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RESEARCH ARTICLE

Enhancing nitrogen removal from wastewater in sequencing batch reactors (SBRs) using additional carbon source produced from food waste acidogenic fermentation at different temperatures

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Abstract

Fermentation slurry from food waste (FSFW) produced at different temperatures (20, 37, and 55 °C) was utilized as external carbon source for promoting nitrogen removal in this study. It was found that high temperatures improved the hydrolysis rate by promoting the hydrolytic enzyme activity. Mesophilic temperature (37 °C) was favorable for organic acid (especially lactic acid) production by selectively enriching the *Lactobacillus* (with a relative abundance of 90.6%), while thermophilic temperature (55 °C) would restrict the acidogenesis rate (18.9%) and result in the accumulation of carbohydrate in the fermented slurry. Organic acids in the FSFW act as easily biodegradable carbon sources, but the macromolecular and particulate organic components can be utilized as slowly biodegradable carbon sources in the denitrification processes. Using the FSFW as carbon sources to enhance nitrogen removal from wastewater in sequencing batch reactors (SBRs) for more than 150 days, the FSFW produced at thermophilic temperature could significantly promote the microbial metabolic capacity of the activated sludge and improve the nitrogen and phosphate removal efficiencies.

Keywords Fermentation slurry from food waste (FSFW) · Organic acid · Denitrification · Microbial community · Metabolic capacity

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Introduction

With the rapid development of cities, more and more food waste (FW) is discharged and has become one of the main organic municipal solid wastes (OMSW) (Braguglia et al. 2018; Nayak and Bhushan 2019). It was reported that approximately 1.3 billion metric tons of FW was generated globally every year (Braguglia et al. 2018; Kwan et al. 2018), and the amount is continuously increasing (Nayak and Bhushan 2019; Xu et al. 2018). Owing to its high content of carbohydrates, proteins, and lipids, FW easily contaminates the air, water, and soil (Zhou et al. 2018). Some traditional methods such as feeding animals, composting, and landfilling are still used to dispose the FW, but these methods may cause serious environmental pollution and health risks (Mehariya et al. 2018; Xu et al. 2018). Hence, looking for the high-efficient and environment-sustainable methods for FW dispose becomes urgent and necessary.

Owing to the less space and energy requirement for recycling the solid organic wastes (Kibler et al. 2018; Zhou et al. 2018), anaerobic digestion (AD) has received more and

more attention in recent years (Braguglia et al. 2018; Kwan et al. 2018). During the AD processes, intermediate organics (e.g., volatile fatty acids (VFAs), lactic acid (LA), and ethanol), biogas (e.g., H_2 and methane gas), and nutrients (nitrogen and phosphate) could be recycled (Luo et al. 2019; Mehariya et al. 2018; Tang et al. 2016; Zhou et al. 2018). However, the rapid acidification would result in the accumulation of organic acids and further restrict the methanogenesis processes (Jiang et al. 2018; Kim et al. 2019). Thus, organic acid production through acidogenic fermentation has recently become a consensus for FW recycling (Kwan et al. 2018; Tang et al. 2016; Zhou et al. 2018). However, owing to the complicated components in FW, it is usually difficult to generate and recycle high-purity organic acids from the fermentation slurry (Hu et al. 2017). Therefore, determining a proper way to reuse the fermented slurry is essential to food waste management.

On the other hand, due to the shortages of biodegradable organics in influent, nutrient (nitrogen and phosphate) removal efficiencies from the wastewater treatment plants (WWTPs) are always unsatisfied (Tang et al. 2017a, 2019). Chemical carbon sources (e.g., acetate, methanol, starch) have been utilized to enhance the denitrification (Pelaz et al. 2018; Tang et al. 2018). However, these chemicals are often unaffordable, which limits their practical application. Thus, using organic wastes (e.g., excess sludge, rice straw, and FW) as supplementary carbon sources to treat the wastewater has become a hot research topic in recent years (Luo et al. 2018, 2019; Tang et al. 2019). In the previous studies, organics in FW fermentation slurry have been successfully utilized as alternative carbon sources to promote nitrogen removal rate from wastewater treatment systems (Tang et al. 2019; Zhang et al. 2016a). It was found that organic acids and other micro-molecular organic matter in the FSFW could be utilized as easily biodegradable carbon sources and increase the microbial diversity (Luo et al. 2019; Tang et al. 2019). The FSFW prepared by thermophilic fermentation was applied in a pilot-scale membrane bioreactor (MBR) to improve the nitrogen removal rate from domestic wastewater, and it was found that the FSFW could significantly reduce the total nitrogen content in effluent and did not cause serious membrane fouling during the practical application (Tang et al. 2017a). Thus, using the FSFW as carbon source for enhancing nutrient removal could be a feasible solution for both organic solid waste (FW) dispose and wastewater treatment. While how to legitimately combine these two parts and optimize the solution should be further considered.

Different organic components in the FSFW would exhibit different nutrient removal performance, due to their effect on microbial metabolism and community diversity (Tang et al. 2019). Fermentation products in the FSFW are significantly influenced by the operational parameters such as pH, temperature, and organic loading rate (Tang et al. 2016). Temperature as one of the important factors for anaerobic fermentation directly affects the microbial communities and bacterial enzyme activities in the fermenter, resulting in different end products at different temperatures (Tang et al. 2016). However, the effect of temperature on carbon source production from food waste during the continuous operation has not been discussed. The information on nitrogen removal performance and activated sludge properties using the FSFW produced at different temperatures was seldom reported.

Therefore, in this study, the characteristics of carbon source production from food waste acidogenic fermentation at various temperatures were firstly observed. Then, the nutrient removal efficiencies and sludge properties using the fermentation slurry were investigated to further optimize the combined route of solid waste disposal and wastewater treatment.

Materials and methods

Food waste substrate

The fresh FW, mainly consisted of rice, noodles, vegetables, and meat, was collected from the student canteen in Chengdu University every 3 days. After sorting out animal bones, tissues, and plastic bags, the FW was crushed using an electrical blender. Thereafter, the FW slurry was sieved (1 mm) and stored in the refrigerator (4 °C). Before adding the FW slurry into the fermenters, the total solid (TS) content was adjusted to approximately 6 ± 0.5% with tap water. The characteristics of fresh FW slurry are shown in Table 1.

Carbon source production at different temperatures

To investigate the effect of temperatures on carbon source production from FW fermentation in the continuous operation, two continuous stirring tank reactors (CSTRs), each with a working volume of 5 L, were conducted at 37 °C (CSTR-37) and 55 °C (CSTR-55), respectively (Fig. S1, Supporting

Table 1 Characteristics of fresh food waste slurry

Parameter	Unit	Value
pH	–	4.9 ± 0.2
SS	%	6 ± 0.5
VS/TS	–	0.92 ± 0.2
COD	g/L	68.6 ± 7.6
SCOD	g/L	28.8 ± 2.3
Carbohydrate	g/L	40.5 ± 2.7
Protein	g/L	8.1 ± 1.6
Lactic acid	g/L	2.9 ± 0.7
VFAs	g/L	0.7 ± 0.3
NH_4^+-N	mg/L	167.6 ± 13.1
TN	g/L	1.6 ± 0.5
TP	g/L	0.2 ± 0.1

information). At the beginning of the fermentation, 5 L of fresh FW slurry was added into each reactor. Temperatures in the CSTRs were controlled using water bath. Another identical fermentor (CSTR-20) operated at ambient temperature (around 20 °C) was conducted as a control test. To simplify the operation procedure, all reactors were operated without pH adjustment. One liter of the fermented slurry was drained from the reactors everyday and replaced with the same volume of fresh FW slurry (TS = 6%) to ensure a solid retention time (SRT) of 5 days.

