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## A viable approach for commercial VFAs production from sludge: Liquid fermentation in anaerobic dynamic membrane reactor

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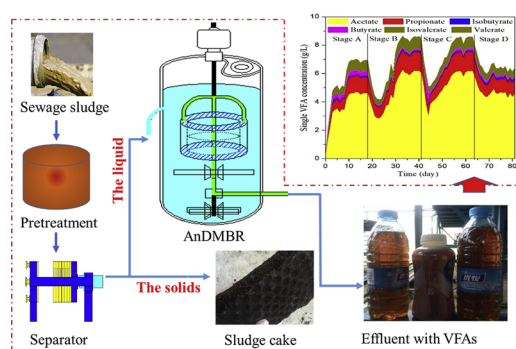
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### GRAPHICAL ABSTRACT



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### ABSTRACT

A novel strategy of liquid fermentation using anaerobic dynamic membrane reactor (AnDMBR) was proposed to enhance volatile fatty acids (VFAs) production from sewage sludge. Results indicated that liquid sludge fermentation in AnDMBR had the potential in commercial VFAs production. VFAs productivity and concentration as well as substrate conversion rate could reach as high as 7.8 kg VFA–COD/m<sup>3</sup> d, 60 g/L and 0.38 kg VFA–COD/kg VS, respectively. Moreover, dynamic membrane was stably operated for approximately 70 days. During the operational period, membrane flux was increased from 6.25 to 25 L/m<sup>2</sup> d and only once online membrane cleaning was implemented. Results of microbial analyses showed bacterial richness and evenness in AnDMBR were increased by membrane separation and organic loading rate (OLR) increase, but reduced by excessive OLR, which should led the variations in the performances of AnDMBR. Furthermore, the necessity of liquid sludge fermentation for VFAs production was further confirmed by economic assessment and the bio-availability analysis of the residual solids in pretreated sludge. The residual solid was proved to be not conducive to enhance VFAs yield. Conversely, the energy consumption for VFAs production could be reduced from over 100 to below 20 kWh/kg VFAs by avoiding the “useless” residual solids entering into fermenters.

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## 1. Introduction

Large amounts of sewage sludge have been produced during biological wastewater treatment. Their disposal often accounts for 50–60% of the total operation cost of wastewater treatment plants [1]. However, the increasing amounts of sewage sludge not only bring challenges but also opportunities. There are abundant organic matters in sewage sludge, such as protein and carbohydrate, which gives it tremendous potential in exploitation for further application. Especially, as VFAs production is recently proved to be more valuable and applicable than methane production [2,3], sludge anaerobic fermentation for VFAs production has been attracting more and more attentions.

However, the full-scale applications of sewage sludge fermentation for VFAs production are still rarely reported, since the current situations of this technology could not fully meet the demands for commercial VFAs production in aspects of weak fermentation intensity, low VFAs concentration and poor substrate conversion rate. To tackle the bottlenecks, several strategies have been examined, such as sludge pretreatment, fermenter configuration optimization and high-solid fermentation, etc. For example, sludge fermentation process could be greatly boosted by mild pretreatments, such as alkaline hydrolysis [4], and both fermentation rate and VFAs yield could be simultaneously improved by high-intensity pretreatments, e.g. the high-pressure thermal hydrolysis [5]. However, the fermentation efficiency of pretreated sludge is still not satisfactory enough. VFAs concentrations in effluent are mostly less than 1% (10 g/L) [6–8] and OLRs of fermenters with pretreated sludge are often not more than 5.0 kg COD/m<sup>3</sup> d [9–11].

Anaerobic dynamic membrane reactor (AnDMBR) had been proved to be promising in promoting VFAs production from fermentation of sewage sludge. By introducing dynamic membrane separation into conventional sludge fermenters, VFAs yield could be improved by 233.3% [12]. However, the low concentration of bioavailable organic matters in the influent made it still difficult to be practically applied. Although high-solid digestion had been confirmed to be feasible for biogas production [13–15], the increase of total solid concentration would deteriorate the dewaterability of fermented sludge and reduce VFAs recovery rate. In AnDMBR, high solids concentration would also cause serious membrane fouling [16].

In fact, the residual solids in the pretreated sludge are barely conducive to VFAs production during the following fermentation process, because almost all of the bioavailable organic matters in sludge has already been released into the supernatant during the pretreatment process [17]. Conversely, the presence of residual solids in fermenter would mitigate mass transfer efficiency, increase mixing energy consumption and hinder fermentative microorganism enrichment [18]. Therefore, in this study, a novel approach of liquid sludge fermentation was proposed to improve VFAs production in AnDMBR. That is, organic matters in sewage sludge were firstly released into the supernatant by pretreatment. Then, after the separation of the residual solids, in the AnDMBR, the biodegradable organics in the liquid were fermented into the permeate containing VFAs.

By using liquid sludge fermentation in AnDMBR, (1) the mass transfer efficiency of substrates and extracellular bio-enzyme could be greatly improved and fermentative bacterial could be doubly enriched, which would then be conducive to the enhancement of fermentation intensity; (2) the concentration of organic matters in the influent could be not limited by the tolerance of fermenter to solids concentration anymore, which would provide the possibility of producing high-concentration VFAs; and (3) the substantially decreased viscosity of the mixture in fermenter would greatly reduce or directly eliminate the energy consumption in stirring. The objective of this study is to provide a novel strategy for commercial VFAs production from sludge fermentation. The potentials of liquid sludge fermentation in improving VFAs production intensity was investigated in AnDMBR, the stability of dynamic membrane and enrichment of fermentative bacterial were

analyzed. By studying the liquid co-fermentation of sludge supernatant and artificial wastewater in AnDMBR, the feasibility of simultaneously realizing high fermentation intensity, high substrate conversion rate and high VFAs concentration were discussed.

## 2. Materials and methods

### 2.1. Substrates and inoculum

Sewage sludge used as the substrate for anaerobic fermentation was taken from the sludge storage tank of a local urban wastewater plant in Wuxi city, China. The fresh sludge was pre-concentrated. The concentrated sludge was with pH of 6.5–7.5, total solid (TS) of  $60.0 \pm 1.4$  g/L, volatiles solids (VS) of  $27.0 \pm 0.8$  g/L, SCOD of  $2.6 \pm 0.4$  g/L, soluble protein of  $131.9 \pm 38.2$  mg/L and soluble polysaccharides of  $256.4 \pm 34.2$  mg/L. All the analyses were conducted in triplicate. Moreover, trace elements contents of Fe, Ni, Co, Cu, Zn, Cr, Hg, Cd and Pb in sewage sludge were 0.094, 0.023, 0.012, 0.412, 1.38, 0.086, 0.0008, 0.002 and 0.06 mg/g dry sludge, respectively. Artificial molasses wastewater was also prepared as a substrate for liquid co-fermentation to produce high-concentration VFAs. The recipe of the molasses wastewater was referred to the study of Zhu et al. [19]. Anaerobic inoculum was harvested from a UASB reactor of a brewery wastewater treatment plant. To enrich acetogenic bacteria, the anaerobic biomass was firstly cultivated according to the method reported by Liu et al [20]. Before inoculating the fermenters, the seeding biomass was rinsed three times using deionized water.

