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Applying fermentation liquid of food waste as carbon source to a pilot-scale anoxic/oxic-membrane bioreactor for enhancing nitrogen removal: Microbial communities and membrane fouling behaviour

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HIGHLIGHTS

- FLFW was applied as an external carbon source in a pilot-scale A/O-MBR system.
- Bacterial metabolic activities and nitrogen removal were improved.
- Microbial community structure with FLFW addition was studied.
- The addition of FLFW did not aggravate membrane fouling.

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ABSTRACT

Fermentation liquid of food waste (FLFW) was applied as an external carbon source in a pilot-scale anoxic/oxic-membrane bioreactor (A/O-MBR) system to enhance nitrogen removal for treating low COD/TN ratio domestic wastewater. Results showed that, with the FLFW addition, total nitrogen removal increased from lower than 20% to 44–67% during the 150 days of operation. The bacterial metabolic activities were obviously enhanced, and the significant change in microbial community structure promoted pollutants removal and favored membrane fouling mitigation. By monitoring transmembrane pressure and characterizing typical membrane foulants, such as extracellular polymeric substances (EPS), dissolved organic matter (DOM), and inorganics and biopolymers in the cake layer, it was confirmed that FLFW addition did not bring about any additional accumulation of membrane foulants, acceleration of fouling rate, or obvious irreversible membrane fouling in the whole operation period. Therefore, FLFW is a promising alternative carbon source to enhance nitrogen removal for the A/O-MBR system.

1. Introduction

Excess discharge of nitrogen from wastewater treatment plants (WWTPs) into receiving waters is in many cases the major reason for water eutrophication. Therefore, effective biological nitrogen removal (BNR) becomes the task for WWTP upgrading in China (Zhang et al., 2016a; Ge et al., 2012), as well as other countries. In general, two distinctive processes are involved in the BNR, namely aerobic nitrification and anoxic denitrification (Coelho et al., 2000). However, the effectiveness of denitrification, in which nitrates and/or nitrites are transformed to gaseous nitrogen under the action of denitrifying bacteria, depends much on the availability of sufficient organic carbon sources (Mohan et al., 2016; Zhang et al., 2016b). Ineffective denitrification often occurs when the influent to a WWTP is with a low C/N ratio, where C represents carbon concentration, usually in terms of COD, while N represents nitrogen concentration, usually in terms of total nitrogen (TN) (Zhang et al., 2016a,b). In order to supplement sufficient carbon source for effective denitrification, various commercial chemicals, such as methanol, ethanol, acetic acid and glucose, have been utilized as external carbon sources (Frison et al., 2013; Ge et al., 2012). This inevitably increases the treatment cost, and meanwhile

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the risk of effluent quality deterioration (Hiraishi and Khan, 2003). Therefore, alternative ways should be explored for solving such a problem.

For domestic wastewater, the organic fractions are mainly from human consumption of food materials (Huang et al., 2010). The wastes of similar properties not only enter the wastewater stream but also the municipal solid wastes. A strategic means is thus suggestible to turn the organic substances in the solid wastes into a form suitable for application as carbon sources to assist BNR in wastewater treatment. In recent years, many studies have been conducted for using anaerobic fermentation products from the organic fractions of municipal solid wastes to enhance nitrogen removal (Zhang et al., 2016b; Frison et al., 2013; Li et al., 2011). Of the municipal solid wastes, food wastes such as those from canteens and restaurants are usually rich in organic contents. It was found that the fermentation liquid from food waste (FLFW) contained various carbohydrates and organic acids, and was a good external carbon source to enhance denitrification (Zhang et al., 2016b). In addition to the improvement of TN removal, the utilization of FLFW also improved the bacterial metabolic activities, and enriched the microbial communities to degrade complicated organics (Zhang et al., 2015; Zhang et al. 2016a). However, few information has been available so far on how the microbial community variation may affect nitrogen removal.

Fermentation products have also been applied in membrane bioreactors (MBRs) for enhancing nitrogen removal and wastewater reuse (Tannock and Clarke, 2016). Although the TN removal efficiency is remarkable, there is a concern on whether or not the application of the fermentation products may aggravate membrane fouling due to the possible increase in fouling-causing substances, such as extracellular polymeric substances (EPS) and dissolved organic matter (DOM) (Trussella et al., 2006; Gao et al., 2014; Mannina et al., 2016; Hao et al., 2016). It was reported that more soluble EPS (SEPS) were released with the increase of organic loading rate (Trussella et al., 2006), while there were also reports on the tendency of EPS increase with decreasing C/N ratio of the influent, along with more serious membrane fouling (Mannina et al., 2016; Hao et al., 2016). In addition to EPS which is recognized as the predominant membrane foulants in MBRs (Mannina et al., 2016; Hao et al., 2016; Tang et al., 2015), the composition of the carbon sources has been found to affect the microbial structure and bacterial metabolism as well (Gao et al., 2014), including the growth of membrane fouling bacterial (MFB) (Miao et al., 2016). All these have indicated the need for a comprehensive study on the specific effect of fermentation product on membrane fouling when it is used as external carbon source for enhancing nitrogen removal in an MBR system in the long term operation.

In the present study, fermentation liquid from the food wastes collected from a university canteen was used as external carbon source to a pilot-scale A/O-MBR system treating the campus wastewater with low C/N ratio. As the food wastes and the wastewater were generated from the same community, the main objective was to investigate the effect of FLFW addition on the performance of the A/O-MBR system under such a specific condition. Attention was paid not only to nitrogen removal but also microbial community and membrane fouling.