Microbial community analysis

To explore the shifts of microbial communities at various temperatures, the raw fresh FW (raw FW) and fermented slurry samples (FSFW-20, FSFW-37, and FSFW-55) from the three CSTRs were obtained for DNA extraction and analysis using the next-generation sequencing techniques. The DNA was amplified by polymerase chain reaction (PCR) using the primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) for the V1–V3 region of the 16S rRNA genes as it was described in the previous study (Tang et al. 2016). The gene amplicon sequencing was then conducted with an Illumina MiSeq platform. The homologous or ambiguous sequences or the sequences with a length shorter than 200 bp were trimmed to obtain high-quality sequences with an average length larger than 500 bp for the taxonomic classification. By comparing to the 16S rRNA sequences in Miseq PE database, taxonomic classification was carried out to assign the optimized sequences into operational taxonomic units (OTUs) at a confidence threshold of 97%.

Nitrogen uptake rate (NUR) tests

To reveal the denitrification properties of the FSFW produced at different temperatures, a series of NUR tests were conducted according to the previous studies (Tang et al. 2017a; Zhang et al. 2016b; Sage et al. 2006). Firstly, the activated sludge was sampled from a sequencing batch reactor (SBR) which was used for wastewater treatment with FSFW as external carbon source for more than 150 days (Tang et al. 2019). After settling for 30 min, supernatant of the activated sludge was discharged. The residual nitrogen compounds and organics in the sludge flocs were then eliminated by washing the sludge with pure water several times. Thereafter, the pretreated activated sludge was added into three flasks and diluted with tap water to make up a volume of 2 L and a mixed liquor volatile suspended solid (MLVSS) content of approximately 3 g/L. Oxygen in mixed liquor was removed by flushing the flasks with nitrogen gas (N_2). Thereafter, sodium nitrate ($NaNO_3$) was added into each flask to generate a final NO_3^--N content of approximately 50 mg/L. The FSFW from the three fermentors (FSFW-20, FSFW-37, and FSFW-55) were then added into the flasks to ensure a chemical oxygen demand (COD) content of approximately 300 mg/L. A control test was simultaneously conducted with NO_3^--N (50 mg/L) but no additional carbon source. The flasks were then sealed, and the mixed liquor was agitated with stirrers. Ten microliters of the mixed liquor was periodically sampled from each flask to analyze the variations of NO_3^--N and NO_2^--N contents. The specific denitrification rate (SDNR) was calculated based on the methods in the previous study (Sage et al. 2006).

Wastewater treatment using the FSFW

To explore the effects of FSFW on nitrogen removal performance during the long-term operation, three lab-scale (each with a working volume of 5 L) sequencing batch reactors (SBRs) were operated using the FSFW-20, FSFW-37, and FSFW-55 as supplementary organic carbon sources, respectively. The schematics and operation sequences of the SBRs are described in the supplementary information (Fig. S2). The inoculated sludge was obtained from the aerobic tank of the pilot-scale membrane bioreactor described in our previous study (Tang et al. 2017a). The FSFW, ammonium salt, and phosphate were added in the influent of the SBRs to adjust the content of chemical oxygen demand (COD), NH_4^+-N and PO_4^{3-}-P to 300–500 mg/L, approximately 50 mg/L and 4–6 mg/L respectively during the operation. The hydrolytic retention time (HRT) was 10 h, and the sludge retention time (SRT) of the SBRs maintained at approximately 30 days by discharging the excess sludge every day. The mixed-liquid suspended solids (MLSS) in the reactors maintained between 3 and 5 g/L with a mixed-liquid volatile suspended solids (MLVSS) to MLSS ratio of 0.7–0.8.

Bacterial metabolic capacity analysis

To analyze the effect of FSFW addition on the bacterial metabolisms, the metabolic characteristics of microorganisms in activated sludge from the three SBRs were tested using the Biolog-ECO plates (Biolog, Inc., Hayward, CA, USA) according to the previous studies (Tang et al. 2018, 2019). Firstly, the raw sludge and activated sludge from the three SBRs were immediately diluted to 1:1000 and washed several times with sterilized NaCl solution (0.9%, w/v). Secondly, 1 mL of the resulting suspension was diluted to achieve an optical density (OD) close to 0.05 at 600 nm with the saline solution to ensure that sample solutions contained approximately the same biomass concentration. Thirdly, 150 μL of the diluted mixture was added to the Biolog plate well using the eight channel pipettes. Thereafter, the ECO plates were inoculated at 25 °C in darkness. The absorbance at 590 nm (OD 590 nm) and 750 nm (OD 750 nm) of the wells was recorded using an ELISA plate reader every 24 h. The data

analysis processes were according to the previous studies (Tang et al. 2017a, 2018).

Analytical methods

The organic components (chemical oxygen demand (COD), carbohydrate, protein, lactic acid (LA), and volatile fatty acids (VFAs)) in the fermented slurry were analyzed according to the methods described in our previous study (Tang et al. 2016). pH value in the fermentors was measured everyday using the pH meter (PHS-3C, China). The α-glucosidase activity was analyzed according to the previous study (Tang et al. 2017b). Samples from the influent, effluent, and mixed liquor of the SBRs were periodically obtained, and the nitrogen compounds (NH_4^+-N, NO_3^--N, NO_2^--N, and total nitrogen (TN)), COD, PO_4^{3-}-P, MLSS, MLVSS, biomass yield, and sludge volume index (SVI) were measured according to the standard methods (APHA 2012).

Results and discussion

Effect of temperatures on carbon source production from FW fermentation

Variations of soluble organics during the fermentation

Variations of soluble organics during the FW fermentation are shown in Fig. 1. SCOD in all reactors gradually increased during the first 10 days, indicating an obvious hydrolysis during this period (Fig. 1a). In the reactor at 20 °C, SCOD gradually increased from 28.3 to 33.6 g/L and then fluctuated between 30 and 35 g/L, while higher SCOD content was observed at higher temperatures (35–45 g/L at 37 °C and 42–50 g/L at 55 °C). It was reported that higher temperature was favorable for enriching functional bacteria, keeping higher hydrolytic bacterial enzyme activity, and further promoting the hydrolysis rate (Kim et al. 2003; Tang et al. 2016), which can be explained by the variations of hydrolytic enzyme activity in Fig. 1f. The highest hydrolytic enzyme activity was detected in the reactor at 55 °C, while the lowest value was found in the reactor at 20 °C. It is well-known that hydrolysis is the rate-limiting step in anaerobic FW fermentation (Tang et al. 2016). The stronger hydrolysis at mesophilic and thermophilic temperatures indicated the higher substrate transformation rate from particulate into soluble organics.

