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# Effect of organic load regulation on anaerobic digestion performance and microbial community of solar-assisted system of food waste

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## ABSTRACT

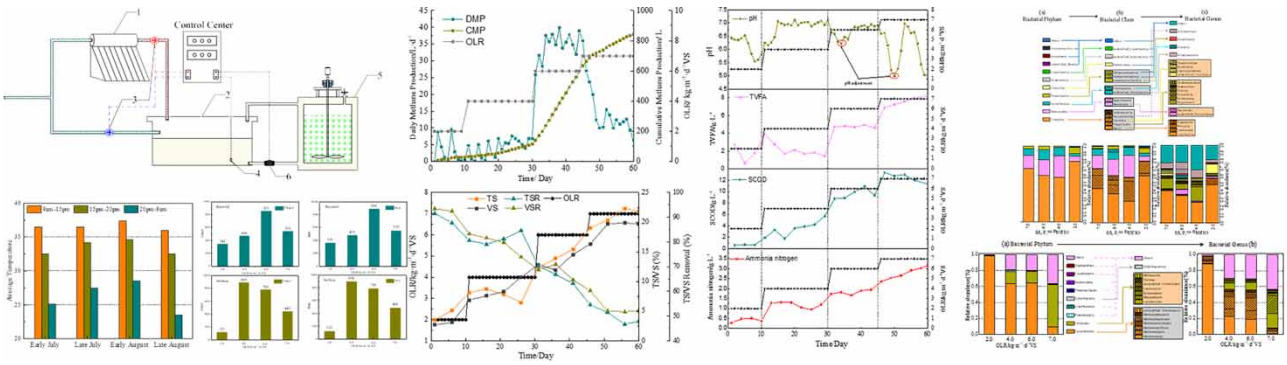
The semi-continuous digestion experiment of food waste was carried out based on a solar-assisted heat anaerobic digestion reactor. The effects of organic load regulation (OLR of 2.0, 4.0, 6.0 and 7.0 kg $m^{-3}$ $d^{-1}$ VS (volatile solid)) on methane production, physical and chemical parameters, and microbial community structure were studied. The results showed that 6.0 kg $m^{-3}$ $d^{-1}$ VS could achieve the optimal methane production as the anaerobic digestion limit OLR of kitchen waste. At this stage, the accumulation of organic acids in the reactor led to a great fluctuation of pH. Artificial alkali regulation could restore the stable operation of the reactor, but the reactor could not withstand the OLR shock of 7.0 kg $m^{-3}$ $d^{-1}$ VS. In addition, Qualcomm sequencing results showed that microorganisms showed high functional concentration and poor community richness under low OLR, and increasing OLR could promote microbial richness. At the same time, the *Methanosaeta* of acetic acid methanogens was relatively abundant in the low OLR stage, while the hydrogen trophic methanogenic bacteria *Methanoregula* and *Methanospirillum* showed high activity in the high OLR stage, but the excessive reproduction of acidogenic bacteria in the digestive system would affect the stability of the archaea community when the OLR was too high.

**Key words:** food waste, microbial community structure, organic load rate regulation, solar energy

## HIGHLIGHTS

- Electric energy was saved by the solar system with a slight decrease of methane yield.
- A low organic loading rate suppresses the succession of microbial structure.
- Unstability caused by organic acid can be restored by artificial control of pH.

## GRAPHICAL ABSTRACT




## INTRODUCTION

With the acceleration of urbanization and the improvement of people's living standard, food waste production in China increased from 37.82 million tons (2011) to 42.22 million tons (2015). According to statistics, about 1.3 billion tons of garbage

will be generated globally in 2021. Food waste was characteristic of high water content, high organic matters and high oil and salt content. The resource utilization of food waste could be realized by the anaerobic digestion (AD) technique, which effectively solved the problems of deposit, treatment and environment pollution, wherein organic load regulation (OLR) and temperature directly influenced the operation of the anaerobic digestive reactor as well as the methane production efficiency. Zhang *et al.* (2014a, 2014b) studied the smooth operation of OLR between 4.33 and 11.40 kg $m^{-3}$ $d^{-1}$ VS at the medium temperature. The average COD removal rate was as high as 85%. However, the ammonia nitrogen content increased with the increase of organic load. Zhang *et al.* (2014a, 2014b) found out the maximum OLR of 3.9 kg $m^{-3}$ $d^{-1}$ VS for the stable and efficient operation born by the reactor. However, Gou *et al.* (2014) found out that the methane production efficiency was the best in the digestive reactor with the OLR of 5 kg $m^{-3}$ $d^{-1}$ VS. The further increase of OLR led to the rancidity phenomenon in the reactor. Although the low OLR was favorable for the stable operation of the reactor, the low utilization rate of organic matter increased the operation cost of biogas engineering, whereas the too high OLR shock inhibited the activity of the methanogenic bacteria, decreased the anaerobic digestive efficiency and even destroyed the microbial community structure. Thus, with the prerequisite of the non-destruction of the balance between organic material and microbes, it was vital to investigate the OLR concentration limit of the anaerobic digestive reactor of food waste.

Temperature was one of the most important factors which influenced the operation efficiency of anaerobic digestion as well as microbial activity. Wu & Sun (2006) studied the influence of the sharp temperature decreasing from 55 to 20 °C on anaerobic digestion of urban organic life waste when simulating the heating failure, showing that the sudden decrease of temperature severely influenced the gas production. Volatile fatty acids (VFAs) rapidly accumulated and pH decreased. In addition, the recovery time prolongs accordingly with the elongation of the duration time of low temperature. Traditional biogas engineering used electricity as an additional energy supply to maintain the fermentation temperature, resulting in the waste of resources. To realize sustainable development, it was valuable to investigate the implementation of saving energy and decreasing emission in anaerobic digestive reactions (Liu & Sun 2019; Kurade *et al.* 2020). Recently, the solar photovoltaic power generation technique has grown mature (Dong *et al.* 2012).

Solar energy has been widely applied as clean energy. However, its use in the field of anaerobic digestion is not in-depth. Among the many influencing factors of anaerobic digestion, temperature is the easiest and most intuitive one to control. Thus, in this test, the solar system and the anaerobic digestive technique were combined to decrease energy consumption and increase the economic benefit of biogas engineering. However, solar radiation was influenced by factors such as climate. There were temperature fluctuations during the real operation of the reactor due to unstable heat radiation. To evaluate the influence of solar-assisted heat system on food waste treatment efficiency, the feeding material OLR of food waste was adjusted. Besides, the optimum OLR concentration of anaerobic digestion of food waste was obtained by simultaneous analysis of the methane production, the physical and chemical parameters and the microbial community structure. Thus, the application value of the solar-heated system in real biogas engineering was investigated. The purpose is to explore the benefits of the combination of solar energy and anaerobic digestion for the current food waste treatment method.

## MATERIALS AND METHODS

### Experimental materials

The main fermentation material is food waste from the canteen in Shenyang Aerospace University, Liaoning, Shenyang, and inoculum is sludge from the concentration tank of Northern Wastewater Treatment Plant, Shenyang, Liaoning. Food waste is mechanically treated and broken to paste by grinder HX-J3022, then put in a freezer at −18 °C in a vacuum bag, characteristics of food waste and inoculum in the study were shown in Table 1. An extraction part of the mechanically treated materials for the domestication of inoculum at 37 ± 0.5 °C for 15 d when the sludge itself no longer produces gas, it is regarded as the completion of domestication.