2. Materials and methods

2.1. Fermentation liquid from food waste (FLFW)

Food wastes were collected from the student canteen in a university campus in Xi'an, China. They were applied for producing the FLFW under 55 °C using an anaerobic semi-continuous stirring tank reactor (110 L) at uncontrolled pH in a way reported in the authors' previous study (Tang et al., 2015) and details are given in the Supporting information. Table 1 shows the chemical properties of the FLFW. Its organic fractions mainly included carbohydrates, proteins and organic acids. The TN and TP contents were relatively low so that the addition of FLFW into the bioreactor might not bring about significant increase of nutrient concentration.

2.2. A/O-MBR operation with FLFW as external carbon sources

A pilot-scale A/O-MBR system was used for the experimental study (Fig. S1). Its total working volume was 8 m^3, with equally partitioned volumes for the anoxic, oxic and membrane tanks. The membrane tank was equipped with a Polyvinylidene Fluoride (PVDF) hollow fiber membrane module (FP-AI, Tianjin MOTIMO Membrane Technology Co., Ltd, China). The specific area of the membrane module was 8 m^2, with a nominal pore size of 0.2 μm. The influent was the domestic wastewater from the same campus with average COD and TN concentrations as 150 mg/L and 26.5 mg/L, respectively, showing a C/N ratio only about 5.5 which was insufficient to provide a favorable BNR condition. To supplement external carbon sources, FLFW was mixed with the influent at the inlet to the A/O-MBR system. Permeate was extracted from the MBR tank by suction pumps (WB70/055-B, Guangdong Yuehua pump Co., Ltd, China) at fixed flow rates under an intermittent operation mode (8 min on/2 min off) for membrane relaxation. Continuous aeration was supplied to the oxic tank to maintain the dissolved oxygen (DO) concentration at 2–4 mg/L. The MBR tank was also aerated for membrane scouring. The transmembrane pressure (TMP) was recorded using an on-line pressure meter (GLP-2000, Beijing). Membrane cleaning was conducted when TMP reached 25 kPa or for particular needs (Hu et al., 2013). The membrane was manually washed with tap water, and then chemical cleaning was performed using sodium hypochlorite (3000 mg/L) for 8 h and citric acid (1%) for 2 h. A perforated plate was placed between the oxic tank and the membrane tank so that similar oxic condition could be maintained. Recirculation was provided for circulating the mixed liquor between the oxic tank and anoxic tank with a ratio of 2. Excess sludge was discharged daily from the membrane tank to maintain the SRT at 30–40 days in the whole experimental period.

The A/O-MBR system was operated for 150 days which included five phases with steady flow rate but varied FLFW dose or HRT as shown in Table 2. In Phase I, the system was operated with HRT=6 h and without FLFW addition (C/N = 5.5), while in Phases II and III, C/N ratio was adjusted to 9.5 and 13.7, respectively by dosing FLFW. In Phases IV and V, HRT was increased to 8 h and C/N ratio was adjusted to 9.3 and 14.2, respectively. The mixed liquor suspended solids (MLSS) in all these operation phases was 4500–4800 mg/L.

Table 1
Physical and chemical parameters of the FLFW.

Parameters	Units	FLFW
pH	–	3.5 ± 0.2
COD	g/L	82.6 ± 7.9
SCOD	g/L	32.1 ± 4.5
VFAs	%COD	6.8 ± 1.2
Lactate	%COD	9.5 ± 0.8
Protein	%COD	18.4 ± 3.1
Carbohydrates	%COD	50.4 ± 2.6
TN	g/L	0.9 ± 0.6
TP	g/L	0.2 ± 0.1

Note: VFAs: volatile fatty acids, ±meant standard deviation.

2.3. Analytic methods

2.3.1. Water quality measurement

Influent wastewater, permeate and mixed liquor were periodically sampled from the A/O-MBR system. Nitrogen compounds, COD, PO_4^{3-}-P, and MLSS were measured following the standard methods (APHA, 1998).

2.3.2. EPS extraction and analysis

EPS was extracted from the bulk sludge in the membrane tank once per ten days by thermal treatment method (Hu et al. 2013). The contents of the extracted EPS samples were analyzed in terms of proteins and polysaccharides. The polysaccharides content was determined by anthrone method with glucose as the standard reference (Frølund et al., 1996), and the proteins content was quantified by a modified Lowry method with bovine serum albumin (BSA) as the standard reference (Lowry et al., 1951).

2.3.3. Three-dimensional excitation-emission matrix fluorescence spectra (3D-EEM)

To characterize DOM components, samples were collected from the influent, anoxic tank, oxic tank, membrane tank and effluent. After centrifuging (5000 r/min for 10 min, 4 °C) and filtering through 0.22-μm filters, the filtrate was analyzed using a FP-6500 spectroflurometer (Jasco Corporation, Japan). The emission spectra of the EEM were scanned with the excitation wavelength varied from 220 to 550 nm in 5 nm increment. Subsequently, the EEM spectra were assessed using Origin Pro 8.0 software (Origin Lab Corporation, USA) and elliptical shape contours were obtained.

2.3.4. Fourier transform infrared (FTIR) spectroscopy

To identify the organic foulants, bulk sludge and cake layer sludge sampled from the fouled membranes were oven-dried at 60 °C for 24 h (Hu et al., 2013). The dried sample was analyzed with a Fourier transform infrared (FTIR) spectrometer (Nicolet 4700 FTIR, Thermo Electron Corporation, Madison, WI) using the attenuated total reflection (ATR) method with the wave number ranging from 4000 to 400 cm^{-1}.