Carbohydrates, accounting for approximately 60% of organic content in the fresh FW, are the main substrates for acid production. Soluble carbohydrate in the fermentation slurry was produced from the substrate hydrolysis and utilized by the acidogenic bacteria in the fermentor; thus, its concentration in the fermented slurry was determined by the rate of hydrolysis and acidification (Tang et al. 2016). In the reactor at 20 °C, due to the suitable pH in the slurry and higher bacterial activity, the concentration of soluble carbohydrate gradually decreased. After 25 days (Fig. 1b), soluble carbohydrate achieved the steady state, indicating that the hydrolysis rate was almost equal to the acidogenesis rate. However, in the reactor at 37 °C, soluble carbohydrates sharply decreased from 19.4 to 4.5 g/L in the first 13 days and finally stabilized at approximately 6.3 g/L, showing a higher degradation rate than that at 20 °C. However, in the reactor at 55 °C, soluble carbohydrate gradually accumulated and increased to 25–28 g/L, indicating a higher hydrolysis rate than acidification rate, which is consistent with the results in our previous study (Tang et al. 2016) and might result from two causes. First, due to the enhanced hydrolysis processes, more particulate carbohydrates were transformed into a soluble form. Second, the activity of acidogenic bacteria was inhibited by the thermophilic temperature and the utilization rate of carbohydrate decreased.

LA was the main acidogenic product in all reactors. In the reactor at 37 °C, LA concentration gradually increased to 18.7 g/L, and maintained stable thereafter (Fig. 1c), showing a high LA yield and productivity during the acidogenesis process. Although high concentrations of residual soluble carbohydrates were detected in the fermented slurry (Fig. 1b), no more LA was produced, which was probably attributable to the inhibition of bacteria by acids or low pH in the reactor (pH value was around 3.3) (Fig. 1e). In the reactors at 20 °C and 55 °C, LA content slightly increased and approached to around 11 g/L and 10 g/L respectively. The low LA yield at 55 °C mainly resulted from the inhibition of bacterial activity by high temperatures, which is consistent with the findings in our previous study (Tang et al. 2016).

VFAs in the fermented slurry were also significantly influenced by the fermentation temperatures (Fig. 1d). In the reactor at 37 °C, the content of VFAs sharply increased from 1.4 to 6.0 g/L in the first 6 days, which was owing to the higher pH value and bacterial activity during this period. After that, the VFAs slowly increased and finally maintained at approximately 7 g/L. However, VFAs in the reactor at 55 °C remained almost constant and were much lower than those at mesophilic temperature during the whole fermentation period, which further testified the negative impact of high temperature on acidification process.

Variations in pH value during fermentation further explained the differences in hydrolysis and acidification processes (Fig. 1e). Accompanied with the accumulation of organic acids in the fermentors, the pH gradually decreased and stabilized at 3.3 and 3.8 at 37 °C and 55 °C respectively, which in turn inhibited the hydrolysis and acidification processes. However, the restriction was relieved by replacing the fermented slurry with fresh FW slurry every day, finally resulting in the stable organic acid content in the reactors. The activity of α-glucosidase in reactor at 20 °C was 19.4 U/L, while it increased to 27.7 U/L and 32.6 U/L in the

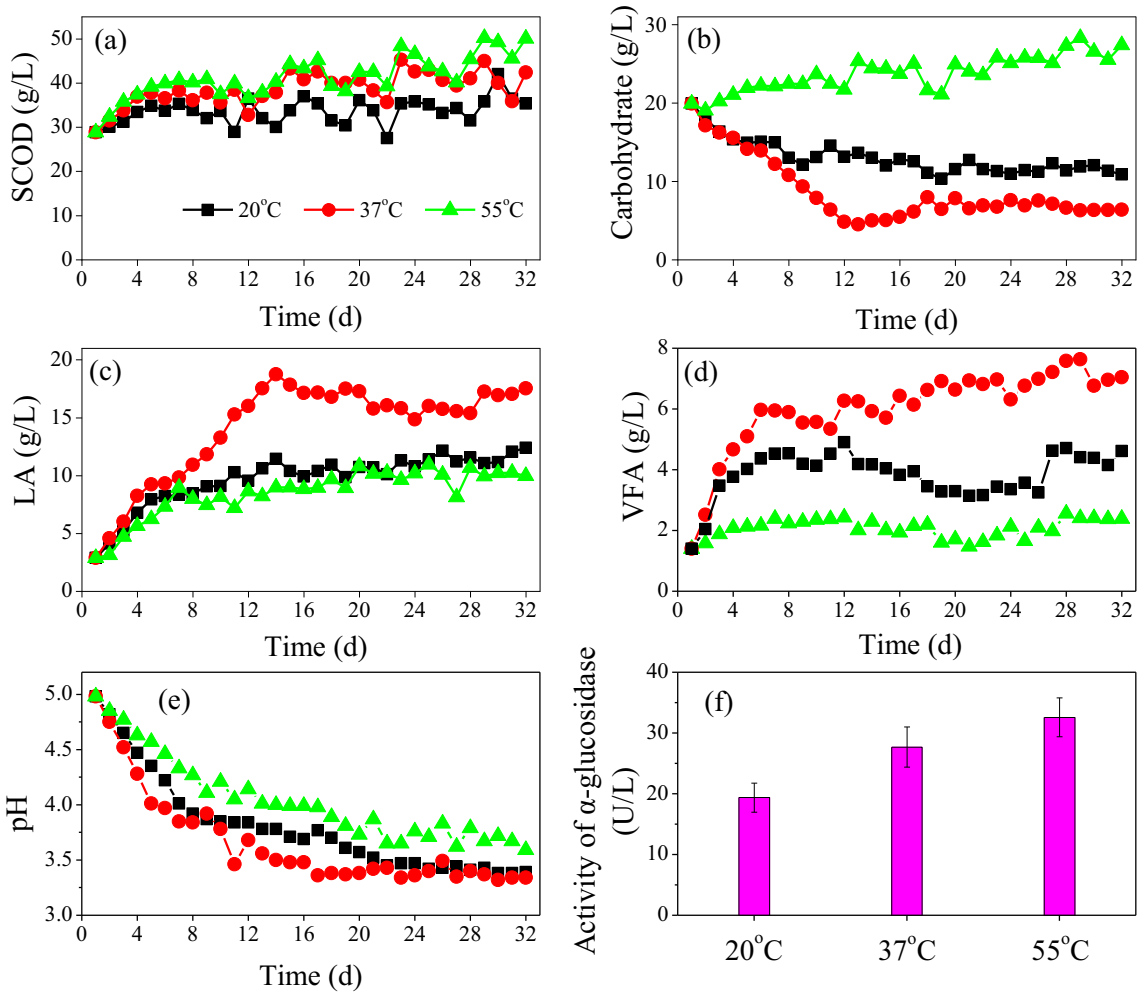

Fig. 1 Variations of SCOD (**a**), soluble carbohydrate (**b**), lactic acid (**c**), VFAs (**d**), pH (**e**), and activity of α-glucosidase (**f**) during the fermentation at different temperatures

reactor at 37 °C and 55 °C, respectively (Fig. 1f), which further explained the variations of SCOD content in the reactors (Fig. 1a).