### Experimental setup

The experiment was conducted between July 1st and August 31st. The experimental design was based on a real solar biogas engineering project. The detailed experimental apparatus is shown in Figure 1. A solar photothermal utilization system (1) is set out to provide a heat source. Cold and hot water enter the water tank (2) through the solenoid valve (3) as a heat transfer medium. Through the feedback adjustment of the temperature probe (4), the appropriate warm water enters the double-layer water bath anaerobic digestion tank (5) through the circulating pump (6).

**Table 1** | Characteristics of food waste and inoculum in the study

| Characteristics | Units | Food waste | Inoculum sludge |
|---|---|---|---|
| pH | – | 4.6 | 6.07 |
| C/N | – | 16.86 | 7.26 |
| Total solid | % | 31.61 | 15.33 |
| Volatile solid | % | 30.79 | 5.36 |
| Total chemical oxygen demand | g/L | 114.08 | |
| Solubility chemical oxygen demand | g/L | 47.63 | 6.21 |
| Total volatile acids | mg/L | 1,009.27 | / |
| Total ammonia nitrogen | mg/L | 467.33 | 243.67 |
| Moisture | % | 69.37 | 80.01 |
| Cellulose | % | 5.02 | / |
| Fat | % | 18.31 | / |
| Protein | % | 10.12 | / |
| Starch | % | 31.27 | / |

/: Untested.

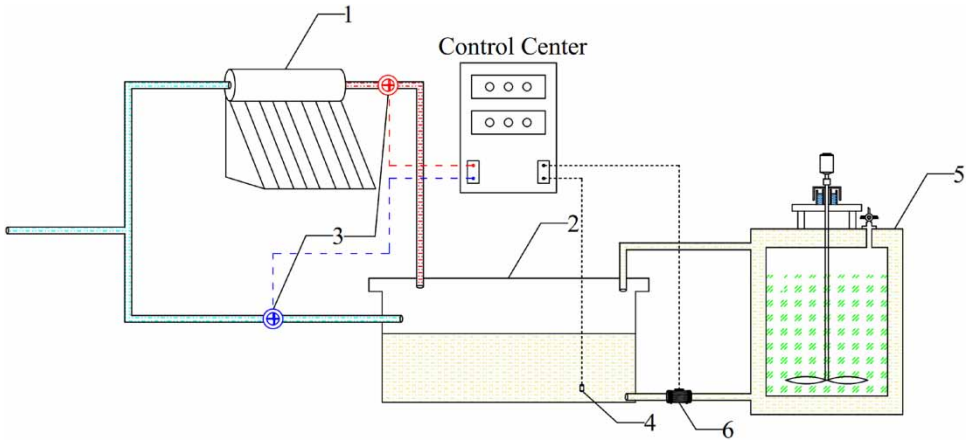



**Figure 1** | Experimental system of anaerobic digestion for solar heating.

Affected by the weather, the alternation of day and night and other natural conditions, the intensity of solar radiation will change, resulting in temperature fluctuations. According to the sunshine situation, it is roughly divided into three periods as shown in Figure 2, and the temperature changes in the water tank in the corresponding period are recorded. Except for some special weathers, maintaining the temperature under sunlight can basically ensure a stable temperature during the day.

The anaerobic digestive system used the lab-made continuous stirred tank reactor (CSTR) with the volume of 12.0 L and the effective volume of 8.0 L, which was made from polymeric methyl methacrylate (PMMA). The 4/17 top of the reactor used the water-sealed structural cap of U-shape to ensure the air tightness of the apparatus during the test. The gear motor (51K40RGN-C) was used for intermittent stirring with the rotation speed of 80 rpm. Stirring for 5 min was conducted every 3 h. The heating of the inner circulating water bath with 'down in and up out' was used in the test. The outlet water temperature of the circulating water bath pot (XT5618-GP) was set as 37 $\pm$ 0.5 °C. There was an air evacuation valve in the reactor lid. The biogas was recorded by the gas flow meter.

## Initiation and operation of the test

The test was selected to be initiated by the wet method. The test cycle was 60 d. The period with the OLR of 2.0 kg $m^{-3}$ $d^{-1}$ VS was reached by the one-time input of food waste, the inoculum and the deionized water. The blowing off $N_2$ for 5 min was

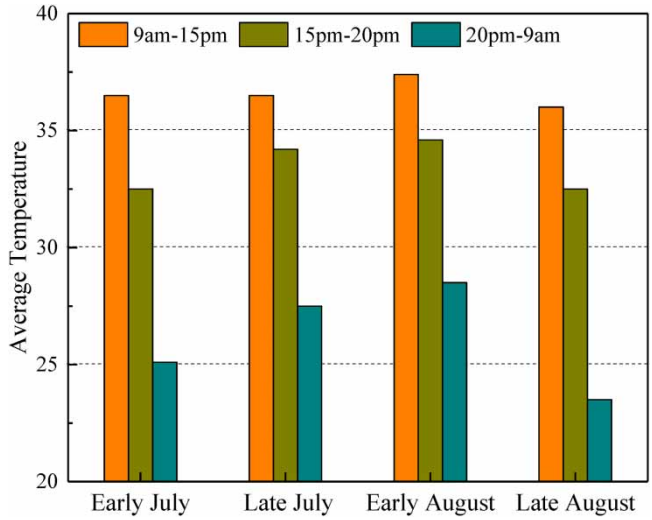



**Figure 2** | The change of temperature in the solar reactor with time.

used to remove air. According to the methane gas change principle, the feeding and discharging of material in the reactor was reasonably controlled to maintain the OLR of 2.0, 4.0, 6.0 and 7.0 kg $m^{-3}$ $d^{-1}$ VS in the corresponding periods of 1 (1–10 d), 2 (11–30 d), 3 (31–45 d) and 4 (45–60 d), respectively, during the stable operation of the reactor in the test stage. The physical and chemical parameters in the reactor were determined at 9 am every day. The sampling time of microbes was the last day of each OLR stage (10, 30, 45 and 60 d).

### Normal parameters in the analysis method

Total solid (TS), volatile solid (VS), $NH_4^+$-N and dissolved chemical oxygen demand (SCOD) were determined by international standard methods. The biogas yield was determined by the wet gas flow meter (LML-1) and its composition was determined by gas chromatography (GC). pH was determined by the portable pH meter (PHSJ-5). Total volatile fatty acid (TVFA) was determined by standard methods (APHA 2008).

### Analysis method of microbial community

DNA sample was extracted by the hexadecyltrimethylammonium bromide (CTAB) method. Subsequently, the purity and concentration of DNA was determined by agarose gel electrophoresis. A proper amount of DNA sample was put in the centrifuge tube and diluted to 1 ng/μL by the sterilized water. Polymerase chain reaction (PCR) amplification was conducted with the template of the diluted gene DNA, the specific primer with Barcode, namely phusion® High-Fidelity PCR master Mix with GC Butter from New England Biolabs, as well as the enzyme with high efficiency and high fidelity. Bacteria diversity and archaea diversity were identified by primers of 16SV4 region and 16SV8 region, respectively. According to the concentration of PCR products, PCR products were purified by 2% agarose gel electrophoresis with 1× TAE. The target band was sliced and recovered. The GeneJET gel recovery kit of Thermo Scientific Corp. was used to recover products. The library was established by the Ion plus Fragment library kit 48 rxns. The established library was sequenced by Ion S5TMXL. The bioanalysis software used for data processing was shown in references (Rognes *et al.* 2016).