### 2.2. Preparation of supernatant from pretreated sludge

The concentrated sewage sludge of  $60.0 \pm 1.4$  g TS/L was pre-treated at 105 °C and pH 12.0 for 8 h. The pretreatment was carried out in a reactor of 30 L that was continuously stirred at 100 rpm. Then, the supernatant with abundant organic matter could be obtained by centrifuging the pretreated sludge at 5000 rpm for 15 min. The supernatant was characterized with the parameters of pH of  $9.8 \pm 0.2$ , TS of  $4.1 \pm 0.6$  g/L, SCOD of  $38.7 \pm 1.0$  g/L, soluble protein of  $12.0 \pm 0.9$  g/L, soluble polysaccharides of  $3.8 \pm 0.2$  g/L and VFAs of  $1.0 \pm 0.1$  g/L.

### 2.3. Anaerobic fermentation of the supernatant from sludge for VFAs production

#### 2.3.1. Fermentation in AnDMBR

The setup of the AnDMBR with a working volume of 14 L employed for liquid sludge fermentation (Fig. 1) was established by modification of an AnDMBR for direct sludge fermentation [12]. In the study, the experimental period of liquid sludge fermentation was divided into 4 stages, namely stage A, B, C and D, whose operating parameters were listed in Table 1, respectively. During stage A, AnDMBR was started by inoculation with 1.4 L seeding sludge and 12.6 L pretreated sludge supernatant. During stage B, C and D, AnDMBR was operated under the help of dynamic membrane in separation of solids and effluent. In this study, just like that in direct sludge fermentation [12], the silk with an aperture of approximately 0.1 mm was still used as the separation layer. But, much higher membrane fluxes were implemented to the dynamic membrane subassembly (Table 1).

Besides, co-fermentation of the supernatant and artificial molasses wastewater for production of high-concentration VFAs was also carried out in the AnDMBR. During the co-fermentation, the OLR that was maintained at 5.0 kg COD/m<sup>3</sup>.d and the other operational conditions of the AnDMBR were the same as the conditions of the fermentation of pretreated sludge supernatant as the sole substrate. SCOD concentration in the influent was stepwise increased from 40 to 60, 85 and 150 g/L, which were corresponded with the SCOD ratios of sludge supernatant to artificial molasses wastewater, 100:0, 50:50, 30:70 and 20:80,

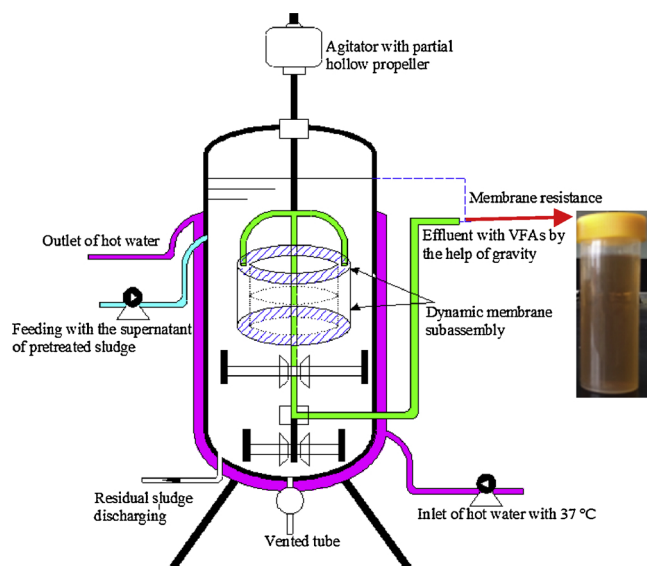


Fig. 1. Set-up of the AnDMBR for liquid sludge fermentation.

**Table 1**  
Operating parameters of the liquid sludge fermentation in AnDMBR.

Parameters	Stage A	Stage B	Stage C	Stage D
Temperature (°C)	37 ± 1	37 ± 1	37 ± 1	37 ± 1
pH	10	10	10	10
Organic loading rates (kg COD/m <sup>3</sup> .d)	5.0	5.0	11.0	17.0
Operation modes	Batch	Continuous	Continuous	Continuous
Membrane flux (L/m <sup>2</sup> .d)	–	6.25	13.89	25

respectively.

### 2.3.2. Anaerobic fermentation of pretreated sludge, residual solids and the supernatant

Sewage sludge of 600 ml were pretreated at 105 °C and pH 12.0 for 8 h, and then evenly divided into two fractions. One fraction was centrifuged at 5000 rpm for 15 min, yielding residual solid of 40 ml and supernatant of 260 ml. Three serum bottles of 500 ml with seeding sludge of 100 ml were employed and filled with 300 ml pretreated sludge (R1), 260 ml supernatant (R2), and 40 ml residual solid with 260 ml distilled water (R3), respectively. The batch fermentation method and operational conditions were referred to the previous study [20]. All the batch assays were carried out in triplicates.

## 2.4. Analytical methods

### 2.4.1. Measurements of conventional indices

For basic measurements, 20.0 mL of the sample was collected and measured immediately after sampling. Conventional indices, including pH, COD, suspended solid (SS), volatile solids (VS), volatile suspended solids (VSS) and total solids (TS), were measured according to the standard methods issued by the State Environmental Protection Administration of China [21]. The concentration of soluble carbohydrate was measured by the phenol-sulfuric method using glucose as the standard [22]. The concentration of soluble protein was determined by the Lowry-Folin method using bovine serum albumin as the standard [23]. VFAs were analyzed by a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with an auto-injector (AOC-20i, Shimadzu) [12]. To measure SCOD, soluble protein, soluble polysaccharides and VFAs, samples were firstly centrifuged at 10,000 rpm for 10 min, and then filtered with 0.45 μm syringe filters.

### 2.4.2. Microbial community analysis

During liquid sludge fermentation in AnDMBR, microbial communities in the sludge at different phases were analyzed when the performance of the AnDMBR was stable particularly in term of the stable concentrations of total VFAs in each phase. That is, samples were respectively taken from the reactor at stage A on day 18 (W1), stage B on day 31 (W2) and day 41 (W3), stage C on day 63 (W4), and stage D on day 81 (W5). The microbial analysis was performed according to the methods published by previous paper [12]. Each sample was analyzed in triplicate and the standard deviations of all analyses were always less than 5%.