Temperatures showed significant effect on the LA yield (Table 2). It was found that the LA yield at 20 °C was around 0.17 g/g-TS which was lower than that at 37 °C (0.27 g/g-TS) and a bit higher than that at 55 °C (0.14 g/g-TS). The higher LA yield and productivity under mesophilic condition were probably attributed to the higher microbial activity and richness of LAB in the slurry (Tang et al. 2016). Although thermophilic temperature was beneficial for hydrolysis, the LA yield was much lower than that in other reactors, demonstrating the inhibition of acidification by high temperatures.

The hydrolysis rate at 55 °C was approximately 37.6% (Table 2), which is much higher than that at 20 °C (13.9%) and 37 °C (27.8%). It was reported that higher temperature was beneficial for promoting the activities of hydrolytic enzymes. The reason was presumably owing to the accelerated growth rate and lower substrate affinity of some thermophilic bacteria (Kim et al. 2002). Moreover, the thermophilic bacteria

Table 2 Organic transformation rate at different temperatures

Temperature °C	SCOD g/L	Carbohydrate	LA	VFAs	Hydrolysis rate %	Acidogenesis rate	LA yield g/g-VS	LA productivity g/h
20	34.77	11.57	11.27	3.85	13.9	34.8	0.17	0.35
37	40.73	6.86	16.24	6.97	27.8	48.4	0.27	0.56
55	45.09	25.76	10.09	2.05	37.6	18.9	0.14	0.30

exhibit higher activity than mesophilic bacteria (Kim et al. 2003), which also contributed to the hydrolysis. However, the highest acidogenesis rate (48.4%) was found in the reactor at 37 °C, indicating that most of the soluble carbohydrates or substrates were utilized by the acidogenic bacteria to produce organic acids. The acidogenesis rate at 55 °C was the lowest (18.9%), even lower than that in the reactor at 20 °C (34.8%), which was mainly related to the effect of temperature on the activities of microbial enzymes of acidogenic bacteria and further proved the lower content of acids in the fermentation slurry (Kim et al. 2003; Tang et al. 2016).

Organic components in the fermentation slurry

Total COD (TCOD) content in the fermentation slurry was lower than that in the substrates, which might be owing to the fact that a part of organics were transformed into biogas by anaerobic bacteria during the fermentation. However, the loss amount of TCOD in all reactors was very low (3–4 g/L), which indicated that the acidogenic fermentation was beneficial for conserving organics in the FSFW. Additionally, TCOD content in the FSFW was very close in the three reactors, showing that temperatures had slight influence on the COD loss. In raw FW slurry, the total particulate COD content was 39.8 g/L, in which particulate carbohydrate (22.9 g-COD/L) and protein (10.5 g-COD/L) were the dominate components. Soluble carbohydrates (20.1 g-COD/L), followed by the protein (2.4 g-COD/L) and LA (3.1 g-COD/L) were the main dissolved organics. LA in the raw FW was owing to the existence of indigenous lactic acid bacteria (LAB) as it was described in our previous study (Tang et al. 2016).

After fermentation, particulate organics decreased to 24.0 g-COD/L at 37 °C and 20.2 g-COD/L at 55 °C. The concentrations of particulate proteins in the fermented slurry slightly decreased, which indicated that proteins were hardly degraded during the acidogenic fermentation. This might be caused by the lack of protease under such a low pH condition (Calsamiglia et al. 2002). Particulate carbohydrates at 55 °C (11.3 g/L) were higher than those at 37 °C (8.5 g/L), which might be owing to the feed-back inhibition of the high content of soluble carbohydrate on the bacteria under thermophilic conditions (Lee and Jin 2017).

LA and carbohydrate, accounting for 60.4% of the SCOD content, are the main soluble organics in the slurry at 37 °C. Probably owing to the existence of heterolactic fermentation, acetic acid was largely detected in the fermented slurry (Castillo Martinez et al. 2013). Other soluble organics such as ethanol, long-chain fatty acid, and amino acid were approximately 6.3 g/L in the fermented slurry.

However, carbohydrate with a proportion of 60.6% of the SCOD was the dominant soluble component in the slurry fermented at 55 °C. The organic acids (LA and VFAs) were also found in the slurry, but only accounted for 29.3% of the SCOD, which further proved the lower acidogenesis rate under thermophilic condition. The main soluble organics in fermentation slurry at 20 °C were carbohydrate (12.3 g/L) and LA (12.0 g/L). Moreover, particulate organic content in this reactor was the highest, which further testified the lowest hydrolysis rate. The different organic components in the fermentation slurry are important for their utilization as carbon sources in wastewater treatment, because different organics exhibit distinct nutrient removal performance, which will be discussed in the o"Characteristics of nutrient removal using the FSFW as carbon sources" section.

Variations of microbial community during the fermentation

According to the above analysis, temperatures showed significant influence on the fermentation process and product components, which might result from different microbial communities in the fermenters. To investigate the relationships between the microbiota and fermentation processes, the microbial community structures in the substrate and fermented slurry were analyzed using the high-throughput sequencing technology.

As it was shown in Fig. 3a, Firmicutes was the dominant phylum in all samples. The relative abundance increased from 75.2% (raw FW) to 98.8% (at 20 °C) and 96.5% (at 37 °C) after fermentation, indicating a selective enrichment of this phylum during the fermentation. It was reported that the Firmicutes could exist under extreme conditions and digest complicated organics (Wang et al. 2018). The significant accumulation of Firmicutes during fermentation is favorable for digesting the complicated organic matter such as polysaccharides and fibers under acidified conditions (Li et al. 2017). However, in the reactor at 55 °C, Firmicutes slightly decreased (from 75.2 to 67.4%), accompanied with an obvious enrichment of Proteobacteria (from 8.4 to 29.5%) in the fermented slurry. Other bacteria such as Bacteroidetes (1.2%), Actinobacteria (1%), and Chloroflexi (0.2%) were also found in the reactor at 55 °C. Bacteroidetes have the capacity to digest proteins and other complicated organics (Li et al. 2017; Rivière et al. 2009), and Chloroflexi and Actinobacteria could consume the complicated substrates (Miura et al. 2007; Zhou et al. 2015). The accumulation of these phyla provided suitable conditions for digesting particulate organics and further testified the higher hydrolysis rate and soluble organic content in the fermented slurry (Fig. 2).