2.3.5. Scanning electron microcopy (SEM) and energy-diffusive X-ray (EDX)

Membrane fibers before and after chemical cleaning were cut from the membrane model and pretreated according to Hu et al. (2013). Briefly, the membrane samples were fixed with a 2.5% glutaraldehyde solution; carefully washed in the 0.1 M phosphate buffer at pH 7.2; sequentially dehydrated with 50%, 70% and 100% ethanol; and subsequently washed three times with tert-butyl alcohol for 10 min each time. After vacuum freeze-drying using a freeze drier (2.5 L, Labconco, USA), the samples were gold-coated using a sputter coater (JFC-1100E, JEOL, Japan) and subsequently observed using scanning electron microcopy (SEM) (Quanta 600 FEG, FEI Corporation, USA). The elements on the membrane surface were measured using NORAN System SIX X-ray spectroscopy (Thermo Scientific, USA).

2.3.6. Biolog plate analysis

The metabolic characteristics of microorganisms in the sludge were assessed using the Biolog-ECO plates (Biolog, Inc., Hayward, CA, USA) as described by Kong et al. (2013). Two activated sludge samples were collected from the aerobic tank: one before the FLFW addition (Day 25) and another after the FLFW addition (Day 130). Each sample was diluted to 1:1000 with sterilized NaCl solution (0.9%, w/v) and shaken four times for 15 s. The resulting suspension of 1 mL was diluted with saline solution to control the optical density (OD) close to 0.05 at 600 nm to ensure that the two sample solutions contained approximately the same biomass concentration. Then 150 μL of the diluted mixture was added to the Biolog plate well using eight channel pipettes and the plates were inoculated at 25 °C in darkness. The absorbance (OD = 590 nm) of the wells was recorded for 168 h using an ELISA plate reader at 24-h interval. Data were processed according to Zhang et al. (2016a) and Kong et al. (2013).

2.3.7. Microbial community analysis

The sludge samples described in Section 2.3.6 were also sent to Sangong, Inc. (Shanghai, China) for DNA extraction and next-generation sequencing. The extracted DNA was amplified by PCR using the primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′ -GWATTACCGCGGCKGCTG-3′) targeting the V1-V3 region of the 16S rRNA genes (Luo et al., 2017). Pyrosequencing was conducted using an Illumina MiSeq platform. The homologous or ambiguous sequences, or those 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.

2.3.8. Additional analyses

As an indicator of sludge settleability, the sludge volume index (SVI) was measured according to standard methods (APHA, 1998), and dewaterability was analyzed using a capillary suction timer (294-50, OFI Testing Equipment, Inc., USA). The particle size distribution of the sludge flocs in the membrane tank was analyzed using a laser granularity distribution analyzer (LS 230/SVM+, Beckman Coulter Corporation, USA) with a detection range of 0.4–2000 μm. The viscosity of the mixed liquor was measured with viscosity meter (NDJ-79, Shanghai precision instrument co., LTD, Shanghai, China).

3. Results and discussion

3.1. Pollutants removal

Prior to applying the FLFW as external carbon source into the A/O-MBR, the denitrification characteristics of the FLFW were analyzed by a series of nitrate uptake rate (NUR) tests (Fig. S2, Supporting Information). It was found that the FLFW showed a high denitrification rate and potential (Table S1, Supporting Information). Using specific denitrification rate (SDNR) as a parameter, the FLFW prepared in this study apparently showed higher denitri-

Table 2
Operational parameters and influent characteristics of the pilot-scale A/O-MBR.

Phase	Operation period (d)	HRT (h)	FLFW dose (L/m^3)	C/N ratio	Influent (mg/L)				
					COD	TN	NH_4^+-N	TP	PO_4^{3-}
I	1–31	6	0	5.5	150 ± 20	26.5 ± 3.8	25.0 ± 3.7	2.5 ± 0.2	2.3 ± 0.1
II	32–52	6	1.5	9.5	250 ± 22	24.9 ± 2.9	23.7 ± 2.8	3.1 ± 0.5	3.0 ± 0.2
III	53–83	6	2.5	13.7	350 ± 33	21.5 ± 2.7	20.7 ± 2.7	2.4 ± 0.8	2.3 ± 0.3
IV	84–112	8	1.5	9.3	250 ± 11	30.8 ± 4.6	29.1 ± 4.2	2.7 ± 0.5	2.6 ± 0.1
V	113–150	8	2.5	14.2	350 ± 33	22.5 ± 4.3	21.4 ± 3.4	2.6 ± 0.6	2.1 ± 0.4

fication rate (Table 3). Rich in biodegradable organic fractions might be the reason for this.

Table 4 shows the pollutants removal when FLFW was used as carbon source to the A/O-MBR system and Fig. 1 shows the variations of NH_4^+-N, NO_3^--N and TN in the whole operation period. Regardless of the fluctuation of NH_4^+-N in the influent (Fig. 1a), very low NH_4^+-N concentration (less than 0.5 mg/L) was detected from the effluent, and the removal of NH_4^+-N were very stable under all conditions (>98%). However, NO_3^--N concentration varied much in different operation phases. Prior to FLFW addition (Phase I), due to insufficient organic carbon in the influent, NO_3^--N in the anoxic tank could not be denitrified and remained a concentration as high as about 13 mg/L (Fig. 1b), resulting in a still higher NO_3^--N concentration in the MBR effluent (about 20 mg/L). The addition of FLFW in Phase II obviously enhanced denitrification for the reduction of NO_3^--N concentration in the anoxic tank to approximately 7 mg/L, along with a lower NO_3^--N concentration in the MBR effluent (about 12 mg/L). When FLFW dose was increased (Phase III), NO_3^--N concentration in the anoxic tank was further reduced to about 3 mg/L, indicating that sufficient carbon source could result in higher denitrification capacity. Under longer HRT (Phases IV and V), the effect of higher FLFW dose for enhancing denitrification was also remarkable.