## 2.5. Calculation methods

Calculation methods for energy consumption in sludge pretreatment and sludge fermentation were referred to the studies [12,18]. VFAs productivity ( $P_{VFA}$ ) was computed by using Eq. (1) (kg/m<sup>3</sup> d), VFAs yield ( $Y_{VFA}$ ) was calculated by using Eq. (2) (kg VFA – COD/kg VS), and sludge consumption capacity ( $R_{VSS}$ ) was based on Eq. (3) (kg/m<sup>3</sup> d).

$$P_{VFA} = Q \times (VFA_t - VFA_0) / V \quad (1)$$

$$Y_{VFA} = (VFA_t - VFA_0) / VS_0 \quad (2)$$

$$RVSS = Q \times (VSS_0 - VSS_t) / V \quad (3)$$

where  $VFA_t$  and  $VFA_0$  were the concentrations of VFAs in the fermented sludge at the beginning and end of the fermentation process, respectively (kg/m<sup>3</sup>).  $Q$  was the flow of the effluent (m<sup>3</sup>/d).  $V$  was the volume of the reactor (m<sup>3</sup>).  $VS_0$  was the concentrations of VS at the beginning of sludge pretreatment process (kg/m<sup>3</sup>).  $VSS_0$  was the concentrations of VSS at the beginning of the sludge pretreatment process (kg/m<sup>3</sup>).  $VSS_t$  was the concentrations of VSS at the end of sludge fermentation process (kg/m<sup>3</sup>).

## 3. Results

### 3.1. Enhanced fermentation intensity of sewage sludge for VFAs production

Both of VFAs concentration and productivity could be greatly enhanced by the application of sludge liquid fermentation in AnDMBR. Firstly, comparing stages A and B, it was found that the conversion rate of organics in sludge could also be improved by the application of dynamic membrane separation during sludge liquid fermentation (Fig. 2A). Under the same OLR of 5.0 kg COD/m<sup>3</sup>.d and the same COD concentration of 38.69 g/L in the influent, the produced VFAs concentration in the effluent could be enhanced from 6.92 g/L in stage A to 8.59 g/L in stage B. Secondly, by comparing stages B and C, it could be found that VFAs productivity was improved by increasing OLR from 5.0 to 11 kg COD/m<sup>3</sup>.d, and the concentrations of VFAs in the effluent were comparable, ranging from 8.5 to 8.6 g/L. Thirdly, the results obtained from stages C and D indicated that excessive OLR would lower the conversion rate of organics in sludge and reduce VFAs concentration in effluent (Fig. 2A). In conventional processes of sludge fermentation for VFAs production, the SCOD concentrations of the pretreated sludge are often in the range of 5–25 g/L and fermentation periods are about 5–10 days, that is, the OLRs are around 1–5 kg COD/m<sup>3</sup>.d [9–11]. In this current study, it is proven that the AnDMBR could be stably operated under the OLRs as high as 11 and even 17 kg COD/m<sup>3</sup>.d, indicating that the fermentation intensity for VFAs production from sewage sludge was greatly enhanced by applying liquid fermentation in AnDMBR.

Furthermore, the compositions of the produced VFAs would be not significantly influenced by the application of sludge liquid fermentation. As shown in Fig. 2B, the VFAs composition in effluent was comparable to that of the conventional fermentation processes. Acetate was still the main component and accounted for about 65–75% of the total VFAs, followed by propionate and then isovalerate. Moreover, elevated

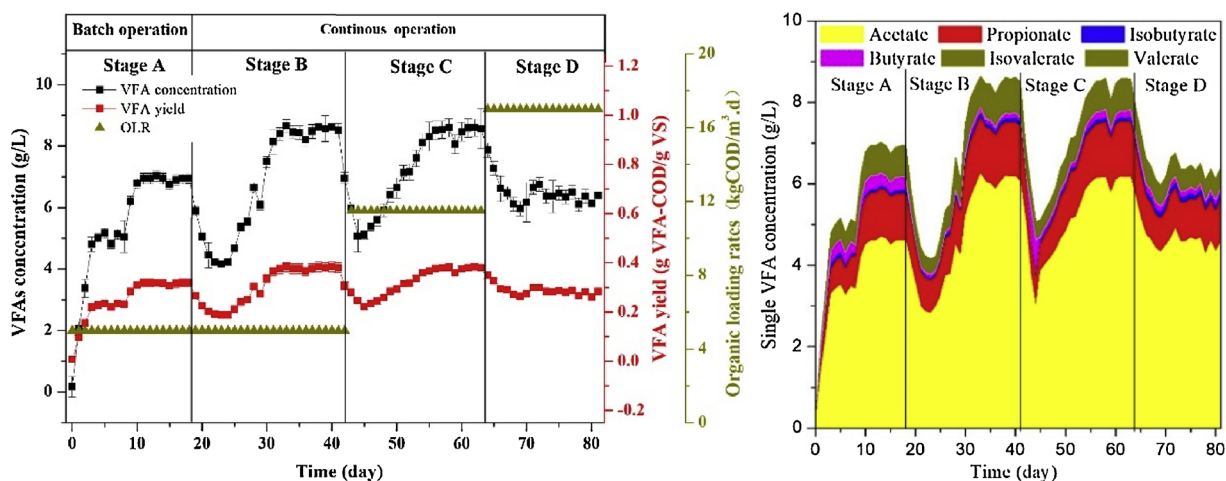


Fig. 2. VFAs production by liquid sludge fermentation under different OLRs in AnDMBR (Left) and VFA profile (Right).

OLRs presented no substantial influences on the compositions of the produced VFAs, though the proportion of acetate content in the total VFAs were slightly increased from 66% to 71% when the OLRs were increased from 5.0 to 11 kg COD/ m<sup>3</sup> d.

### 3.2. Performances of different sludge anaerobic fermentations for VFAs production

Liquid fermentation could substantially speed up sludge fermentation process but hardly improve the yield of VFAs or the conversion rate of organic matters. As shown in Table 2, according to OLRs, VFAs productivity and sludge consumption capacity, it could be concluded that compared with conventional fermentation processes, liquid fermentation could substantially improve the efficiencies of VFAs production and sludge treatment. Especially, after application of dynamic membrane separation, the efficiency of VFAs production could be improved by about 3–5 times while the efficiency of sludge treatment could even be elevated by 10–20 times. However, the advantages of liquid fermentation in increasing VFAs concentration and yield were not evident. The VFAs concentration is positively relative to the concentration of SCOD in the influent. As mild hydrolysis method was adopted in this study and the dewaterability of pretreated sludge would deteriorate with the increase of sludge concentration, thus the limited TS of the used sludge resulted in the fact that the SCOD concentration in the influent of AnDMBR could not reach very high levels. Correspondingly, mild hydrolysis was just able to release organic matters from biomass into supernatant, but the biodegradability of organic matters could not be improved. Actually, this situation could be completely reversed by adopting high intensity sludge pretreatments. For example, Morgan-Sagastume et al. [5] obtained VFAs of 15–20 g/L from sludge fermentation with the pretreatment of high-pressure thermal hydrolysis.

Table 2  
Efficiencies of anaerobic fermentation of sewage sludge [24–26].