## RESULTS AND DISCUSSION

### Influence of OLR regulation on methane production

Figure 3 shows the change of methane production in the semi-continuous anaerobic digestive reactor. The OLR was maintained between 2.0 and 6.0 kg $m^{-3}$ $d^{-1}$ VS. Although daily methane production (DMP) fluctuated, the reactor could stably operate and methane production demonstrated an increasing trend with the increase of OLR. The OLR of 2.0 kg $m^{-3}$ $d^{-1}$ VS was the initiation stage of the reactor, whose methane production was below 4.0 L/d. However, at this stage, the methane concentration is relatively high, maintaining above 60% according to the GC. Since it was difficult for microorganisms to obtain sufficient nutrition, the gas production presented low and fluctuated with the addition of the substrate. When the

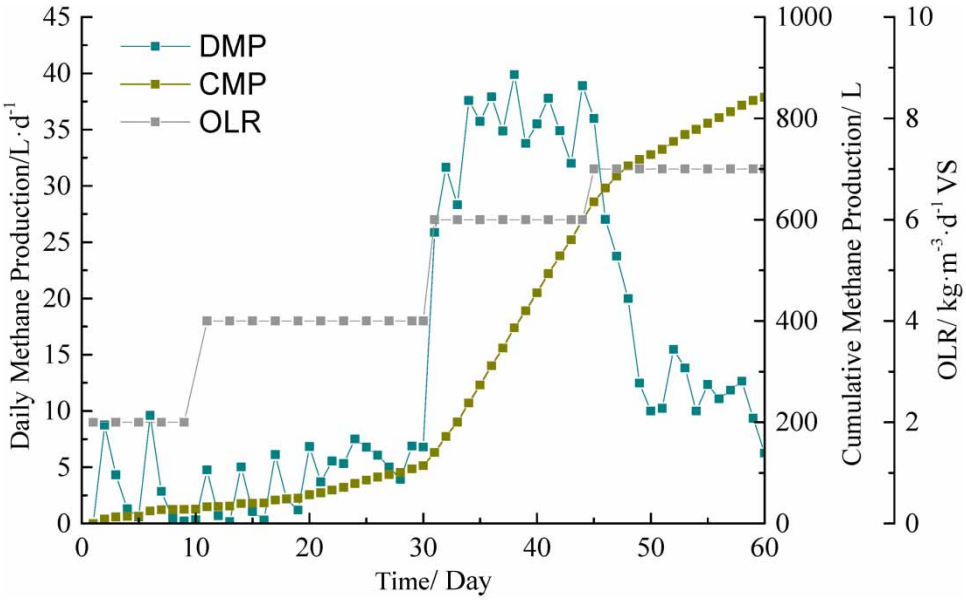



**Figure 3** | The relationship of methane production with the OLR.

OLR was increased to 4.0 kg $m^{-3}$ $d^{-1}$ VS, the methane production change in the reactor was similar to that in the previous stage. The daily production increased rapidly with the addition of food waste. In this stage, TVFA concentration demonstrated a slow decreasing trend, illustrating that a small addition of food waste could not sufficiently satisfy the nutrition requirement of the methanogenic bacteria. It found out that maintaining the OLR between 0.7 and 1.5 kg $m^{-3}$ $d^{-1}$ VS could result in the insufficient nutrition supply to the methanogenic bacteria. Thus, the methane production fluctuated, which was consistent with the results with the OLR of 2.0 kg $m^{-3}$ $d^{-1}$ VS in this test (Lv *et al.* 2019). The fluctuation of DMP ceased until the increase of OLR to 6.0 kg $m^{-3}$ $d^{-1}$ VS. In this stage, the optimum methane production efficiency was realized and the methane content was stable at 55–60%. The highest methane production was 39.89 L/d (38 d). The average methane production approached a stable state with the value of 34.72 L/d. However, during the further increase of OLR to 7.0 kg $m^{-3}$ $d^{-1}$ VS, methane production fell off the cliff with the average methane production of 13.75 L/d, and most of them was $CO_2$ (>45%) and a small amount of $H_2S$, $H_2$ and CO (<10%). Those results showed that the OLR of 2.0–4.0 kg $m^{-3}$ $d^{-1}$ VS could maintain the stable operation of the reactor. However, the low substrate concentration resulted in the deficiency of nutrition material, which could be utilized by the methanogenic bacteria and could not satisfy the requirement of growth and proliferation and lead to the hungry phenomenon. It was the stage of 6.0 kg $m^{-3}$ $d^{-1}$ VS that realized the highly efficient utilization of food waste with the optimum methane production. During this stage, the reactor operation exhibited stable and highly efficient production efficiency. However, the reactor could not bear the shock of too high organic matters and hence, the operation failed. In addition, temperature fluctuations will lead to a certain degree of reduction in methane production, but compared with the energy saved, it is still a preferable method.

### Influence of OLR regulation on physical and chemical parameters

pH was one important index evaluating the stability of the anaerobic digestive system. It showed that the value of pH between 6.5 and 7.5 was most favorable for the methane production. Too high or too low would influence the activity of microbes (Liu *et al.* 2007; Li *et al.* 2018). As shown in Figure 4, the initial pH value in the initiation stage was about 6.52. It sharply decreased to 5.53 on the 5th day (the 8th day). However, during the sludge acclimation stage, pH decreased rapidly on the following day after the addition of food waste, which deviated from the formal experimental phenomenon. The sludge acclimation was conducted in the thermostatic water bath pot (37 °C), where the microbial community adapted to the thermostatic environment. However, in the formal test, the digestive temperature fluctuated due to the unstable influence from the solar heat radiation. As shown in Figure 2, the temperature difference in 1 d was even larger than 10 °C, resulting in that microbes could not adapt to the environment with the fluctuated temperature in the initial stage of the test. Thus, the active expression was inhibited and the hydrolytic acidification efficiency was influenced, leading to the apparent lagging. When the