The variation of the effluent TN further shows the overall results of nitrogen removal in different operation phases (Fig. 1c). In Phase I the average TN removal was merely 18.1% due to the low C/N ratio without FLFW addition, while in Phases II and III, the average TN removals increased to 47.8% and 58.1%, respectively, due to FLFW addition. Under higher HRT (Phases IV and V), the TN removals were 44% and 67.8%, respectively, indicating that the effect of FLFW addition on nitrogen removal was positive regardless the change in HRT. The high organic content of the FLFW could provide enough carbon sources to denitrifying bacteria, so that denitrification process was enhanced and the TN removal was much improved (Mohan et al., 2016).

In the whole operation period, the effluent COD was relatively stable (between 18 and 27 mg/L) and COD removal was over 90% except for Phase I as 86.7% (Table 4), indicating that the organics supplemented to the A/O-MBR system by FLFW addition might be more biodegradable. Regarding phosphorus in terms of PO_4^{3-}-P and TP (Table 4), FLFW addition also brought about their enhanced removal from about 40% (Phase I without FLFW addition) to 52–69% (Phases II-V). The higher TP removal might be resulted from sufficient organics for phosphate release and substrate storage under anoxic conditions and subsequently increased phosphate uptake under oxic conditions (Mannina et al., 2016).

3.2. Variations of microbial communities

3.2.1. Microbial metabolic characteristics

To characterize the microbial metabolic activity of the microorganisms in the activated sludge before and after FLFW addition, the developments of average well-color development (AWCD) during incubation were investigated. As shown in Fig. 2a, AWCD rapidly increased in the first 48 h, and approached constant values after 96 h for both the samples before and after FLFW addition. However, with FLFW addition, the final AWCD value (about 1.03 cm^{-1}) was higher than that without FLFW addition (about 0.89 cm^{-1}), indicating more rapid bacterial growth and higher microbial metabolic activity due to the availability of sufficient organic carbon from the added FLFW (Kong et al., 2013). Higher diversity of microbial communities might be the reason for the higher metabolic activity (Zhang et al., 2016a).

Instead of using data for each well individually, the substrates can be assigned to different organic groups such as alcohols, amines, amino acids, carbohydrates, carboxylic acids, esters and polymers. The average absorbency fraction at 120 h for each group could be calculated in the same way as that for all the plates. As shown in Fig. 2b, no obvious difference was found between the distributions of the substrates for the two sludge samples, indicating that the organic components in the wastewater and the added FLFW were very similar, possibly because the wastewater and the raw materials for producing the FLFW, namely the food wastes, were from similar origin. The higher microbial metabolic activity after FLFW addition might be mainly due to the increasing quantity of available organic carbon, rather than any change in the organic components.

3.2.2. Microbial community structure

Microbial communities in the activated sludge samples were investigated using the next-generation sequencing technique. As shown in Fig. 3a, four predominant phyla identified from the two samples were Proteobacteria, Chloroflexi, Bacteroidetes and Acidobacteria. The sum of these phyla accounted for 86.9% in the sludge sample before FLFW addition, while it increased to 94.6% after FLFW addition, which provided explanation for the higher substrate utilization capacity (Fig. 2). After FLFW addition, Chloroflexi increased from 7.5% to 23.4%, while the relative abundance of other phyla apparently decreased (from 61.2% to 56.1% for Proteobacteria, 16.2% to 14.3% for Bacteroidetes, 1.9% to 0.8% for Acidobacteria), indicating a significant change in microbial communities. Chloroflexi are recognized as a phylum responsible for the degradation of soluble microbial products (SMP) such as carbohydrates and cellular materials, so that its increase might benefit membrane fouling control (Miura et al., 2007). On the other hand, Proteobacteria has been found to be a dominant phylum of fouling causing bacteria in MBRs to play the role to induce biocake formation (Ishizaki et al., 2016). Therefore, the increase of Chloroflexi and decrease of Proteobacteria after FLFW addition might be advantageous for mitigating membrane fouling. With the higher proportion of heterotrophic bacteria, the relative abundance of nitrifying bacteria (Nitrospirae and Planctomycetes) decreased after FLFW addition, while little influence was noticed to the nitrification results (Table 4). Other phyla, such as Verrucomicrobia, Firmicutes, Actinobacteria and Gemmatimonadetes, were also detected but their relative abundances were below 1%.

Selective enrichment was noticed at the family level for the bacteria. As shown in Fig. 3b for the top 22 families with relative abundance higher than 1%, within the phylum of Proteobacteria, Xanthomonadaceae decreased from 19.0% to 11.2%, while

Table 3
Comparison of SDNR for different carbon sources.

Carbon source	C/N ratio	Main components	Denitrification rate $mgNO_3$-N/(gVSS·h)	References
Fermented primary sludge	10.0	VFAs, carbohydrate, protein	1.4	Soares et al., 2010
Disintegrated surplus activated sludge	9	Carbohydrate, protein, VFAs	1.9	Soares et al., 2010
Acidogenesis from food waste	4	VFAs	3.6	Lim et al., 2008
Sodium acetate	4	Acetate	4.7	Lim et al., 2008
FLFW	6	Carbohydrate, protein, VFAs, Lactate	5.1	This study

Table 4
Effluent quality and pollutants removal in different operation phases.