Fermentation types	Conventional fermentation with TS (kg/m <sup>3</sup> )			Liquid fermentation	Liquid fermentation with DM separation		
	15	33.8	70		5.00 (stage A)	5.00 (stage B)	11.00 (stage C)
Organic loading rates (kg COD/m <sup>3</sup> d)	3.35	3.32	2.20	5.00 (stage A)	5.00 (stage B)	11.00 (stage C)	17.00 (stage D)
Sludge consumption capacity (kg/m <sup>3</sup> d)*	18.75	21.14	23.33	124.45	124.45	273.75	422.65
VFAs productivity (kg/m <sup>3</sup> d)	0.78	1.10	0.50	2.89	3.55	7.80	8.56
VFAs yield (kg VFA-COD/kg VS)	0.29	0.41	0.26	0.31	0.38	0.38	0.27
VFAs concentrations (kg/m <sup>3</sup> )	3.10	8.70	7.53	6.92	8.59	8.56	6.28

\* The water content of sludge was 80%.

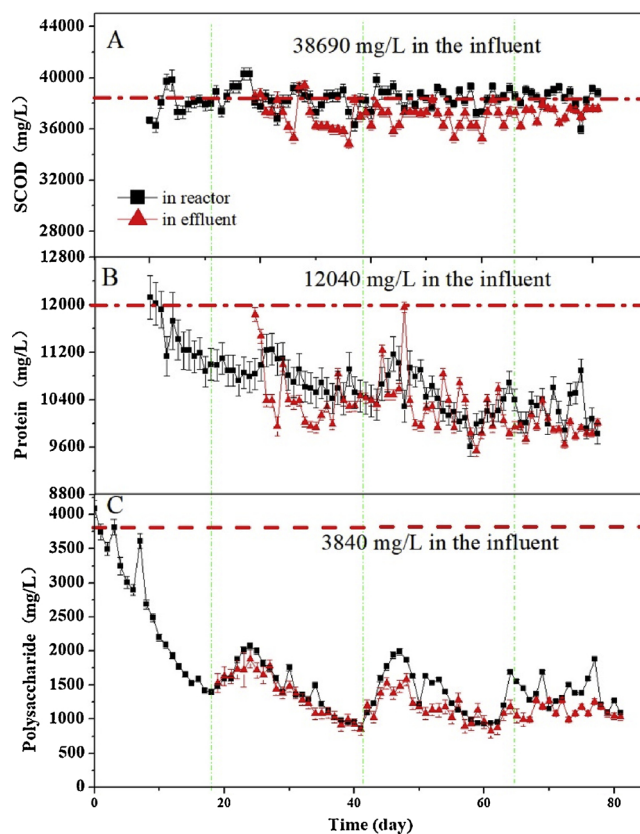


Fig. 3. Degradation and distribution of the main substrates during sludge liquid fermentation.

### 3.3. Degradations of the main substrates during sludge liquid fermentation

Main fractions of organics in substrates could be stably degraded during liquid fermentation in the AnDMBR with the increase of OLRs. As shown in Fig. 3, though the concentrations of the residual substrates, protein and polysaccharide, were very high, their accumulations in AnDMBR were not observed with the increase of OLRs. Even during stage D, the produced VFAs concentration decreased (Fig. 2) while the concentrations of residual substrates still remained stable. Presumably, some non-VFAs matters were produced, such as long-chain fatty acids. However, by comparing the concentrations of substrates in the reactor and in the effluent, the contribution of dynamic membrane separation on substrates retentions was observed to be negligible, the result of which was very different from the direct sludge fermentation in AnDMBR for VFAs production [12]. The observed phenomenon most likely was related to the reduced particle size of substrates by sludge pretreatment. Finally, as shown in Fig. 3A, throughout the whole fermentation process, SCOD concentration could keep very stable and no obvious loss of SCOD was observed, which indicated methanogenesis was effectively suppressed.

### 3.4. Stability of dynamic membrane operation

Compared with that in direct sludge fermentation in AnDMBR [12], the performance of dynamic membrane could be much more stable in sludge liquid fermentation. During about 70 days operation (Fig. 4), online membrane cleaning was only implemented once and membrane resistances could be kept at low levels, about  $1.0\text{--}3.0 \times 10^{11} \text{ m}^{-1}$  at the stable stages. Moreover, the influence of membrane flux on membrane fouling seemed not to be substantial. As membrane flux was improved from 6.25 to 13.89 and then to  $25.0 \text{ L/m}^2\text{.d}$ , the increase of membrane resistance was not seriously accelerated, which was probably linked to the reduced solids content in the reactor. Although membrane flux of about  $2.0 \text{ L/m}^2\text{.d}$  was suitable for direct sludge fermentation in AnDMBR [12], the membrane flux of dynamic membrane could reach as high as  $20\text{--}100 \text{ L/m}^2\text{.d}$  during wastewater treatment by DMBR [27]. Obviously, the conditions of the latter are comparable to that of sludge liquid fermentation. Furthermore, dynamic membrane also presented high efficiency in SS retention in AnDMBR with liquid sludge fermentation. As shown in Fig. 4, the SS in the effluent was below  $3 \text{ mg/L}$  under normal conditions, which should be one of the main reasons to the strong capacity of AnDMBR in VFAs production from sludge fermentation.

### 3.5. Microbial community analysis

Bacterial communities of the five samples were analyzed using Illumina MiSeq sequencing. As shown in Table 3, bacterial richness and

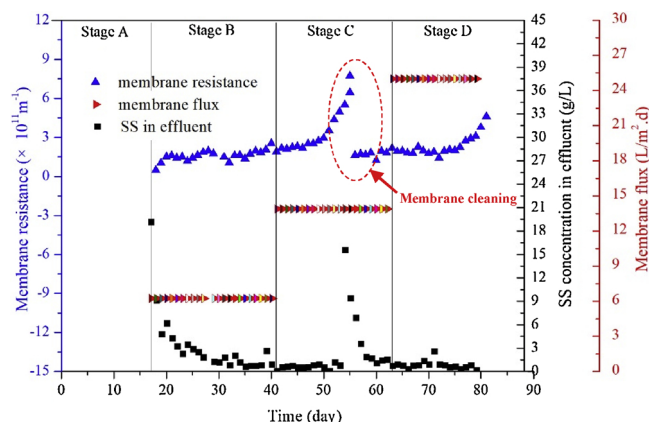


Fig. 4. Operation of dynamic membrane during liquid sludge fermentation.