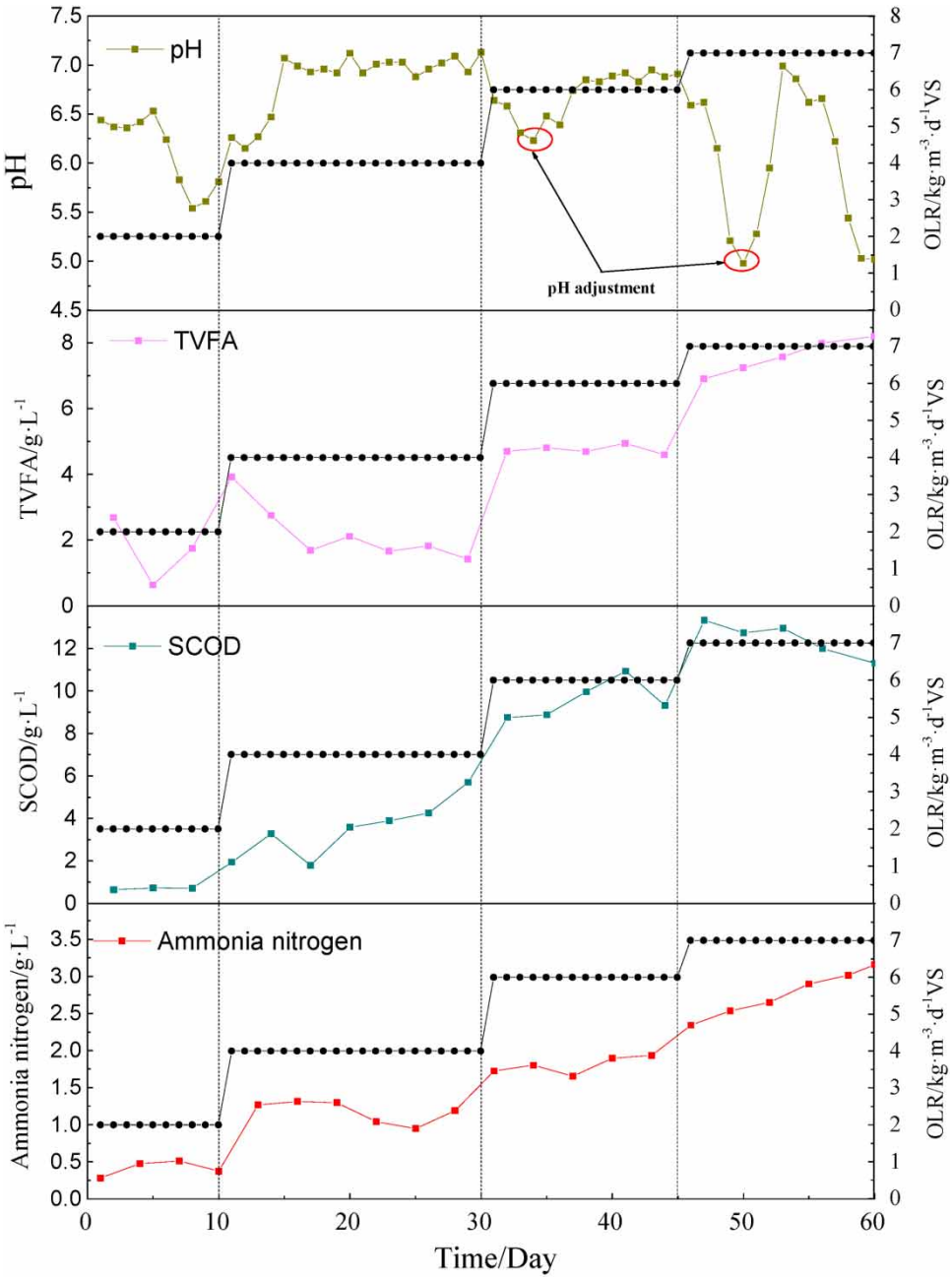



**Figure 4** | The relationship between physical and chemical parameter changes with the OLR.

OLR was increased to 4.0 kg $m^{-3}$ $d^{-1}$ VS and pH was maintained around 6.94–7.23, the operation of the reactor was stable, illustrating that the anaerobic digestive system had a good ability to maintain the acid–base balance of the digestive system during the operation of the reactor with a low OLR (≤4.0 kg $m^{-3}$ $d^{-1}$ VS). However, in the stage with the OLR of 6.0 kg $m^{-3}$ $d^{-1}$ VS, the average concentration of TVFA increased from 1.7 g/L in the previous stage to 4.0 g/L. The occurred acidification phenomenon resulted in the rapid decrease of pH to 6.23. Due to the decreased pH, which was unfavorable for the existence range of the methanogenic bacteria, a proper amount of NaOH solution (2 mol/L) was added for artificial regulation. With the gradual recovery of pH, a dynamic balance between acid production by hydrolysis and methanation was recovered. The methane production was not influenced dramatically. After further increasing of the OLR to 7.0 kg $m^{-3}$ $d^{-1}$ VS, TVFA concentration in the reactor increased sharply with the accumulation amount exceeding 8.0 g/L. The acidification

phenomenon occurred again. The methane production decreased sharply. pH decreased to 5.07. Although a large amount of alkaline solution was added to alleviate the acid inhibition, pH could not be maintained stable. In this stage, the average TVFA concentration reached 6.5 g/L. The reaction system acidified severely, illustrating that 6.0 kg $m^{-3}$ $d^{-1}$ VS was the organic load rate limit born by the anaerobic digestion with food waste. In this stage, acid inhibition occurred in the operation of the reactor, which could still be maintained by artificial regulation. When the OLR exceeded the maximum bearing capacity of the reactor, the internal balance of the digestive liquor was destroyed because the hydrolysis/acidification rate was far higher than the methane generation rate. The sharply decreased pH inhibited the activity of the methanogenic bacteria. The irreversible influence took place. The outside regulation could not change the mechanism change of the digestive liquor (Park *et al.* 2010). The dissolved COD in different OLR stages is shown in Figure 3. The SCOD concentration change was mainly due to the decomposition of cellulose protein and fat in food waste to soluble organic compounds with a low molecular weight such as dimer and amino acids by cellulase and proteinase (Qiao *et al.* 2019; Xu *et al.* 2019). When the OLR was between 2.0 and 7.0 kg $m^{-3}$ $d^{-1}$ VS, SCOD concentration increased from 0.80 to 13.33 g/L. In the initial stage of anaerobic digestion, the growth and proliferation of microbes consumed most of the nutrients, which resulted in no apparent increase in SCOD concentration. When the OLR was increased to 6.0 kg $m^{-3}$ $d^{-1}$ VS, the average SCOD concentration increased to 8.40 g/L, almost three-folds increasing compared with that in the previous stage. The increase of organic matters effectively alleviates the hungry state of the hydrolytic acidifying bacteria and the methanogenic bacteria. The inhibition of the reactor with low efficiency was exempted. However, when the OLR was further increased to 7.0 kg $m^{-3}$ $d^{-1}$ VS, the average SCOD concentration reached 12.47 g/L. Too high concentration destroyed the operation stability of the anaerobic digestive reactor. In the late stage of the test, the decrease of methane production was dramatic, illustrating that the large increase of the SCOD concentration was due to not only the stronger hydrolytic acidification efficiency of the hydrolytic acid-producing bacteria with a high OLR but also the influenced activity of the methanogenic bacteria. The utilization efficiency of nutrition material was inhibited, resulting in not only poor methane production but also the simultaneous huge organic matter accumulation in the digestive liquor.