Phase	Effluent (mg/L)					Removal efficiency (%)				
	COD	TN	NH_4^+-N	TP	PO_4^{3-}-P	COD	TN	NH_4^+-N	TP	PO_4^{3-}-P
I	20 ± 5.0	21.6 ± 2.2	0.5 ± 0.1	1.5 ± 0.5	1.4 ± 0.12	86.7 ± 3.2	18.1 ± 5.9	98.2 ± 1.6	40.0 ± 20	41.7 ± 5.0
II	24 ± 5.3	12.8 ± 1.4	0.3 ± 0.1	1.1 ± 0.2	0.9 ± 0.25	90.4 ± 2.5	47.8 ± 6.3	98.8 ± 0.4	64.5 ± 6.5	69.0 ± 8.6
III	26 ± 6.0	9.0 ± 2.5	0.3 ± 0.1	0.9 ± 0.4	0.8 ± 0.23	92.6 ± 4.1	58.1 ± 9.8	98.3 ± 0.8	62.5 ± 11	65.2 ± 10.0
IV	18 ± 4.6	17.0 ± 2.9	0.4 ± 0.2	1.2 ± 0.5	1.1 ± 0.18	92.8 ± 4.6	44.0 ± 11.2	98.3 ± 0.8	55.6 ± 18.5	57.7 ± 6.9
V	27 ± 4.5	6.2 ± 1.6	0.4 ± 0.2	1.1 ± 0.3	1.0 ± 0.25	92.3 ± 2.6	67.8 ± 8.1	98.2 ± 0.5	52.2 ± 13.0	52.4 ± 11.9

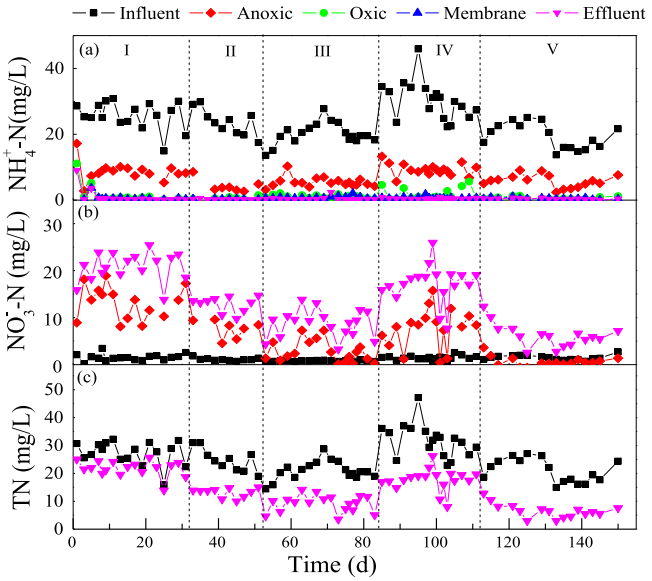

Fig. 1. Variations of NH_4^+-N (a), NO_3^--N (b) and TN (c) in the A/O-MBR system during the operation period.

Rhodocyclaceae and Comamonadaceae increased from 10.7% and 9.7% to 14.8% and 13.0%, respectively. The three families were dominant in both sludge samples. Other families e.g. Pseudomonadaceae, Moraxellaceae and Sphingomonadaceae showed slight increase after FLFW addition. As a member participating in denitrification (Miao et al., 2016), the increase of Comamonadaceae was a proof of the high denitrification potential after FLFW addition. Additionally, Anaerolineaceae could degrade macromolecular organics (e.g. glucose) to provide more available carbon sources for denitrification (Miao et al., 2016). Therefore, the accumulation of Anaerolineaceae could further strengthen microbial metabolic activity and enhance nitrogen removal (Fig. 2). Among the phylum of Bacteroidetes, the relative abundance of Saprospiraceae, Cryomorphaceae and Cytophagaceae varied little, while Chitinophagaceae decreased from 5.4% to 1.5% and Flavobacteriaceae increased from 1.5% to 4.2% after FLFW addition.

At the genus level, as shown in Table 5, the relative abundance of *Ornatilinea* (a genus of Anaerolineaceae) increased from 0.73% to 22.87%, while *Zoogloea* and *Thauera* (genera of Rhodocyclaceae) increased from 0.5% and 3.0% to 2.8% and 8.7%, respectively, after FLFW addition. These genera are believed to be metabolically versatile in wastewater treatment (Ma et al., 2013). Moreover, the relative abundance of *Aquabacterium*, a facultative aerobe capable of using oxygen or nitrate as electron acceptors (Sutton et al., 2009), increased from 0.11% to 2.48%, and *Thermomonas,* a denitrifying bacterium in wastewater treatment (Mcilroy et al. 2016), increased from 0.52% to 2.8%. All these could further explain the reason for the high microbial metabolic activities and high nitrogen removal after FLFW addition (Fig. 2).

3.3. Characteristics of membrane fouling

3.3.1. Membrane filtration performance

Fig. 4 shows the variations of TMP and the calculated fouling rate based on TMP data. In Phase I (Day 1 to Day 31) without FLFW addition, the TMP was approximately 10.4 kPa at the start and increased to 17.3 kPa at the end, so that the fouling rate was calculated as 173 Pa/d. However, in Phase II (Day 32 to Day 52) with FLFW addition, TMP increased from 10.4 kPa to 22.0 kPa, showing