**Table 3**  
Diversity indices used in this study <sup>a</sup>.

Samples	W1 (Stage A)	W2 (Stage B)	W3(Stage B)	W4 (Stage C)	W5 (Stage D)
OTU	268	211	240	250	162
Chao	335.43	314.21	344.69	317.53	240.30
Shannon	4.00	2.92	3.58	4.57	2.88
Simpson	0.84	0.72	0.82	0.90	0.70

<sup>a</sup> An asterisk indicates the values are significantly different ( $P < 0.05$ ). The significant diversity is greater than 0.97. OUT indicates operational taxonomic units.

evenness in the fermentation sludge could be influenced by the operation mode, membrane separation application and OLRs. The results of samples W1 and W2 indicated that the shift from the batch operational mode to the continuous mode would reduce the bacterial richness and evenness of fermentation sludge. However, the results of W2 and W3 implied that the application of dynamic membrane was seemingly conducive to increase the bacterial richness and evenness. Especially, the elevated OLRs could further stimulate the increases of bacterial richness and evenness, which was indicated by the results of W3 and W4. However, with the further increase in OLRs, the bacterial richness and evenness from stage C to stage D were substantially reduced.

Phylogenetic differences in 16S rRNA gene sequences were characterized at phylum and genus levels to investigate the diversity of microbial community. The three dominant phyla, Proteobacteria, Bacteroidetes and Firmicutes, which are able to effectively degrade organic compounds, such as proteins and polysaccharides [28], were all present in the 5 samples but with different relative abundances (Fig. 5A). In particular, under the pressure of high OLR in W5, two phyla of Proteobacteria and Firmicutes presented very high abundances, which were more than 95%. Reportedly, some species of Proteobacteria play important roles in anaerobic fermentation processes [29]. Within the phylum of Firmicutes, some microbes, such as *Clostridia*, are associated with high-rate hydrolysis [29].

Moreover, top 20 abundant genera were selected and their profiles among different samples were shown in Fig. 5B. Comparative analysis revealed the operation mode, membrane separation and OLRs presented evident effects on the bacterial communities and seemingly accelerated the enrichment of a few bacterial genera. However, the dominant genera of the samples presented substantial differences. The heatmap indicates there were four clusters, besides samples of W2 and W3 were clustered together. The high similarity of the samples W2 and W3 was because they were harvested in the beginning and the end of stage B while VFA contents were stabilized, respectively. The significant differences of the four clusters suggested clear distinctions in the community structure between sludge samples, despite the fact their operations were run by one end to another start.

Furthermore, according to canonical correspondence analysis (CCA), substantial differences between the communities of the 5 samples could be observed (Fig. 5C). The principal components 1 and 2 accounted for 62.91% and 22.64%, respectively, of the total community variation. Samples of W1, W2 and W3 presented relatively similar communities which were significantly distinct from those of W4 and W5. These results implied that the application of membrane separation and increase of OLRs were conducive to the selectively enrichments of some particular bacteria in the fermentative sludge.

## 4. Discussions

### 4.1. Technical feasibility of commercial VFAs production from liquid sludge fermentation

As far as commercial production is concerned, satisfactory

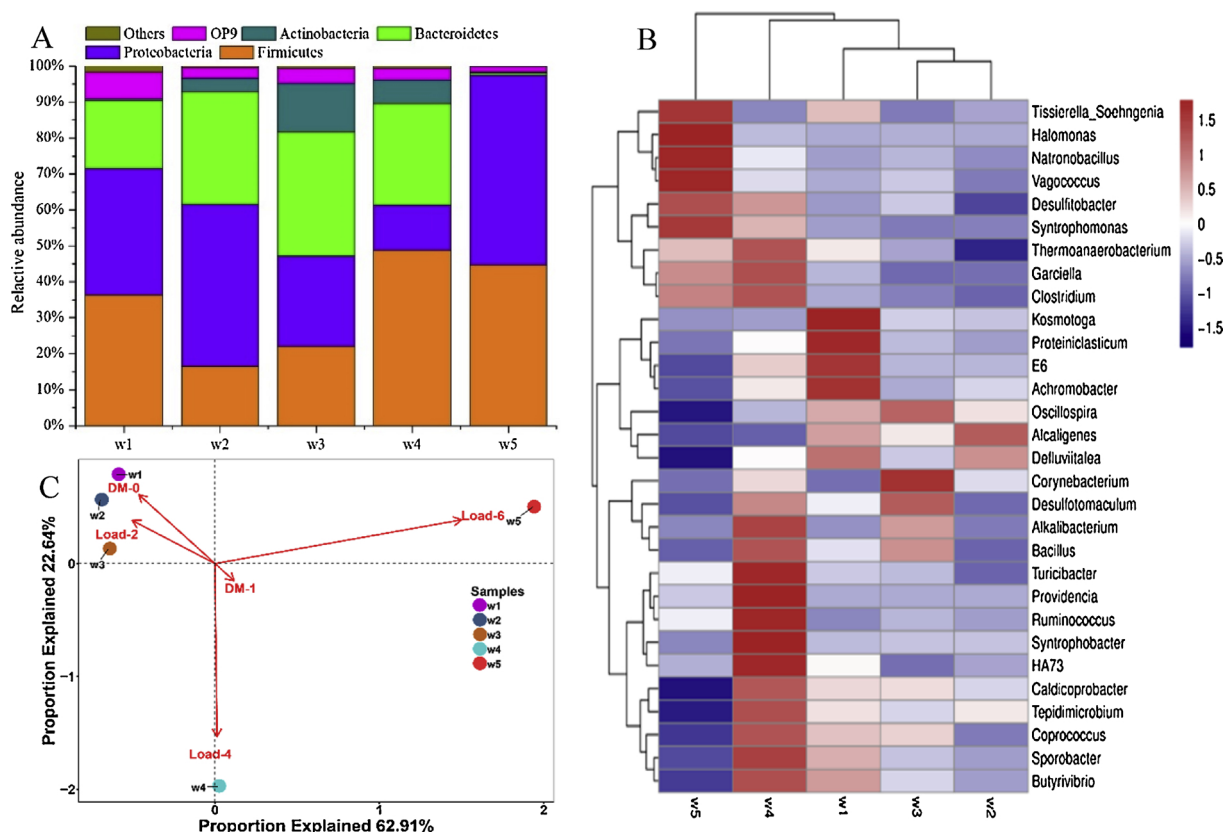


Fig. 5. Taxonomic classification of bacterial communities sequences at the phylum level in the five samples (A), heatmap of the 20 most abundant bacterial genera in the samples (B) and CCA of bacterial communities in the samples (C).

fermentation intensity, sufficient product concentration and efficient substrate conversion rate are often simultaneously demanded. However, as the contents of readily biodegradable matters in sewage sludge are often very low, so fermentation intensities, VFAs concentrations and substrate conversion rates are usually less than 5.0 kg COD/m<sup>3</sup>.d, below 10.0 kg/m<sup>3</sup> and about 0.2–0.4 kg VFA/kg VS, respectively, in the conventional sludge fermentation processes. Recently, although high-solid digestions are widely studied and applied for biogas production, they are definitely not suitable for VFAs production since the recovery of produced VFAs from the high-solid fermented broth would become very difficult.