The ammonium nitrogen included $NH_4^+$ and free $NH_3$. The ammonia nitrogen was from the hydrolysis process of protein by bacteria in the digestive liquor (Xu *et al.* 2019). In the initial anaerobic digestion, the hydrolytic bacteria utilized the large molecule organic compounds in food waste and resulted in the gradual increase of ammonia nitrogen concentration. With the gradual increase of the activity of the methanogenic bacteria, the methanogenic bacteria utilized the ammonia nitrogen in the digestive liquor as the carbon source for its own growth. Thus, the ammonia nitrogen concentration in the digestive liquor gradually decreased. When the OLR increased to 6.0 kg $m^{-3}$ $d^{-1}$ VS, the ammonia nitrogen concentration gradually increased with the average concentration of 1.80 g/L. When the OLR increased to 7.0 kg $m^{-3}$ $d^{-1}$ VS, the accumulated concentration of ammonia nitrogen reached 3.16 g/L. Although the increase of ammonia nitrogen effectively increased the total alkalinity in the system, neutralized VFAs and thus maintained the internal pH stability of the digestive liquor, the low demand of microbes to ammonia nitrogen illustrated that too high concentration of ammonia nitrogen also inhibited the reactor operation. Duan *et al.* (2019) reported that ammonia poisoning occurred in the system to inhibit the activity of anaerobic microbes when the total ammonia nitrogen concentration exceeded 3.0 g/L.

### Influence of OLR regulation on TS and VS

TS and VS represented the change of organic and inorganic contents, respectively. The removal rate of TS and VS represented the organic substrate utilization rate by microbes and the metabolic ability of the acid-producing bacteria in the anaerobic digestive system (Braguglia *et al.* 2018). As shown in Figure 5, TS and VS concentrations in the reactor changed along with the increase of OLR following a positive correlation change trend. However, the removal rates exhibited the opposite change. When the OLR increased from 2.0 to 7.0 kg $m^{-3}$ $d^{-1}$ VS, TS and VS increased from 3.53 and 2.7% to 21.48 and 19.68%, respectively. However, TS and VS removal rates decreased from 91.58 and 93.45% to 47.93 and 52.23%, respectively.

During the reactor operation with a low OLR (4.0 kg $m^{-3}$ $d^{-1}$ VS), the removal rates of TS and VS in the reactor all maintained above 75%, illustrating the better degradation ability of food waste. When the OLR was further increased, the removal rates of TS and VS decreased to 63.16 and 64.66%, respectively. However, the anaerobic digestive system could keep running. When the OLR increased to 7.0 kg $m^{-3}$ $d^{-1}$ VS, the average removal rates of TS and VS were smaller than 60%, which reached the lowest level during the experiment. In this stage, the digestive liquor in the reactor changed gradually from brown to milky white. No matter how high the time and the frequency of the stirring device were increased, the changing into viscous state with the clear solid–liquid separation could not be improved. High organic matter concentration severely

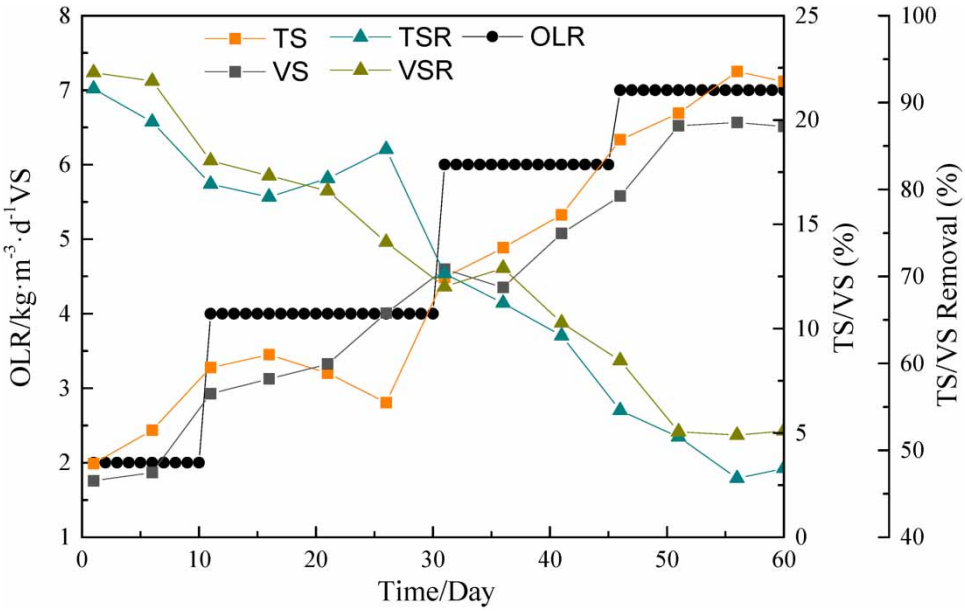



**Figure 5** | The relationship between TS and VS varies with the OLR.

destroyed the balance of anaerobic digestion. The operation of the digestive reaction failed. The above results showed that the degradation efficiency of raw material by microbes was high in the stage with a low OLR. With the continuous increase of OLR, organic compounds such as TVFA in the reactor rapidly accumulated, leading to the decrease of the removal rates of TS and VS. However, 6.0 kg $m^{-3}$ $d^{-1}$ VS was the OLR limit of the digestive reactor operation. In this stage, the removal rates of TS and VS in the reactor could still maintain stable. However, when it was increased to 7.0 kg $m^{-3}$ $d^{-1}$ VS, the excessive accumulation of organic matters in the reactor led to the accumulation of organic acid and ammonia nitrogen. The anaerobic digestive balance was destroyed, leading to the operation failure. In addition, in the late stage of the test, the material feeding and discharging time were decreased due to an increase of OLR in the reactor. The frequent emission of the bioliquid might result in the loss of microbes with the bioliquid, thus influencing the utilization rate of material.

### Influence of OLR regulation and temperature fluctuation on bacterial flora

Hydrolytic acidification was in the speed-limiting stage of the anaerobic digestive system, which was susceptible to the influence of the abundance and the activity of the bacterial community. The succession of microbial community could analyze the differentiated influence of OLR and the temperature change on the anaerobic digestive reaction from a microscopic angle. Stages of S1–S4 corresponded to microbial data on the last day in the stages with the OLR of 2.0–7.0 kg $m^{-3}$ $d^{-1}$ VS, respectively. Figure 6 shows a bacterial community change of three levels of 'phylum–class–genus'. *Firmicutes* dominated the whole process of the digestive reaction. In the initial stage of the anaerobic digestion with the OLR of 2.0 kg $m^{-3}$ $d^{-1}$ VS, the relative abundance of *Firmicutes* exceeded 75%, wherein the OLR change did not dramatically influence the bacterial structure at the phylum level. Only when the OLR increased to 4.0 and 6.0 kg $m^{-3}$ $d^{-1}$ VS, the relative abundance of *Actinobacteria* and *Bacteroidetes* increased to some extent with the increase of the concentration of digestive substrates. It showed that *Firmicutes* could widely utilize the digestive substrate. It could also secrete many hydrolytic enzymes such as proteinase to increase the degradation rate of large molecule organic materials in food waste. It was also responsible for the generation of VFA. Combined with hydrogenotrophic methanogen, it led to the occurrence of syntrophic joint reaction and played a key role in the promotion of methane production in the reactor. Thus, it was the dominant hydrolytic acid-producing bacteria in the anaerobic digestion (Zhou *et al.* 2016; Kurade *et al.* 2020). However, *Actinobacteria* and *Bacteroidetes* could increase VFAs in the digestive liquor by the degradation of protein, lipid and complex organic compounds (Mahdy *et al.* 2020). Meanwhile, there kept a positive correlation between those and Methanobacteriaceae and Methanosarcinaceae (Ohnishi *et al.* 2011). Like the abundance change of Firmicutes, the relative abundance of *Proteobacteria* in the initial stage was relatively high. However, the addition of more digestive substrates resulted in the decrease of the relative abundance to 5%, illustrating that *Proteobacteria* could express activity under the condition with a low OLR, compared with that with a high OLR. Mahdy