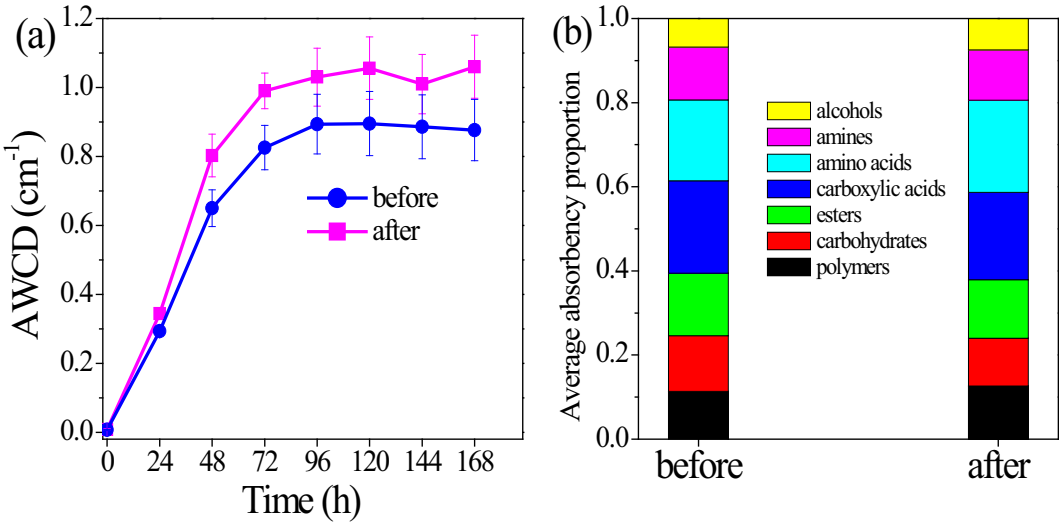

Fig. 2. Microbial metabolic characteristics of the activated sludge before and after FLFW addition.

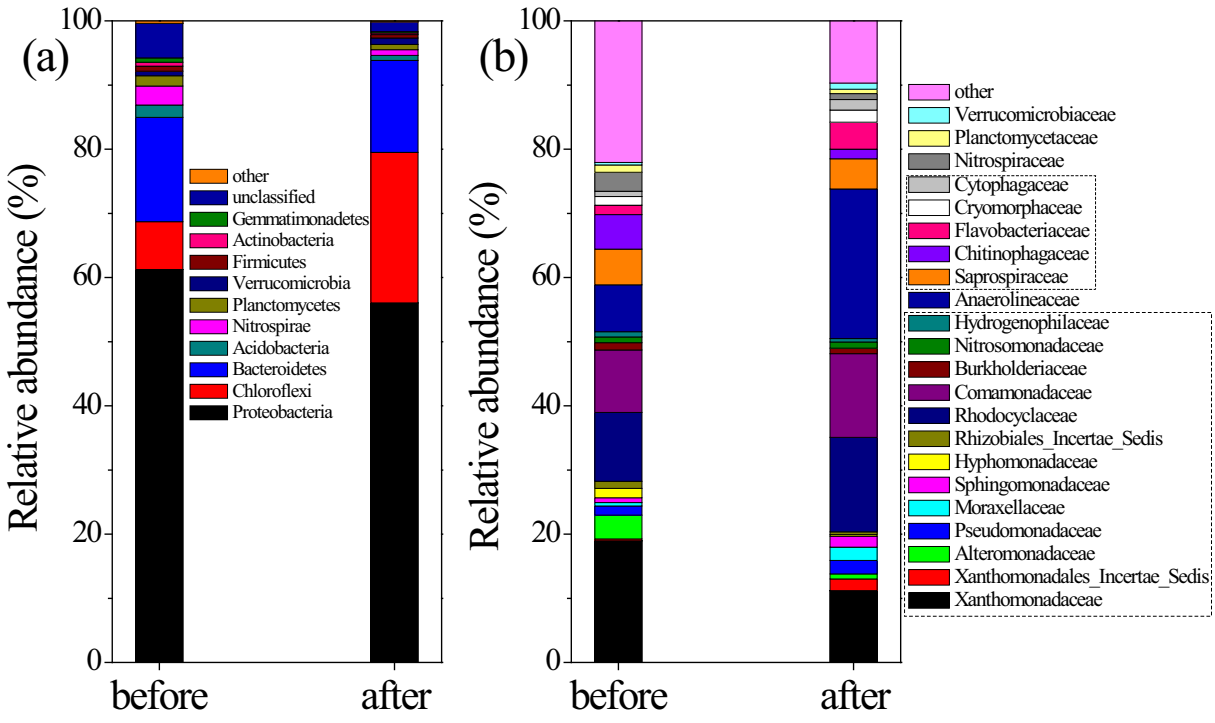

Fig. 3. Microbial community structure at phylum (a) and family (b) levels in sludge samples before and after FLFW addition.

Table 5
The relative abundance of microbial communities at genus level before and after the addition of FLFW.

					Unit ,%	
Phylum	Class	Order	Family	Genus	Before	After
Acidobacteria	Acidobacteria	Subgroup_4	–	*Blastocatella*	1.72	0.69
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	*Ornatilinea*	**0.73**	**22.87**
Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	*Crocinitomix*	1.22	1.83
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	**0.93**	**3.88**
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	*Ferruginibacter*	2.32	0.18
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	*Nitrospira*	3.06	0.91
Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	*Woodsholea*	1.19	0.12
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales_Incertae_Sedis	*Bauldia*	1.12	0.41
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	*Lautropia*	0.83	0.45
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	2.67	0.96
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Aquabacterium*	**0.11**	**2.48**
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas*	1.24	0.79
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Simplicispira*	0.82	1.12
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	0.61	0.69
Proteobacteria	Betaproteobacteria	Hydrogenophilales	Hydrogenophilaceae	*Thiobacillus*	0.63	0.45
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Azoarcus*	2.35	1.19
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Azospira*	2.57	0.37
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Sulfuritalea*	1.6	0.02
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Thauera*	**2.96**	**8.65**
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Zoogloea*	**0.52**	**2.83**
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	*Haliangium*	0.8	0.37
Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*BD1-7_clade*	0.7	0.66
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	0.47	0.94
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Alkanindiges*	0	1.05
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	1.47	2.12
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Arenimonas*	4.42	1.77
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Dokdonella*	4.73	0.15
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Lysobacter*	**0.41**	**3.98**
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	**0.52**	**2.8**

a slightly higher fouling rate of 185 Pa/d. Increasing the dose of FLFW in Phase III (Day 53 to Day 83) did not aggravate membrane fouling and the fouling rate returned back to 173 Pa/d.