The study indicates that liquid fermentation seems to provide a feasible alternative for the commercial VFAs production from sewage sludge. The complex sludge fermentation seriously interfered by the high-concentration solids could be transformed into much simple wastewater-like fermentation. During liquid sludge fermentation, the fermentation intensity could be improved by adopting advanced reactors, such as AnDMBR, the conversion rate of substrates could be enhanced by corresponding pretreatments and VFAs concentration could be increased by improving substrates concentrations.

To further confirm the technical feasibility of sludge liquid fermentation for commercial VFAs production, co-fermentation of pretreated sludge supernatant and artificial molasses wastewater was implemented to obtain high-concentration VFAs under acceptable fermentation intensity and substrate conversion rate. Fig. 6 showed the concentration of VFAs was enhanced to nearly 60 g/L in the effluent. To date, such high-concentration VFAs from sewage sludge fermentation has not been reported yet. Moreover, when the OLR was under a relatively high level of about 5.0 kg COD/m<sup>3</sup>.d, VFAs yields could reach 0.58, 0.53 and 0.42 kg VFA–COD/kg COD with influent SCOD concentrations of 60, 85 and 150 kg/m<sup>3</sup>, respectively. Besides, the purity of VFAs was further enhanced and the proportion of acetate in total

VFAs reached as high as approximately 95%.

#### 4.2. Integrity of liquid sludge fermentation in carbon recovery

From the perspective of the exploitation degree of carbon source in sludge, the necessity of liquid sludge fermentation were investigated and discussed. The performances of SCOD release and VFAs production during anaerobic fermentation of pretreated sludge (R1), the supernatant (R2) and the residual solid (R3) were shown in Fig. 7, respectively. Firstly, SCOD release could be observed during R3 fermentation possibly due to the incomplete hydrolysis during sludge pretreatment, but seemingly the bioavailability of the released SCOD was poor, resulting in the lower VFAs production in R3 and comparable VFAs concentrations in R1 and R2. Results indicate the contribution of the residual solids in pretreated sludge on VFAs production is very limited, and liquid fermentation process is integral enough for carbon recovery from sewage sludge.

#### 4.3. Substrates loss during high concentration VFAs production from liquid sludge fermentation

To simultaneously obtain intensive fermentation and high-concentration product, substrates loss often seems inevitable. As shown in Fig. 8, with the increases of SCOD concentration in the influent by adding artificial molasses wastewater, the concentration of residual protein in AnDMBR could kept stable and even slightly reduced. The result indicated that sludge degradation was possibly not influenced during liquid co-fermentation process (Fig. 8). However, the concentration of residual polysaccharide increased sharply. Though the conversion rate of substrates was not seriously reduced (Fig. 6), the absolute amount of the residual substrates, especially polysaccharide, were still very large due to their high concentrations in influent.

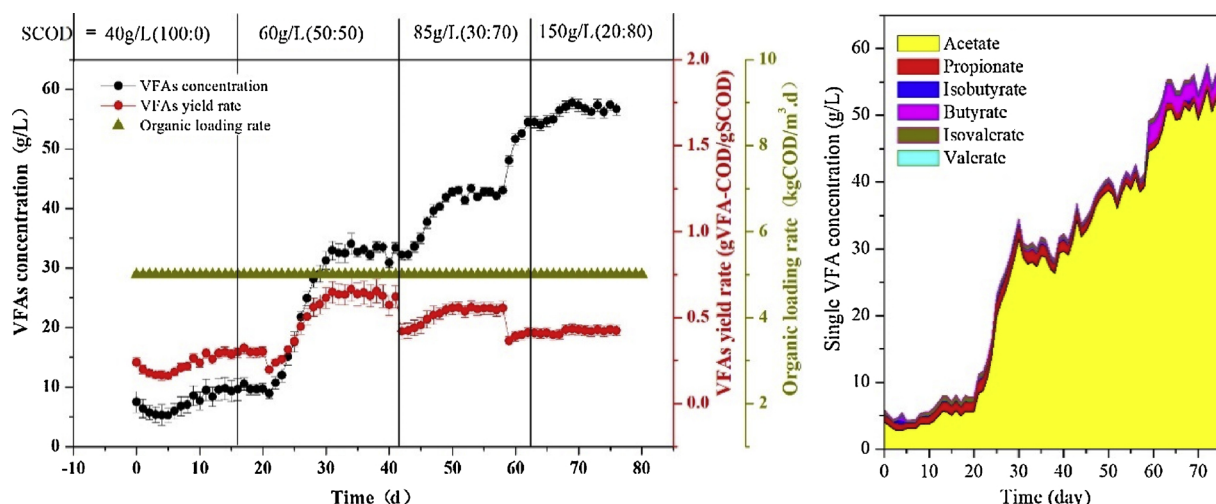


Fig. 6. VFAs production (Left) from liquid co-fermentation of sludge supernatant and artificial molasses wastewater in the AnDMBR and their profile (Right).

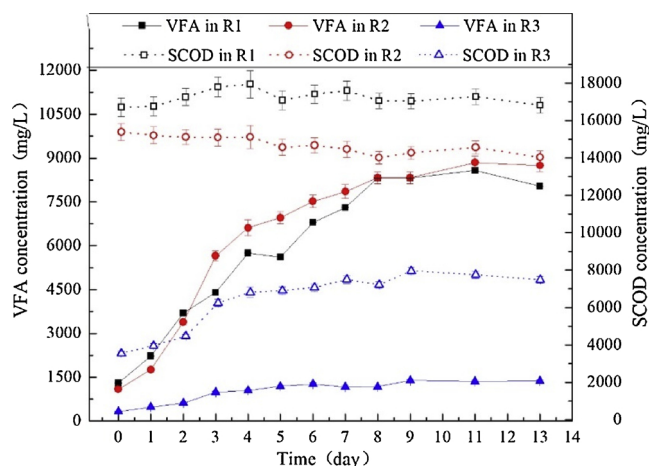


Fig. 7. SCOD release and VFAs production during anaerobic fermentation of pretreated sludge, residual solid and supernatant, respectively.

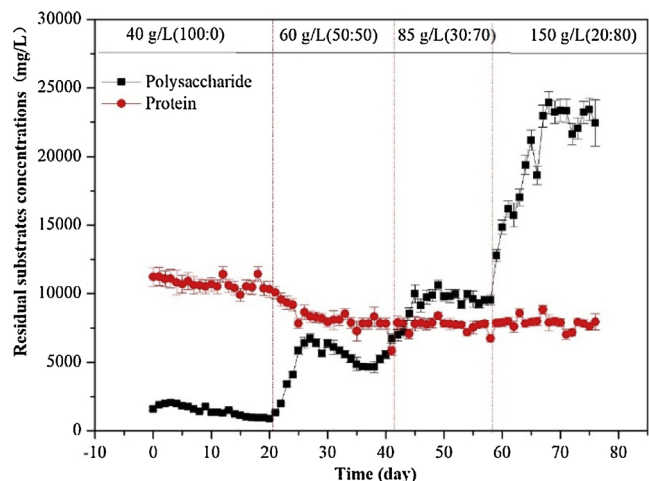


Fig. 8. Accumulations of residual substrates during liquid co-fermentation of sludge supernatant and artificial molasses wastewater.