Lactobacillus, the LA producer, was the dominant genus in all samples (Fig. 3b). With a relative abundance of 43.6%, *Lactobacillus* in raw FW could act as the starters for the acidogenic fermentation (Tang et al. 2016). Additionally, the *Weissella* with a relative abundance of 19.2% was also detected in the substrate. Other bacteria such as *Propionibacterium* (8.1%), *Leuconostoc* (7.2%), *Acetobacter* (3.3%), *Anderseniella* (2.6%), and *Pediococcus* (2.5%) in the raw

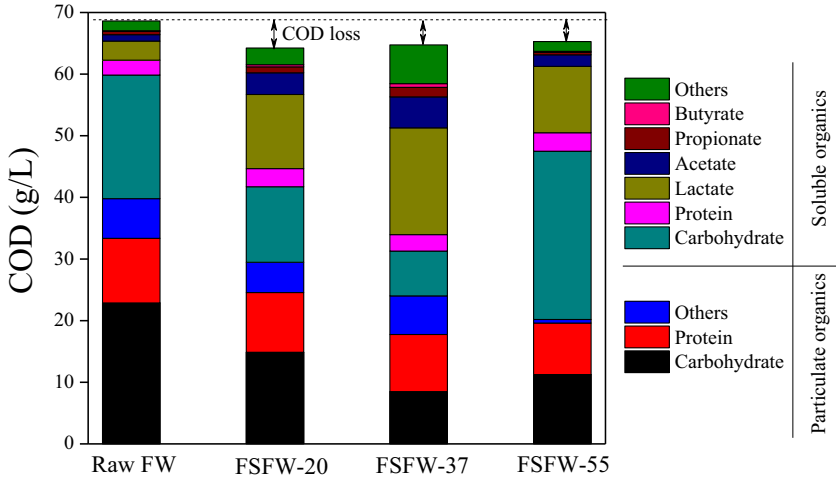

Fig. 2 Organic components in the substrate and FSFW produced at different temperatures

FW indicated the high diversity of bacterial communities in fresh FW substrate.

However, in the reactors at 20 °C and 37 °C, the relative abundance of *Lactobacillus* increased to 84.6% and 90.6%, respectively, which explained the high LA content in the fermented slurry and is consistent with the results in our previous study (Tang et al. 2016). Other LAB genera such as *Pediococcus* (11.4%), *Weissella* (2.1%), and *Leuconostoc* (0.7%) were also detected in the reactor at 20 °C, which contributed to transforming the substrates into lactic acid under the low-pH condition (Liu et al. 2014). *Acetobacter*, with a relative abundance of 0.6%, was found in the reactor, which explained the higher acetate content in the VFAs, while in the reactor at 37 °C, these genera obviously decreased. *Pediococcus* (2.1%), *Weissella* (2.3%), *Leuconostoc* (1.1%), and *Pseudomonas* (1.5%) were the main detectable genera, which might be resulted from the higher LA concentration and lower pH value in the slurry. It was reported that LA can be utilized as bactericide and inhibit the growth of other microorganisms (Odey et al. 2018). In the reactor at 55 °C, although the *Lactobacillus* (with a relative abundance of 57.7%) was the main member, more types of microorganisms were detected. In addition, *Pseudomonas* (12.6%), *Acetobacter* (7.5%), *Pediococcus* (6.7%), and *Serratia* (4.2%) were found in the reactor with a high relative abundance. The abundant bacterial communities in the fermented slurry might be owing to the lower acid content and higher pH value in the reactor, which provided suitable conditions for the accumulation of various bacteria. Additionally, the various microbial communities in the reactor were beneficial for substrate degradation, which further explained the higher hydrolysis rate under this condition. The lower proportion of *Lactobacillus* in the fermentor further testified the lower LA content in the fermented slurry. Overall, the selective enrichment of microbiota in the reactors at various temperatures further explained the differences in fermentation products during the fermentation.

Characteristics of nutrient removal using the FSFW as carbon sources

Denitrification properties of the FSFW in the UNR tests

Denitrification properties of the fermentation slurry were investigated through batch tests (Fig. 4). The NO_3^--N content in

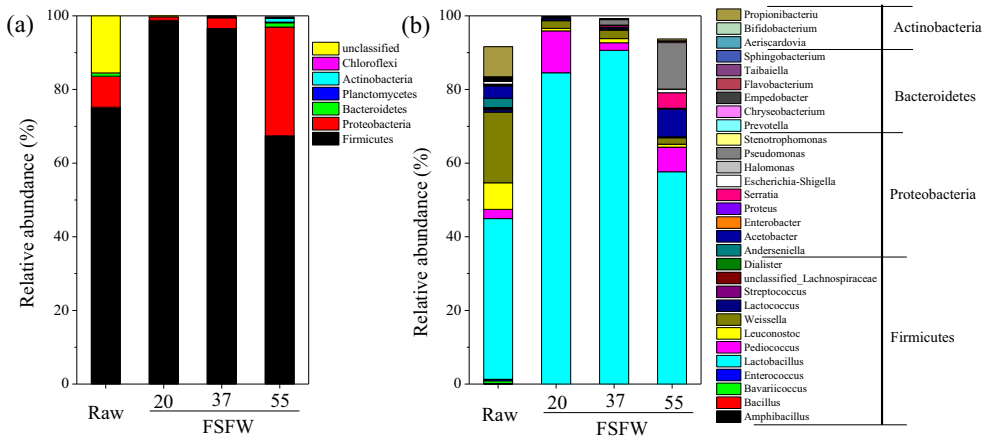

Fig. 3 Microbial communities in the substrate and fermentation slurry at different temperatures. **a** Phylum level and **b** genus level

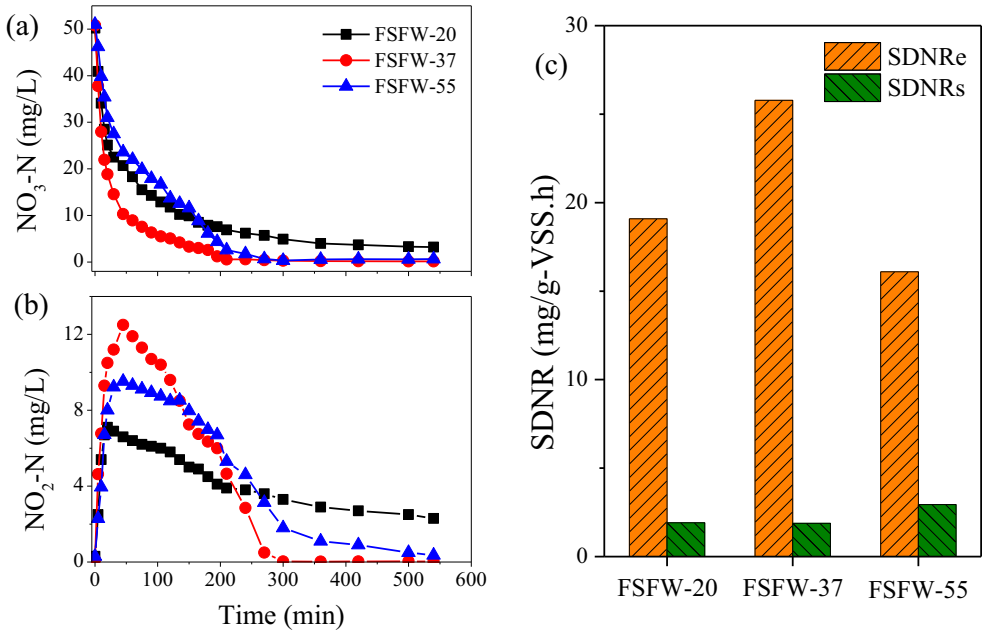

Fig. 4 **a**–**c** Denitrification properties of the FSFW produced at different temperatures. $SDNR_e$ represents the SDNR of easily biodegradable carbon source, and $SDNR_s$ represents the SDNR of slowly biodegradable carbon source