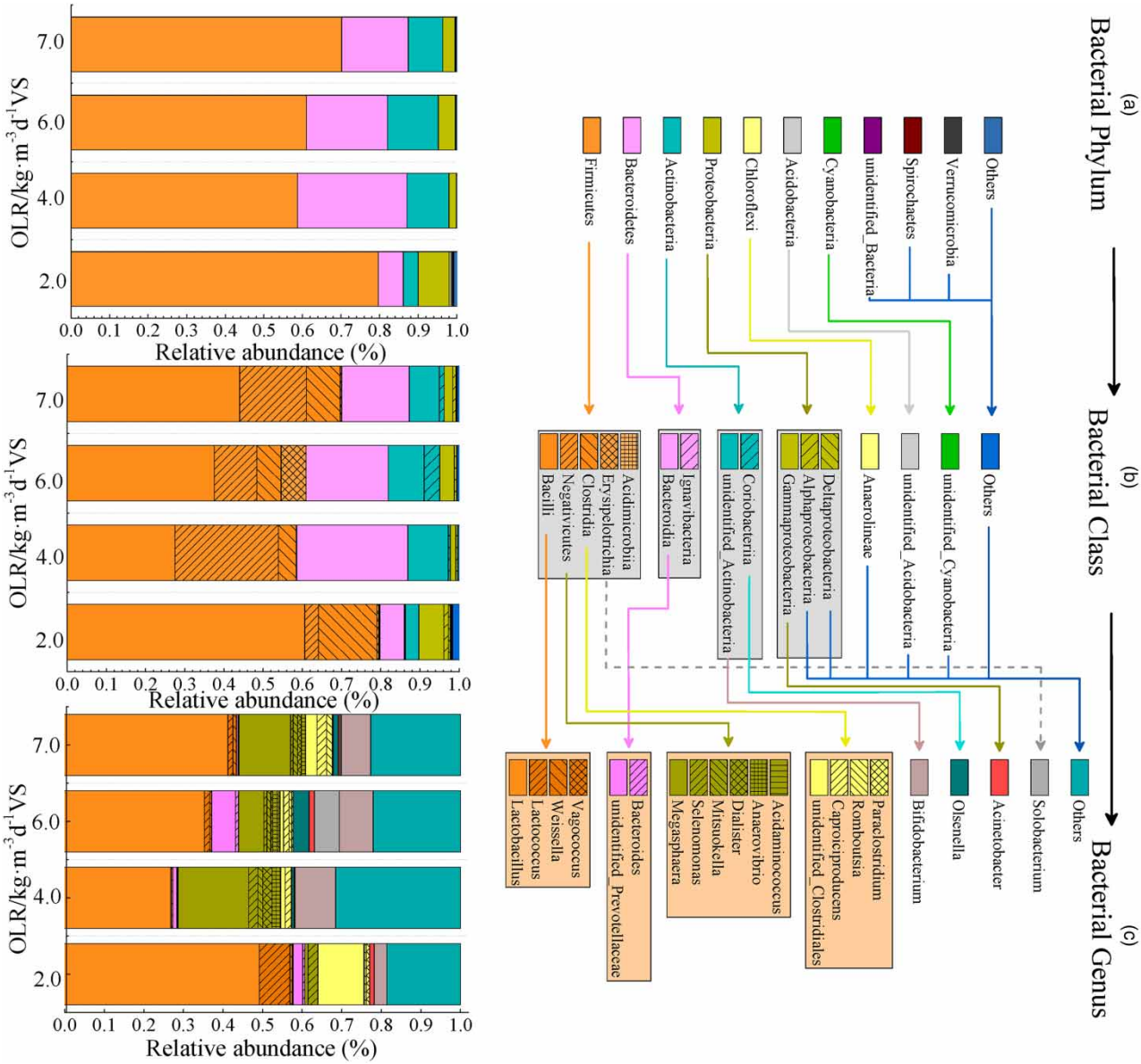



**Figure 6** | The relationship of bacteria with the OLR.

(Liu 2016) obtained the same conclusion during the OLR influence investigation about the bacterial community change in the anaerobic digestive process with chicken manure. In addition, temperature fluctuations have little effect on the microbial community structure. However, it is more likely to affect the activity of microorganisms. According to previous studies on the temperature of anaerobic digestion, microorganisms are obviously more active at a stable temperature.

Although the relative abundance of Firmicutes phylum did not change dramatically, bacteria demonstrated the significant differentiation at the level of class of Firmicutes phylum, wherein the relative abundance of *Clostridia* and *Negativicutes* demonstrated an opposite change trend. When the OLR was 2.0 kg $m^{-3}$ $d^{-1}$ VS, the relative abundance of *Clostridia* was 14.94%, which was the maintained highest relative abundance. However, the relative abundance of *Negativicutes* was 3.46%, whose activity expression was poor. However, with the continuous increase of OLR, the relative abundance of *Clostridia* class decreased 8.76% (6.0 kg $m^{-3}$ $d^{-1}$ VS). In this stage, the relative abundance of *Negativicutes* increased by 7.38%. The relative abundance of *Clostridia* was mainly influenced by the change of *Clostridiales*, which was the important cellulose degradation bacterium in the anaerobic digestive system with food waste. It could not only produce compounds

such as ethanol, butanol and acetone based on the cellulose of cell wall but also be the important producer of $H_2$, butanoic acid and acetic acid in the anaerobic digestive system (Xu *et al.* 2019). The corresponding activity was high in the stage with a low OLR, possibly due to the unbalance between small food addition in the initial stage and the nutrition required by the growth and proliferation of microbes. Easily degradable compounds such as protein, starch and sugar were totally consumed. Subsequently, cellulose and semicellulose degradation were accelerated, providing sufficient nutrition for the stability of microbial community. With the increase of OLR, the supplement of easily degradable organic compounds alleviated the hungry state of microbe. At that time, the hard degradable ability of cellulose dwarfed the dominant role of *Clostridiales*, significantly. However, the relative abundance of *Megasphaera* of *Negativicutes* class increased from 7.54 to 17.89% when the OLR increased from 2.0 to 4.0 kg $m^{-3}$ $d^{-1}$ VS. *Megasphaera* could utilize the organic acid with low molecular weight, which mainly included butanoic acid based on lactic acid. Common lactic acid-producing bacteria such as *Lactococcus* and *Lactobacillus* were the main producer of lactic acid in the digestive liquor. They dominated in the stage of 2.0 kg $m^{-3}$ $d^{-1}$ VS whose relative abundance reached 56.9%, illustrating that the lactic acid accumulation from the utilization of food waste by the lactic acid-producing bacteria provided abundant nutrition for *Megasphaera*, whose activity was activated in the stage of 4.0 kg $m^{-3}$ $d^{-1}$ VS. During the whole test, *Lactobacillus* maintained its relative abundance above 29%. Although *Megasphaera* could generate butanoic acid utilizing lactic acid, which effectively alleviated the lactic acid inhibition, the too low active expressive could not maintain the dynamic balance with *Lactobabacillus*. Considering the poisoning influence of lactic acid on the methangenoic bacteria, in the late test, the severe acidification phenomenon in the reactor with a high OLR was possibly due to the lactic acid accumulation induced by the concentrating of *Lactobacillus*.