Recycling the residual substrates should be a feasible approach to relieve their losses, since the residual substrates actually presented good bioavailability and their remaining are mainly ascribed to the bio-transformation kinetic equilibrium and negative feedback inhibition of

products.

#### 4.4. Economic assessment

By comparing different kinds of sludge fermentation for VFAs production, the economic feasibility of liquid sludge fermentation in AnDMBR is evaluated in aspects of energy consumptions, hydraulic retention time and material demand, as well as sludge biomass reduction and the produced VFAs amount. As shown in Table 4, results indicate energy consumption could be substantially reduced by implementing liquid fermentation. During sludge pretreatment and fermentation, there are mainly three aspects determining the energy consumption, namely biomass concentration, fermentative sludge viscosity and fermentation intensity. With the increase of TS, energy consumption per unit mass of sludge will be reduced. However, high concentration of the fermentative sludge would result in large viscosity which is positively relative to the stirring energy consumption. Liquid sludge fermentation could reduce energy consumption during pretreatment process by adopting high-solid sludge hydrolysis, and avoid high energy consumption for stirring by removing solids before fermentation process. Moreover, as mentioned above, the fermentation intensity could be enhanced for several folds by liquid fermentation, which thus substantially reduced the energy consumption per unit mass of the produced VFAs.

Hydraulic retention time (HRT) is another important factor that influences the costs of investment and operation. Table 4 shows the shift from direct sludge fermentation to liquid sludge fermentation could efficiently reduce HRT. Moreover, in VFA production from the liquid fermentation, the reactors of up-flow anaerobic sludge blanket (UASB), stirred anaerobic sequencing batch reactor (SASBR) and AnDMBR seemed to be suitable candidates. UASB presented slightly lower HRT but due to its influent with low concentration COD, whereas AnDMBR presented much higher VFAs productivity. The amount of the produced VFAs (i.e. VFAs productivity) is comprehensively determined by fermenter OLR and substrates conversion rate. To both of the two parameters, AnDMBR presented better performance.

Finally, Table 4 indicates that the alkali demand is negatively relative to the biomass concentration adopted in the fermentation process but not relative to the application of liquid fermentation. Moreover, sludge reduce is positively relative to the hydrolysis intensity both in pretreatment and fermentation processes. Therefore, under the same pretreatment efficiency, seemingly direct sludge fermentation is otherwise benefit to reduce sludge biomass.



**Table 4**  
Economic comparisons of different sludge fermentation process [2,18,25,26].

Processes	Items	Direct pretreated sludge fermentation with different TS (g/L)			Liquid sludge fermentation in different reactors (SCOD, g/L)					
		15	33.8	70	UASB (16.4)	SASBR (38.7)	AnDMBR (38.7)			
Pretreatment*	Hydraulic retention time (h)	2.0	2.0	2.0	3.0	8.0	8.0	8.0	8.0	8.0
	Alkali demand (kg NaOH /m <sup>3</sup> )	1.56	1.85	2.11	2.05	2.10	2.10	2.10	2.10	2.10
	Heating energy demand (kwh/kg TS)**	7.78	3.42	1.67	1.80	1.95	1.95	1.95	1.95	1.95
	VSS reduce rate (%)	35.83	27.67	38.33	34.52	68.73	68.73	68.73	68.73	68.73
Fermentation	Organic loading rate (kg COD/m <sup>3</sup> d)	3.35	3.32	2.20	6.5	5.0	5.00	11.00	17.00	
	Hydraulic retention time (day)	6.00	7.00	8.00	2.52	7.74	7.74	3.52	2.28	
	VFAs concentrations (g/L)	3.10	8.70	7.53	7.04	6.92	8.59	8.56	6.28	
	Stirring energy demand (kwh/kg VFA)	47.89	55.77	70.78	4.63	6.50	5.29	2.41	2.19	
	Heat preservation (kwh/kg VFA)	16.86	12.00	26.30	2.02	4.55	3.70	1.66	1.54	
	VSS reduce rate (%)	17.62	16.74	13.89	0.00	0.00	0.00	0.00	0.00	
	Hydraulic retention time (d)	<b>6.08</b>	<b>7.08</b>	<b>8.08</b>	<b>2.65</b>	<b>8.07</b>	<b>8.07</b>	<b>3.85</b>	<b>2.61</b>	
In total	VFAs productivity (kg/m <sup>3</sup> d)	<b>0.78</b>	<b>1.10</b>	<b>0.50</b>	<b>2.78</b>	<b>2.89</b>	<b>3.55</b>	<b>7.80</b>	<b>8.56</b>	
	VSS reduce rate (kg/kg VFA)	53.45	44.41	52.22	34.52	68.73	68.73	68.73	68.73	
	Energy demand (kwh/kg VFA)	<b>102.40</b>	<b>81.06</b>	<b>112.60</b>	<b>23.27</b>	<b>27.96</b>	<b>22.61</b>	<b>17.74</b>	<b>22.36</b>	
	Alkali demand (kg/kg VFA)	0.50	0.21	0.28	0.29	0.30	0.24	0.25	0.33	

\* Pretreatment conditions are uniformly set to thermal alkaline pretreatment with pH 12 and 70 °C, and heat recovery was not considered.

\*\* The electricity consumption is the energy demand of sludge heated from 20 to 70 °C and the heat recovery rate is 80.

## 5. Conclusion

In this study, results indicate the novel strategy of liquid fermentation in AnDMBR seems reliable for commercial VFAs production from sewage sludge. Firstly, this strategy achieved a very good performance in aspects of VFAs productivity, OLR, substrate conversion rate and VFAs concentration, which reached as high as 7.8 kg/m<sup>3</sup>.d, 17 kg COD/m<sup>3</sup>.d, 0.4–0.6 and 60 g/L, respectively. Then, it presented considerable operation stability with dynamic membrane under relatively high membrane flux, though the microbial communities seemed to be sensitive to the operation mode, membrane separation application and OLRs increase. Also, this strategy showed outstanding in economy and energy consumption. Residual solids in pretreated sludge could hardly contribute to VFAs yield, and energy consumption was reduced from over 100 to below 20 kwh/kg VFAs by avoiding them entering into fermenters.

