. 7. Like previous studies, the cellulose and hemicellulose contents, which are the main degradation products, decreased after fermentation, whereas the relative lignin content increased.

The degradation rate of cellulose and hemicellulose in the ECS and ESS groups was faster than that in the OCS and OSS groups. The central region of the OCS and OSS groups exhibited a poor degradation effect. Aside from the small-scale central region, degradation in the ECS and ESS groups was relatively more uniform, and degradation of the bottom feedstock was more complete. Following the second peak of gas yield, the order of influence of the spraying method on the degradation rate of cellulose and hemicellulose was, from the highest to the lowest, second layer > first layer > third layer. The stepwise spraying was helpful to improve the degradation rate of the middle layer. The lignin degradation rates of the four groups were evenly distributed, although the lignin content in the ECS and ESS groups was slightly higher than in the OCS and OSS groups, especially in the first layer and third layer. Following the second peak of gas yield, the decrease in spray frequency increased the lignin content of each layer.

The addition of the fortified bacteria agent had a greater contribution to the degradation rate of cellulose and hemicellulose than the spray methods. Therefore, the degradation rate of the ECS and ESS groups was higher than that of the OCS and OSS groups, and the average degradation rate of cellulose and hemicellulose increased 56.14% and 35.75%, respectively. The stepwise spraying method increased the average degradation rate of cellulose and hemicellulose 5.78% and 3.67%, respectively, when compared to the continuous spraying method. On the one hand, the strengthened agent in the enhanced groups was rich in cellulose decomposing bacteria, which greatly increased the degradation rates of these substances. In contrast, the long-term continuous spraying in the continuous groups resulted in increased viscosity and decreased hydrolytic enzyme activity and methanogenic activity, which limited the utilization efficiency of hydrolyzed products (Karthikeyan and Visvanathan 2013; Estevez *et al.* 2014). However, lignin contains a variety of aromatic ring substances with complex and diverse structures, which exhibit strong resistance to microbial corrosion and are difficult to degrade (Jae-Jung *et al.* 2009; Zhang *et al.* 2014b). Thus, the poorer degradation in the central region of the OCS and OSS groups indicates that it is necessary to add an enhanced agent to accelerate the rate of degradation depression areas when dealing with high-density feedstock.

CONCLUSIONS

1. The added microbial fortification agent was able to advance the arrival time of the peak temperature by one day and increase the average fermentation temperature by 8.28%. The stepwise spraying method was able to maintain the stability of the temperature environment (≤ 0.3 °C per day).
2. The microbial fortification agent was able to increase gas production and shorten the start-up time by 3 days. The first yield peak of the enhanced groups was 4 days earlier

than the ordinary groups. The maximum gas yield of the enhanced groups was 13.01% higher than the ordinary groups, and the methane concentration increased 16.98%. After the second gas yield peak, the reducing of spraying frequency had almost no effect on the gas yield.

3. The stepwise spraying method was helpful to improve the moisture distribution and the degradation rate of the middle layer. Therefore, the degradation rate of the ECS and ESS groups were higher than that of the OCS and OSS groups. The average degradation rate of cellulose and hemicellulose increased 56.14% and 35.75%, respectively.

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