In addition, when the OLR was increased to 6.0 kg $m^{-3}$ $d^{-1}$ VS, the relative abundance of *Solobacterium* in two reactors increased suddenly. It had a significant function in the generation of $H_2$ during the electrolysis of the residual sludge digestive liquor in the reactor (Minchin 1987). The relative abundance change of archaea in Figure 4 illustrated that the methanogenic bacterium was mainly hydrogenotrophic methanogen when the OLR was 6.0 kg $m^{-3}$ $d^{-1}$ VS, illustrating that *Solobacterium* was easy to be concentrated during the period with a high OLR. Co-cultivation with hydrogenotrophic methanogen was also generated, providing $H_2$ for the digestive system and promoting the succession from aceticlastic to hydrogenotrophic methanogen in the reactive system. Although the relative abundance of *Acinetobacter* was low, it was expressed in every period with different OLRs. *Acinetobacter*, which was aerobic bacteria that existed in the reactor, verified the mixture of oxygen. It might possibly be due to the frequent addition of food waste in the late period of the experiment, resulting in the mixture of $O_2$ and raw materials which could not be removed. A stable temperature may allow the fermentation system to have good resistance to high OLR shocks, but it also cannot withstand excessive OLR in a long term.

### Influence of OLR regulation on archaea flora

When analyzing the relative abundance of 'phylum–class-level' of archaea, it was found out that the structural changing trend of archaea community was similar to that of bacteria. Namely, it exhibited high functional centrality and poor species diversity with a low OLR. With the gradual increase of OLR, the community diversity gradually became rich. In the initial stage of the test, the relative abundance of Euryarchaeota in two reactors all exceeded 97%. However, with the gradual increase of OLR, the relative abundance of the bacteria of *Firmicutes* in the determination of archaea gradually increased. Even in the optimum stage of methane production, the relative abundance of *Firmicutes* was as high as 37%, illustrating that the excessive proliferation of acid-producing flora in the interior of the digestive system had a significant influence on the stability of microbial community in the reactor. Although alkaline solution was added for artificial regulation, the rancidity phenomenon could not be changed.

The relative abundance of *Methanosaeta* was about 90% which predominated in the stage with an OLR of 2.0 kg $m^{-3}$ $d^{-1}$ VS. *Methanosaeta* was an obligate aceticlastic methanogen, which could produce $CH_4$ and $CO_2$ by the utilization of acetic acid in the digestive liquor when the OLR increased to 4.0 kg $m^{-3}$ $d^{-1}$ VS. The relative abundance of *Methanosaeta* sharply decreased 52.1%. At this time, the relative abundance of *Methanoregula* and *Methanospirillum* gradually increased. They even increased 8.02 and 13.1% in the stage with an OLR of 6.0 kg $m^{-3}$ $d^{-1}$ VS, respectively, compared with those in the initial stage. They were substituted for *Methanosaeta* to be the predominant methanogen. Both *Methanoregula* and *Methanospirillum* belonged to hydrogenotrophic methanogen, which mainly utilized $H_2$ and $CO_2$ and produced methane (Müller *et al.* 2016; Li *et al.* 2020). The above results illustrated that aceticlastic methanogen was easy to express high activity with the low OLR. With the increase of OLR, the increase of $H_2$ and $CO_2$ concentrations elevated the proliferating velocity of hydrogenotrophic methanogen and thus led to the succession of the methanogen category.

During the whole process of the test, with the increase of OLR, the relative abundance ratio of others increased gradually, illustrating that the increase of the feeding amount of the digestive system had a significant promotion effect on the increase of bacteria abundance. In addition, the change of OLR was the main factor of community succession.

### Influence of OLR regulation on alpha diversity of bacteria/archaea

An alpha diversity analysis index with a 97% consistency threshold of different samples is shown in Figure 7. The index change of Chao1 and Ace could feedback the changing status of community abundance in the digestive liquor. The larger the value, the higher the abundance of microbial community.

When maintaining the OLR lower than 6.0 kg $m^{-3}$ $d^{-1}$ VS in the digestive reactor, bacterial indexes of Chao1 and Ace demonstrated increasing trends with the increase of OLR. Especially when the OLR increased from 4.0 to 6.0 kg $m^{-3}$ $d^{-1}$ VS, the index increased more than two-folds. However, with the further increase of OLR to 7.0 kg $m^{-3}$ $d^{-1}$ VS, the community abundance decreased with a large amplitude. Combined with Figure 6, it illustrated that the increase of nutritional material supply could effectively promote the growth and the proliferation of microbial community and thus increase the community abundance. However, in the stage with a low OLR, nutritional material could be centralizedly utilized by the specific bacteria, resulting in the hydrolytic acidification process, which could only be finished by a small part of obligate bacteria. The community structure demonstrated high centrality and low abundance. The index decrease of Chao1 and Ace in the stage with the OLR of 7.0 kg $m^{-3}$ $d^{-1}$ VS was possibly due to the loss of flora with the bioliquid because of the frequent discharging in the late test.

Compared with the change of bacterial alpha diversity, methane-producing archaea demonstrated a totally different change. In the whole process of the digestive reaction, the average levels of Ace and Chao1 indexes were lower than