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## References

- [1] M. Xu, X. Wen, Z. Yu, Y. Li, X. Huang, A hybrid anaerobic membrane bioreactor coupled with online ultrasonic equipment for digestion of waste activated sludge, *Bioresour. Technol.* 102 (2011) 5617–5625.
- [2] H. Liu, P. Han, H. Liu, G. Zhou, B. Fu, Z. Zheng, Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater, *Bioresour. Technol.* 260 (2018) 105–114.
- [3] R. Kleerebezem, B. Joosse, R. Rozendal, M.C.M. Van Loosdrecht, Anaerobic digestion without biogas? *Rev. Environ. Sci. Biotechnol.* 14 (2015) 787–801.
- [4] Y. Chen, X. Jiang, K. Xiao, N. Shen, R.J. Zeng, Y. Zhou, Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH increase—Investigation on dissolved organic matter transformation and microbial community shift, *Water Res.* 112 (2017) 261–268.
- [5] F. Morgan-Sagastume, S. Pratt, A. Karlsson, D. Cirne, P. Lant, A. Werker, Production of volatile fatty acids by fermentation of waste activated sludge pre-treated in full-scale thermal hydrolysis plants, *Bioresour. Technol.* 102 (2011) 3089–3097.
- [6] H. Liu, L. Wang, B. Yin, B. Fu, H. Liu, Deep exploitation of refractory organics in anaerobic dynamic membrane bioreactor for volatile fatty acids production from sludge fermentation: performance and effect of protease catalysis, *J. Environ. Manage.* 217 (2018) 478–485.
- [7] H. Ma, X. Chen, H. Liu, H. Liu, B. Fu, Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: alkaline or neutral pH? *Waste Manage.* 48 (2016) 397–403.
- [8] Q.L. Wu, W.Q. Guo, X. Bao, H.S. Zheng, R.L. Yin, X.C. Feng, H.C. Luo, N.Q. Ren, Enhanced volatile fatty acid production from excess sludge by combined free nitrous acid and rhamnolipid treatment, *Bioresour. Technol.* 224 (2017) 727–732.
- [9] Q.L. Wu, W.Q. Guo, H.S. Zheng, H.C. Luo, X.C. Feng, R.L. Yin, N.Q. Ren, Enhancement of volatile fatty acid production by co-fermentation of food waste and excess sludge without pH control: the mechanism and microbial community analyses, *Bioresour. Technol.* 216 (2016) 653–660.
- [10] L. Huang, Z. Chen, D. Xiong, Q. Wen, Y. Ji, Oriented acidification of wasted activated sludge (WAS) focused on odd-carbon volatile fatty acid (VFA): regulation strategy and microbial community dynamics, *Water Res.* 142 (2018) 256–266.
- [11] J. Garcia Aguirre, E. Aymerich, J. González-Mtnez, de Goñi, M. Esteban-Gutiérrez, Selective VFA production potential from organic waste streams: assessing temperature and pH influence, *Bioresour. Technol.* 244 (2017) 1081–1088.
- [12] H. Liu, Y. Wang, B. Yin, Y. Zhu, B. Fu, H. Liu, Improving volatile fatty acid yield from sludge anaerobic fermentation through self-forming dynamic membrane separation, *Bioresour. Technol.* 218 (2016) 92–100.
- [13] J. Zhang, Y. Xue, N. Eshtiaghi, X. Dai, W. Tao, Z. Li, Evaluation of thermal hydrolysis efficiency of mechanically dewatered sewage sludge via rheological measurement, *Water Res.* 116 (2017) 34–43.
- [14] B. Sajjadi, A.A.A. Raman, R. Parthasarathy, Fluid dynamic analysis of non-Newtonian flow behavior of municipal sludge simulant in anaerobic digesters using submerged, recirculating jets, *Chem. Eng. J.* 298 (2016) 259–270.
- [15] Y. Tang, X. Li, B. Dong, J. Huang, Y. Wei, X. Dai, L. Dai, Effect of aromatic re-polymerization of humic acid-like fraction on digestate phytotoxicity reduction during high-solid anaerobic digestion for stabilization treatment of sewage sludge, *Water Res.* 143 (2018) 436–444.
- [16] Y.F. Zhu, H.B. Liu, H. Liu, S. Huang, H.J. Ma, Y. Tian, Filtration characteristics of anaerobic fermented sewage sludge for fatty acids production, *Sep. Purif. Technol.* 142 (2015) 8–13.
- [17] B. Yin, H. Liu, Y. Wang, J. Bai, H. Liu, B. Fu, Improving volatile fatty acids production by exploiting the residual substrates in post-fermented sludge: protease catalysis of refractory protein, *Bioresour. Technol.* 203 (2016) 124–131.
- [18] L. Zhang, H. Liu, Z. Zheng, H. Ma, M. Yang, H. Liu, Continuous liquid fermentation of pretreated waste activated sludge for high rate volatile fatty acids production and online nutrients recovery, *Bioresour. Technol.* 249 (2018) 962–968.
- [19] L.-p. Fan, D.-d. Xu, C. Li, S. Xue, Molasses wastewater treatment by microbial fuel cell with MnO<sub>2</sub>-Modified cathode, *Pol. J. Environ. Stud.* 25 (2016) 2356–2359.
- [20] H. Liu, Y. Wang, L. Wang, T. Yu, B. Fu, H. Liu, Stepwise hydrolysis to improve carbon releasing efficiency from sludge, *Water Res.* 119 (2017) 225–233.
- [21] State Environmental Protection Administration of China, Standard Methods for the Analysis of Water and Wastewater, China Environmental Science Publishing House, Beijing, 2002.
- [22] M. DuBois, E. Gilles, K.A.J.K. Hamilton, P.A.J. Rebers, F. Smith, Calorimetric method for determination of sugar and related substances, *Anal. Chem.* 28 (2002) 350–356.
- [23] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [24] H. Liu, P. Han, H. Liu, G. Zhou, B. Fu, Z. Zheng, Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater, *Bioresour. Technol.* 260 (2018) 105–114.
- [25] X. Huang, C. Shen, J. Liu, L. Lu, Improved volatile fatty acid production during waste activated sludge anaerobic fermentation by different bio-surfactants, *Chem. Eng. J.* 264 (2015) 280–290.
- [26] X. Li, G. Liu, S. Liu, K. Ma, L. Meng, The relationship between volatile fatty acids accumulation and microbial community succession triggered by excess sludge

- alkaline fermentation, *J. Environ. Manage.* 223 (2018) 85–91.
- [27] Y. Hu, X.C. Wang, H.H. Ngo, Q. Sun, Y. Yang, Anaerobic dynamic membrane bioreactor (AnDMBR) for wastewater treatment: a review, *Bioresour. Technol.* 247 (2018) 1107–1118.
- [28] S. Jaenicke, C. Ander, T. Bekel, R. Bisdorf, M. Dröge, K.H. Gartemann, S. Jünemann, O. Kaiser, L. Krause, F. Tille, M. Zakrzewski, A. Pühler, A. Schlüter, A. Goesmann, Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing, *PLoS One* 26 (2011) 1–15.
- [29] J. Guo, Y. Peng, B.J. Ni, X. Han, L. Fan, Z. Yuan, Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing, *Microb. Cell Fact.* 14 (2015) 33.