all reactors sharply decreased during the first 20 min (Fig. 4a), showing a high denitrification rate. This was mainly attributed to the low molecular organic acids such as lactate and VFAs in the fermented slurry which could be easily utilized by microorganisms, and enhance the denitrification processes (Sage et al. 2006; Zhang et al. 2016a). After 20 min, NO_3^--N content in the three reactors showed some differences. The fastest decline rate of NO_3^--N was observed in the reactor with FSFW-37 as carbon source. NO_3^--N content in the reactor decreased from 50.2 to 18.9 mg/L in 20 min and then to 10.3 mg/L in 45 min followed by a slower decreasing rate, which might be due to the fact that the easily biodegradable carbon sources were almost exhausted, and the bacteria could only use particulate organics or macromolecular substrates (e.g., carbohydrate and protein) for denitrification. The NO_3^--N content in the reactor with FSFW-20 and FSFW-55 decreased more slowly. In the first 180 min, NO_3^--N content in the reactor using the FSFW-20 was relatively lower than that in the reactor with FSFW-55, which mainly resulted from the higher organic acid content in the fermentation products. But after 180 min, the NO_3^--N content in the reactor with FSFW-55 was much lower, and finally decreased to approximately 0.6 mg/L at the end of the experiment. However, in the reactor supplied with FSFW-20, the final NO_3^--N concentration maintained at 3.5 mg/L, which might be due to the fact that the high content of particulate organics in the FSFW-20 should be firstly hydrolyzed into soluble forms prior to the denitrification; thus, longer time was needed to reduce the residual NO_3^--N in the mixed liquor.

Variations of NO_2^--N were analyzed (Fig. 4b). It was found that NO_2^--N obviously accumulated in the first 20 min and increased to 7.1 mg/L, 10.5 mg/L, and 8.0 mg/L in the reactor with FSFW-20, FSFW-37, and FSFW-55, respectively. The accumulation of NO_2^--N might be owing to the fact that denitrifying bacteria intensively utilized the easily biodegradable organics in the FSFW (e.g., lactate and VFAs) and transformed the NO_3^--N into NO_2^--N in a short period (Tang et al. 2019). However, after a sharp increase, the NO_2^--N content gradually decreased and reached a low level in reactors with FSFW-37 and FSFW-55, which demonstrated that the FSFW produced at 37 °C and 55 °C could be effectively utilized as carbon source for denitrification. But in the reactor supplied with FSFW-20, the residual NO_2^--N maintained at around 2.5 mg/L, indicating an incomplete denitrification using this slurry as carbon source.

Specific denitrification rate (SDNR) in the reactors with the FSFW was calculated by simulating the variations of nitrogen content during the batch tests (Fig. S3, Supporting information). According to the previous studies (Sage et al. 2006; Zhang et al. 2016a), the SNDR is composed of two parts, the first one ($SDNR_e$) is resulted from the easily biodegradable carbon sources (e.g., lactic acid, VFAs) and the second part ($SDNR_s$) was caused by the slowly biodegradable carbon sources such as carbohydrates, proteins, and particulate organics. The $SDNR_e$ of FSFW-37 was the highest (25.8 mg/g-VSS·h), which was much higher than that using FSFW-20 (19.1 mg/g-VSS·h) and FSFW-55 (16.1 mg/g-VSS·h) (Fig. 4c). The higher denitrification rate using FSFW-37 was mainly attributed to the higher content of organic acids in the fermentation slurry. It is reported that the low molecular organics could be easily degraded by the denitrifying bacteria and exhibited higher denitrification rate than that of soluble

carbohydrate and protein (Tang et al. 2018). Thus, the higher proportion of lactic acid and VFAs in the FSFW-37 and FSFW-20 than that of FSFW-55 further explained the higher $SDNR_e$ value in Fig. 4c. After the fast decline stage, easily biodegradable carbon source was almost depleted, denitrifying bacteria could only use the slowly biodegradable carbon source for nitrogen removal, and the denitrification processes entered into the slow stage. Interestingly, the FSFW-55 exhibited higher $SDNR_s$ (2.94 mg/g-VSS·h) than that of FSFW-37 (1.88 mg/g-VSS·h) and FSFW-20 (1.91 mg/g-VSS·h), which might be owing to the higher content of soluble slowly biodegradable carbon source (e.g., carbohydrates and proteins) in the fermentation slurry.

From the above analysis, it was concluded that the three types of fermentation slurry contained different organic compounds and exhibited different denitrification properties. The FSFW-37 contains higher organic acids and exhibited a higher $SDNR_e$, while the FSFW-55 composed of larger amount of soluble carbohydrate and showed a higher $SDNR_s$, which is important for their application in wastewater treatment.

Nitrogen removal performance with the FSFW during long-term operation

To explore the nitrogen removal performance of the fermentation products during the practical application, the fermentation slurries (FSFW-20, FSFW-37, and FSFW-55) were respectively utilized as external carbon sources in the SBRs to treat the low C/N ratio wastewater (C/N = 4–5). With the addition of FSFW, the COD content in influent increased to 300–500 mg/L, but low value was detected in the effluent (15–35 mg/L). All reactors showed satisfactory COD removal rate (> 90%) (Table 3), which demonstrated that the organics in influent could be effectively and completely utilized by the microorganisms in the activated sludge. This further indicated that the FSFW can be safely supplied as carbon sources in wastewater treatment systems (Tang et al. 2019).