Chemical cleaning was conducted on Day 71 to recover TMP from 25.2 kPa to 16.2 kPa. For the preparation of operation under higher HRT (Phases IV and V with HRT increased from 6 h to 8 h),

chemical cleaning was conducted again and TMP was further recovered to 11.1 kPa, indicating an effective removal of foulants from the membrane and restoration of membrane permeability (Mannina et al., 2016). The membrane fouling rate was calculated as 105 Pa/d in Phase IV (Day 84 to Day 112) and 99 Pa/d in Phase V (Day 113 to Day 150). FLFW addition and HRT increase did not result in any additional membrane fouling.

Based on the TMP data, total membrane resistance (R) could also be calculated (Table S2, Supporting information). R increased from $19.4 \times 10^{10}\ m^{-1}$ to $28.2 \times 10^{10}\ m^{-1}$ in Phase I, and further increased to $40.4 \times 10^{10}\ m^{-1}$ before the first chemical cleaning. After chemical cleaning on Day 71 and Day 83, R decreased to $25.5 \times 10^{10}\ m^{-1}$ and $21.9 \times 10^{10}\ m^{-1}$, respectively. Afterwards, R slightly increased from $21.9 \times 10^{10}\ m^{-1}$ to $34.8 \times 10^{10}\ m^{-1}$ in Phase IV, and from $33.9 \times 10^{10}\ m^{-1}$ to $41.8 \times 10^{10}\ m^{-1}$ in Phase V.

3.3.2. EPS and DOM

Fig. 5 shows the EPS contents in terms of polysaccharides and proteins for both soluble EPS (SEPS) and bound EPS (BEPS) in each operation phase. Comparing with Phase I without FLFW addition, no significant accumulation of SEPS was found in the membrane tank in the following operation phases (Fig. 5a). As SEPS may easily attach onto the membrane and lead to membrane fouling (Trussella et al., 2006; Mannina et al., 2016; Pan et al., 2010), the relatively low SEPS in the whole operation period was an indication of low level of foulants in the MBR. Comparing with polysaccharides, the protein concentration was higher. This provided another evidence for the low level of foulants because polysaccharides are found to show higher fouling potential (Trussella et al., 2006; Pan et al., 2010).

BEPS content in the membrane tank was more stable in the whole operation period and the protein fraction was much higher than the polysaccharides fraction (Fig. 5b). Furthermore, the total BEPS was about one order higher than SEPS. Generally speaking, the fouling potential of BEPS was lower than that of SEPS (Trussella et al., 2006; Pan et al., 2010). Therefore, BEPS as the main component of EPS in the MBR was also an indication of low level of membrane foulants.

Fluorescent organic matter was also important membrane foulant (Shao et al., 2014), and should be focused on its variation after FLFW addition. Fig. 6 shows the 3D-EEM fluorescence spectra along the A/O-MBR array. For the influent, there were three distinctive

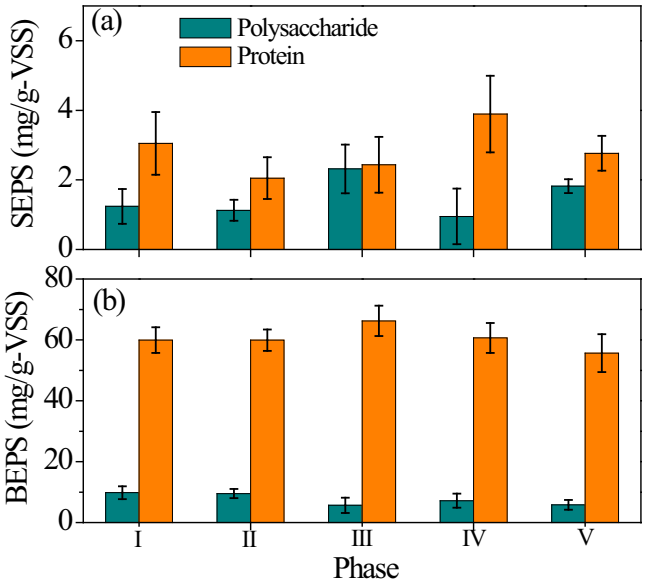

Fig. 5. EPS content in the membrane tank during operation: (a) SEPS; (b) BEPS.

peaks at excitation/emission wavelengths (Ex/Em) of 230/334 nm (peak A), 285/340 nm (peak B) and 355/436 nm (peak C). Peak A and peak B were associated with tyrosine aromatic protein-like substances and tryptophan protein-like substances, respectively (Chen et al., 2003), while peak C could be described as visible humic acid-like fluorescence. For the samples from the anoxic, oxic, and membrane tanks, the three distinctive peaks were still visible, but the fluorescence intensity (FI) of the protein-like substances (peak A and peak B) apparently decreased along the array (Table 6), possibly due to their biodegradation. The decrease of these protein-like substances might have much benefited membrane fouling control. However, comparing with the FI of the tryptophan protein-like substances (peak B) which decreased from 926.2 in the influent to 530.1 in the membrane tank, the reduction in FI for the tyrosine aromatic protein-like substances (peak A) was slight (from 220.7 in the influent to 155.5 in the membrane tank), indicating that the tyrosine aromatic protein-like substances were less biodegradable. Nevertheless, for the effluent sample both peak