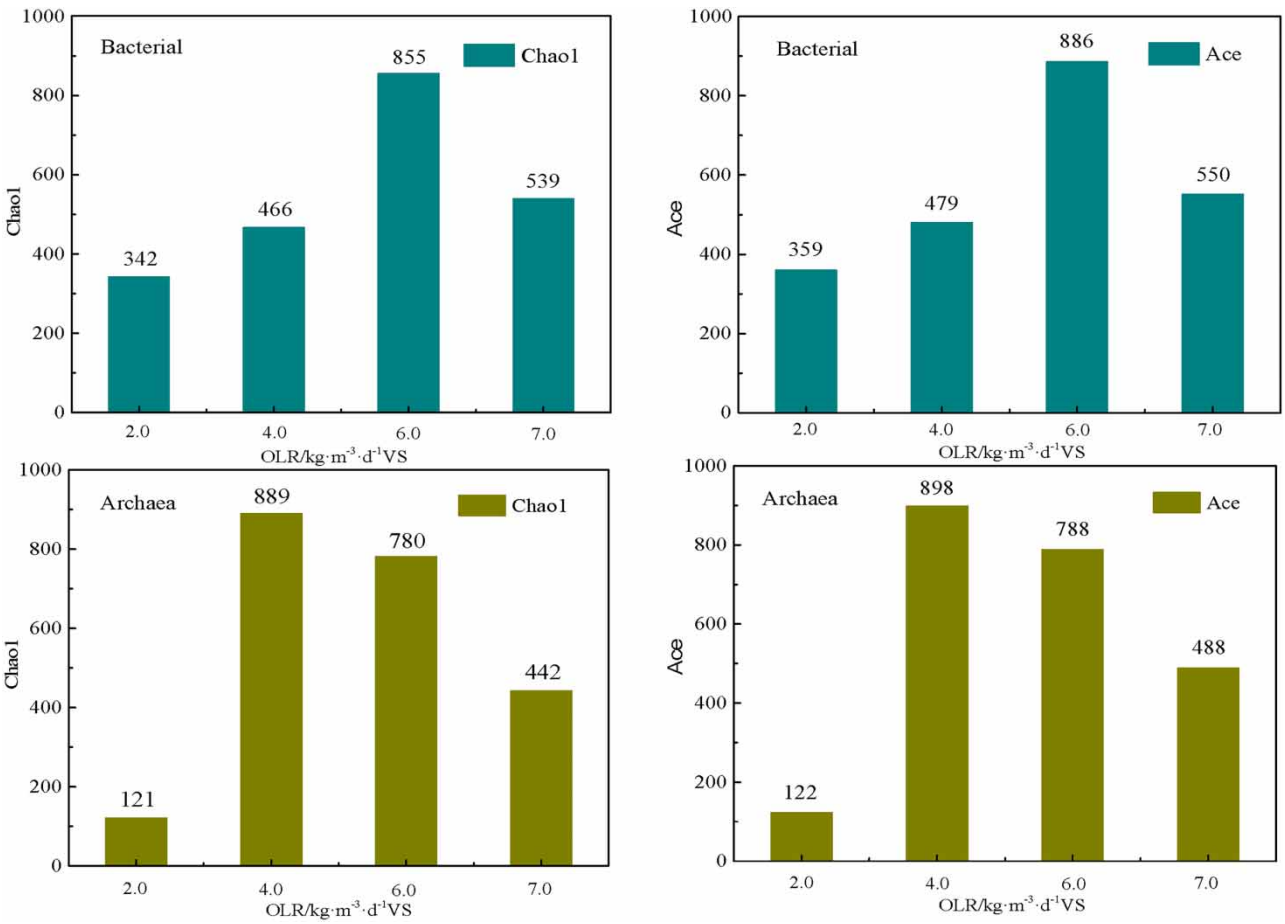



**Figure 7** | Changes of microbial community alpha diversity index with the OLR.

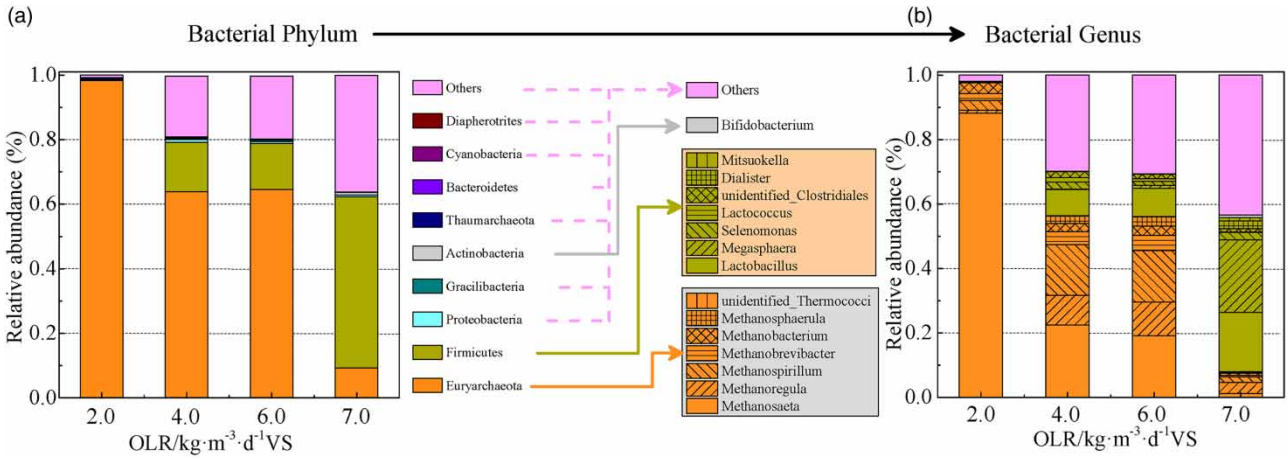



**Figure 8** | The relationship of archaea with the OLR.

those of bacteria. In the stage with the OLR of 4.0 kg $m^{-3}$ $d^{-1}$ VS, the Chao1 index reached 889.39. When the OLR was increased to 6.0 kg $m^{-3}$ $d^{-1}$ VS, the Chao1 index was 13.93% or so, which also occurred in the change of Ace index. Diversity in the stage with the OLR of 6.0 kg $m^{-3}$ $d^{-1}$ VS was lower than that of 4.0 kg $m^{-3}$ $d^{-1}$ VS, possibly due to the organic acid accumulation, which has occurred in the reactor in this stage. Although the pH stability was maintained by the regulation of artificial alkaline solution, a part of methanogen which had a low tolerance to the environmental change could not bear the sharp pH decreasing in a short time and lost activity. Figure 8 shows that a lot of bacteria were mixed in the change of archaea community, illustrating that acid accumulation resulted in dramatic inhibition.

## CONCLUSIONS

1. The OLR limit of 6.0 kg $m^{-3}$ $d^{-1}$ VS in the anaerobic digestion with food waste realized the optimum methane production. During this stage, organic acid accumulation in the reactor resulted in pH fluctuation with a large amplitude. The stable operation of the reactor could be recovered by the adjustment of the artificial alkaline solution.
2. The reactor could not bear the OLR shock of 7.0 kg $m^{-3}$ $d^{-1}$ VS. Both organic acid accumulation and ammonia inhibition occurred simultaneously. The unbalance of the digestive reaction process resulted in the operation failure.
3. Microbes exhibited high functional centrality and poor community richness under the condition of low OLR. Increasing OLR could promote microbial abundance. The relative abundance of *Methanosaeta*, which belonged to acetic acid methanogenic bacteria, was relatively large in the stage with a low OLR. However, *Methanoregula* and *Methanospirillum*, which belonged to hydrotrophic methanogenic bacteria, exhibited high activities in the stage with a high OLR.
4. When the OLR was too large, the excessive proliferation of acid-producing flora in the interior of the digestive system had a significant influence on the stability of the archaea community.

Based on the above results, it is not difficult to see that the main factor affecting anaerobic digestion is OLR. However, temperature fluctuation has a certain impact on microbial activity and AD system shock resistance. In the long run, the use of solar-assisted anaerobic digestion in the summer period is completely feasible as long as the dosage of the substrate is well controlled and calculated. As for the cold environment, solar energy and electric heating can be considered for anaerobic digestion, which can save energy consumption to a certain extent while ensuring the fermentation environment.

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## AUTHOR CONTRIBUTIONS

X.Z. contributed to the conception of the study and performed the data analyses and wrote the manuscript; M.L. contributed significantly to analysis and manuscript preparation; H.D. helped perform the analysis with constructive discussions; L.F. performed the experiment; W.W. helped perform the analysis with constructive discussions; L.F. performed the experiment; Q.Y. contributed significantly to analysis and manuscript preparation.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information. All data generated or analysed during this study are included in this published article.

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