NH_4^+-N in influent was around 50 mg/L and stabilized below 1.0 mg/L in effluent during the whole operation period (Fig. 5), showing a removal efficiency above 97% in all reactors (Table 3). This indicated that the fermentation products in the FSFW have negligible influence on the ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), which is consistent with the results in our previous studies (Tang et al. 2018, 2019). However, NO_3^--N in effluent was obviously influenced by the COD content in influent. Before adding the FSFW, NO_3^--N in effluent was above 30 mg/L, reactors showed a total nitrogen (TN) removal around 35%, which was mainly resulted from the low organics content in influent. After adding the FSFW, the C/N ratio in influent increased to about 6, NO_3^--N content in effluent gradually decreased to approximately 25 mg/L, resulting in a TN removal efficiency around 50%. This was because with the addition of the FSFW

Table 3 Pollutant removal efficiencies in the three SBRs before and after adding the FSFW as external carbon source. Unit: %

	Before adding FSFW(C/N = 4–5)				After adding FSFW (C/N = 6)				After adding FSFW (C/N = 10)			
	COD	NH_4^+-N	TN	PO_4^{3-}-P	COD	NH_4^+-N	TN	PO_4^{3-}-P	COD	NH_4^+-N	TN	PO_4^{3-}-P
FSFW-20	94.2 ± 4.4	98.5 ± 1.1	35.2 ± 5.5	10.2 ± 2.5	93.2 ± 8.3	98.6 ± 1.0	51.7 ± 7.8	15.2 ± 7.5	91.2 ± 5.3	98.3 ± 0.8	74.2 ± 5.9	27.2 ± 12.7
FSFW-37	90.5 ± 3.7	98.5 ± 1.2	35.1 ± 7.9	11.9 ± 6.9	94.5 ± 5.6	98.5 ± 0.9	52.8 ± 10.6	24.2 ± 9.5	93.5 ± 6.9	98.3 ± 0.9	82.9 ± 6.1	41.8 ± 28.3
FSFW-55	91.7 ± 8.2	98.0 ± 1.1	33.8 ± 7.6	13.9 ± 3.5	90.7 ± 10.1	97.5 ± 5.7	56.0 ± 14.4	27.2 ± 12.6	92.7 ± 10.1	98.0 ± 1.9	85.9 ± 5.0	91.2 ± 17.4

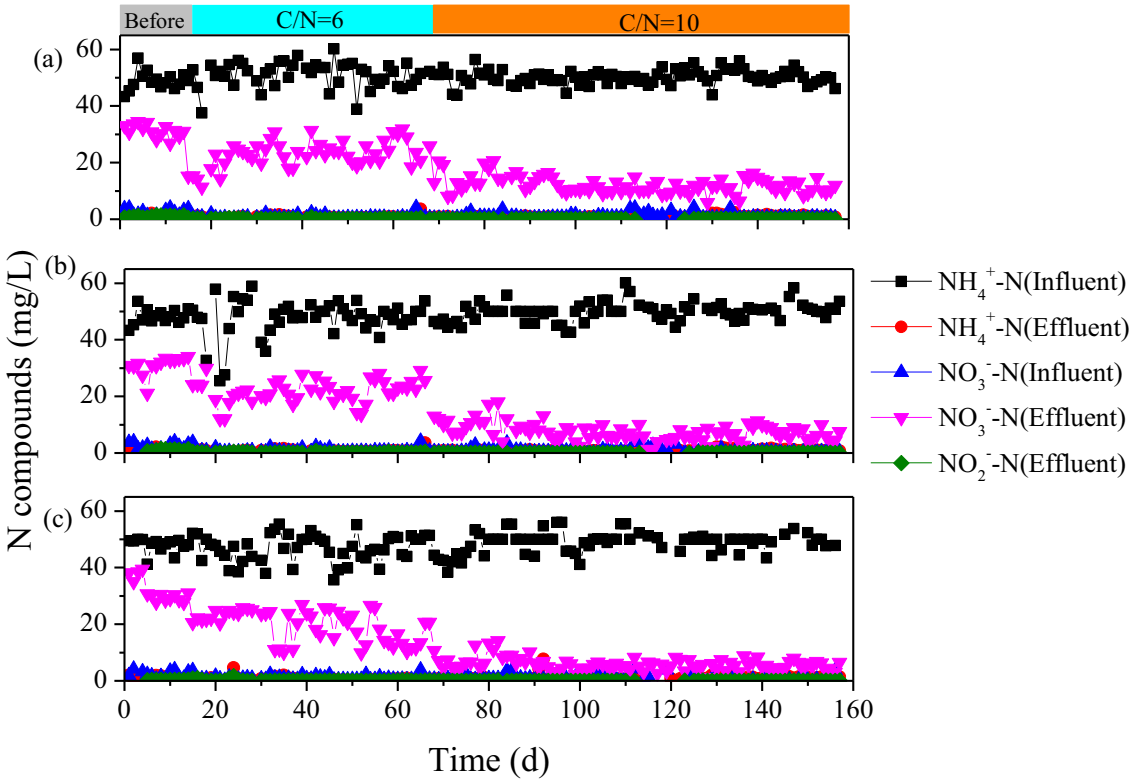

Fig. 5 Variations of nitrogen compounds in the SBRs before and after using the FSFW as external carbon sources. Reactor fed with FSFW-20 (**a**), FSFW-37(**b**), and FSFW-55(**c**)

in influent, more carbon sources were supplied to denitrifying bacteria, which enhanced the denitrification processes and promoted the nitrogen removal rate (Tang et al. 2017a, 2019). Further increasing the FSFW amount, C/N ratio in influent was elevated to approximately 10, which further improved the denitrification processes. NO_3^--N in effluent finally maintained at approximately 6.2 mg/L and 5.1 mg/L in the reactor with FSFW-37 and FSFW-55, respectively, which indicated that both of the two types of fermentation slurry could be effectively utilized as carbon source for nitrogen removal. However, NO_3^--N content in effluent of the reactor with FSFW-20 was much higher (approximately 11.3 mg/L), resulting in a lower TN removal efficiency (74.2%). This was mainly attributable to the higher content of slowly degradable organics (particulate organics) in influent; thus, longer time was needed to realize denitrification as it was shown in Fig. 4.

Phosphate removal efficiency before adding the FSFW was very low in all reactors (Table 3), which was mainly resulted from the insufficiency of organics in influent. After adding the FSFW and increasing the C/N ratio to 6, phosphate in effluent was still very high. Reactors exhibited a lower phosphate removal (approximately 20%). By further promoting the C/N to 10, the reactor with FSFW-37 showed a phosphate removal rate of 41.8%, but the reactor with FSFW-55 obtained a nitrogen removal rate around 91.2%, which indicated that the FSFW produced under thermophilic temperature was favorable for phosphate removal during the long-term operation. It has been found that the FSFW-55 could achieve high phosphate removal efficiency through aerobic phosphate accumulation and anoxic denitrifying phosphate accumulation processes by enriching phosphate accumulating organisms (PAOs) and denitrifying phosphate accumulating organisms (DPAOs) (Tang et al. 2019). Thus, it could be concluded that both the fermentation products with higher concentrations of organic acids (FSFW-37) and soluble carbohydrate (FSFW-55) could achieve satisfactory nitrogen removal efficiencies. However, the FSFW-55 was favorable for phosphate removal, which might be related to the differences in organic compounds in the fermentation slurry and distinct microbial communities in the activated sludge, and will be further studied in the future.

Microbial metabolic properties of the activated sludge using FSFW as external carbon sources

To investigate the metabolic activity of the microorganisms in the activated sludge cultivated by different FSFW, the variations of average well-color development (AWCD) in the Biolog ECO plate during the incubation of the microorganisms in the activated sludge were investigated. The AWCD value of raw activated sludge increased from 0.01 to 0.78 cm^{-1} during the first 72 h (Fig. 6a), but it increased to 0.84 and 0.90 cm^{-1} for the activated sludge cultured with FSFW-20 and FSFW-37,

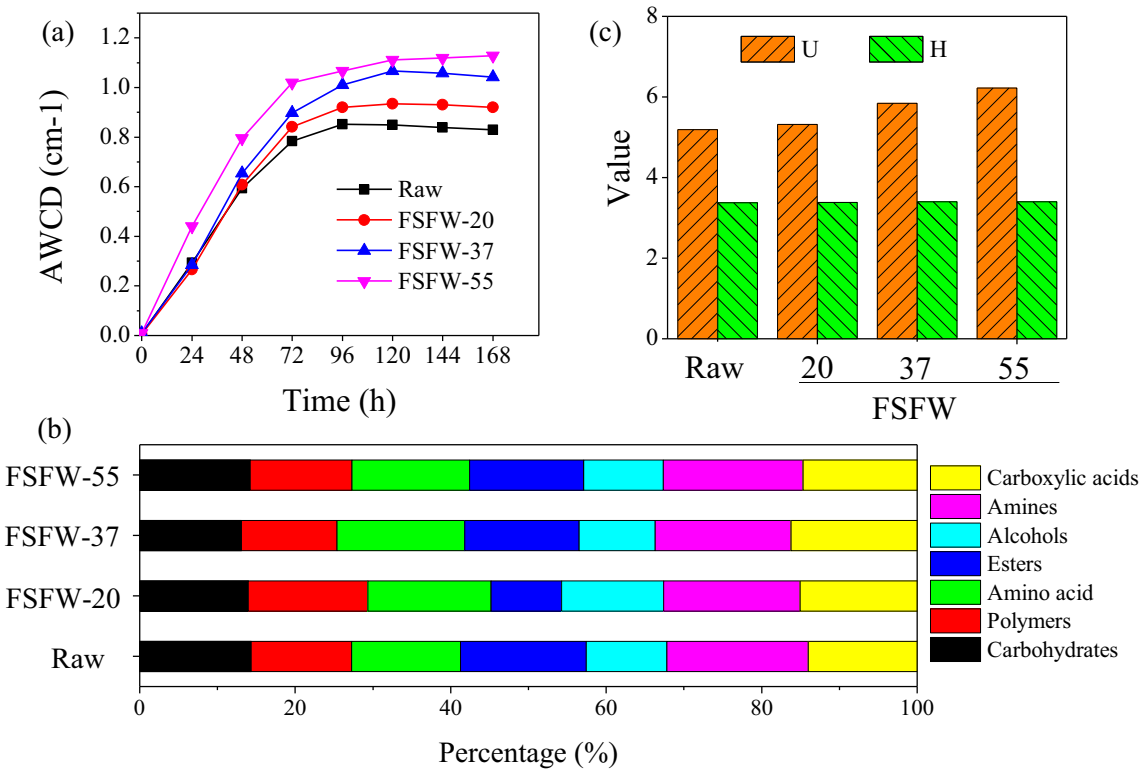

Fig. 6 **a**–**c** Carbon source metabolic activity of the activated sludge cultured with the FSFW

respectively, which indicated that the sludge cultivated by FSFW had a slightly higher metabolic capacity. While the microorganisms fed with FSFW-55 showed a much higher metabolic capacity as the AWCD increased to 1.02 cm^{-1}. With a linear increase, the AWCD value approached to the constant values after 96 h, which indicated that most of the carbon sources in the ECO plate were utilized by the bacteria. The higher AWCD value of the sludge cultivated with the FSFW indicated the more rapid bacterial growth and higher microbial metabolic activity (Kong et al. 2013; Tang et al. 2019), which might be caused by the higher bacterial diversity in the activated sludge (Zhang et al. 2016b). It was reported that hydrolysates (e.g., soluble carbohydrate and protein) and acidogenesis products (e.g., VFAs, lactate) in the FSFW can provide more types of carbon sources for the bacteria. Thus, higher microbial diversity was established to degrade the substrates in the reactors (Tang et al. 2017a, 2019).

Although microorganisms in the four activated sludge samples could utilize all the 7 types of carbon sources in the ECO plates, some differences could also be found (Fig. 6b). Bacteria cultured with FSFW-20 showed higher utilization rate for carboxylic acid (15.1%) and polymers (15.4%), which might be owing to the higher content of organic acids and polysaccharide in the fermentation products. The microorganisms cultivated with FSFW-37 exhibited higher degradation capacity on ammonia acid (16.4%) and carboxylic acid (16.2%), which might be due to the fact that the organic acids such as VFAs and lactate were the dominant organics in the FSFW-37 and changed the metabolic processes of the microorganisms (Tang et al. 2018, 2019). The activated sludge fed with FSFW-55 showed higher capacity for utilizing the carbohydrates (14.2%), which might be associated with the higher content of carbohydrate from the FSFW. Based on the above analysis, the activated sludge cultured with various carbon sources showed different organic digestion capacity, which further explained the nitrogen removal properties of the FSFW.

The McIntosh index (U) and Shannon's diversity (H) were introduced to further evaluate the diversity of metabolism and richness of the microorganisms in the activated sludge (Tang et al. 2018, 2019). The U values of sludge cultivated with FLFW-55 and FSFW-37 were 6.2 and 5.8 respectively and were higher than those of sludge fed with FSFW-20 (5.3) and the raw activated sludge (5.2). This indicated that the bacteria in the sludge cultured with FSFW-37 and FSFW-55 could digest more diverse carbon sources, which further verified the higher AWCD value in Fig. 6a. Thus, it could be deduced that organics in the FSFW-55 and FSFW-37 could be effectively utilized as carbon sources by microorganisms, or more kinds of bacteria were accumulated in the sludge to utilize the organics together. The H value is related to the number of utilizable carbon sources in the ECO plate (Yang et al. 2011; Zhang et al. 2016b). No evident difference is found in Fig. 6c, indicating that the carbon sources in the plate could be equally utilized by the four activated sludge samples.

Conclusions

Temperatures showed obvious effect on the carbon source production from food waste. Thermophilic temperature could enhance hydrolysis by promoting the hydrolytic enzyme activity and enriching more diverse microbial communities, but higher acidogenesis rate and LA yield were observed at mesophilic temperature. Higher relative abundance (90.6%) of *Lactobacillus* was found at mesophilic temperature (37 °C) than at thermophilic temperature (55 °C). FSFW showed combined properties of easily and slowly biodegradable carbon sources during the denitrification processes. Total nitrogen removal efficiencies increased with the increment of FSFW amount and achieved above 80% at a C/N ratio of 10. Both FSFW-37 and FSFW-55 could obviously improve the microbial metabolic capacity and achieve satisfactory nitrogen removal efficiency, but the reactor supplied with FSFW-55 exhibited higher phosphate removal potential during the long-term operation of the SBR, indicating that using the FSFW as carbon source for enhancing nitrogen removal is a feasible solution for organic solid waste dispose and wastewater treatment.

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