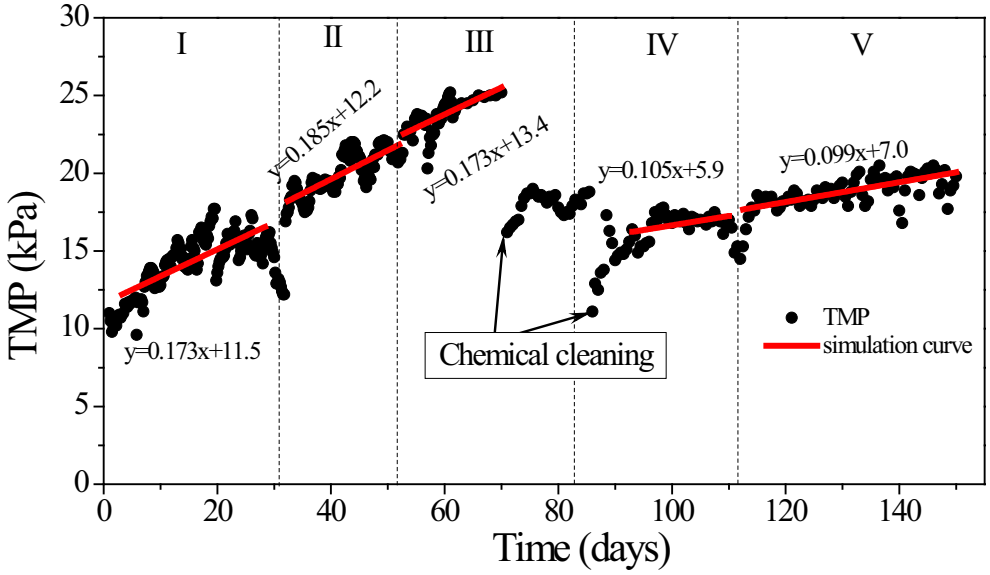

Fig. 4. Variations of TMP and fouling rate during operation period.

A and peak B were no longer distinctive, possibly because most of the protein-like substances in the membrane tank were retained by the cake layer and membrane fiber (Hu et al., 2013). On the contrary, peak C was visible in all the samples and the corresponding FI slightly increased along the array. This might be due to the production of humic-like substances of low molecular weights as biological byproducts (Hu et al., 2013).

3.3.3. Foulants retained in the cake layer

To investigate the membrane foulants retained in the cake layer, FTIR analysis was conducted for identifying the main functional groups of biopolymers (Fig. S3, Supporting Information). As a result, a number of regions of adsorption and peaks were identified on the FTIR spectra including a broad region of adsorption at 3285 cm^{-1} due to the stretching of the O–H bond in hydroxyl functional groups (Kumar et al., 2006), peaks near 2923 and 2852 cm^{-1} representing aliphatic methylene groups in fats and lipids (Bell et al., 2016), peak at 2923 cm^{-1} due to the stretching of the C–H bonds (Hu et al., 2013; Kumar et al., 2006). Many protein related ranges were also identified, such as amide I (1700–1600 cm^{-1}), amide II (1600–1500 cm^{-1}), and amide III (1300–1200 cm^{-1}) and two peaks at 1640 cm^{-1} and 1530 cm^{-1}, all indicating that proteins were accumulated in the cake layer. Polysaccharides or polysaccharide-like substances were identified from the cake layer sludge as shown by the adsorption range of 1200–900 cm^{-1} (Badireddy et al., 2008), the sharp peak around 1020 cm^{-1} (Bell et al., 2016) and the absorption peaks less than 1000 cm^{-1} (Chu et al., 2014). Based on the FTIR spectra, the cake sludge showed much higher absorbance than the bulk sludge, indicating that many potential membrane foulants such as proteins and polysaccharides were accumulated in the cake layer so that direct contact of these foulants with the membrane surface could be alleviated.

3.3.4. Membrane surface observation

As shown in Fig. 4, after chemical cleaning TMP increased in much slower rate. To investigate the reason underlying this phenomenon, the outer surface of the membrane before and after chemical cleaning was observed by SEM (Fig. S4, Supporting Information). It was seen that prior to chemical cleaning, the membrane was covered with a cake layer of rough surface and uneven structure (Fig. S4A), and the pores on the membrane surface were hardly visible, while after chemical cleaning, the surface tended to be smooth with the pores clearly observable (Fig. S4B). This clearly indicated the effect of chemical cleaning for removing the fouled layer on the membrane surface and bringing about TMP reduction (Fig. 4).

The EDX spectra further revealed the effect of chemical cleaning. The main elements detected from the fouled membrane surface included C, F, Cl, Mg, Al, Si, Ca and Fe (Fig. S4C). Metal ions could easily precipitate with biopolymers or carbonates on the membrane surface and significantly impact the formation of the cake layer (Meng et al., 2007). However, after chemical cleaning using NaClO and citric acid, metal ions were no longer detectable, and F and C became the main elements detected from the membrane (Fig. S4D). It was reported that NaClO could effectively remove organic foulants, while citric acid primarily removed inorganic foulants from the membrane (Le-Clech et al., 2006). Both the SEM observation and EDX analysis proved that in this study FLFW addition did not cause serious irreversible membrane fouling, and the foulants could be easily eliminated by chemical cleaning.

Table 6
The fluorescence intensity (FI) of the DOM based on the 3D-EEM.

Sample	Peak A (Ex/Em = 230/334)	Peak B (Ex/Em = 285/340)	Peak C (Ex/Em = 355/436)
Influent	220.7	926.2	612.5
Anoxic tank	190.1	572.2	628.1
Oxic tank	159.1	531.8	651.0
Membrane tank	155.5	530.1	668.8
Effluent	69.9	291.5	722.1

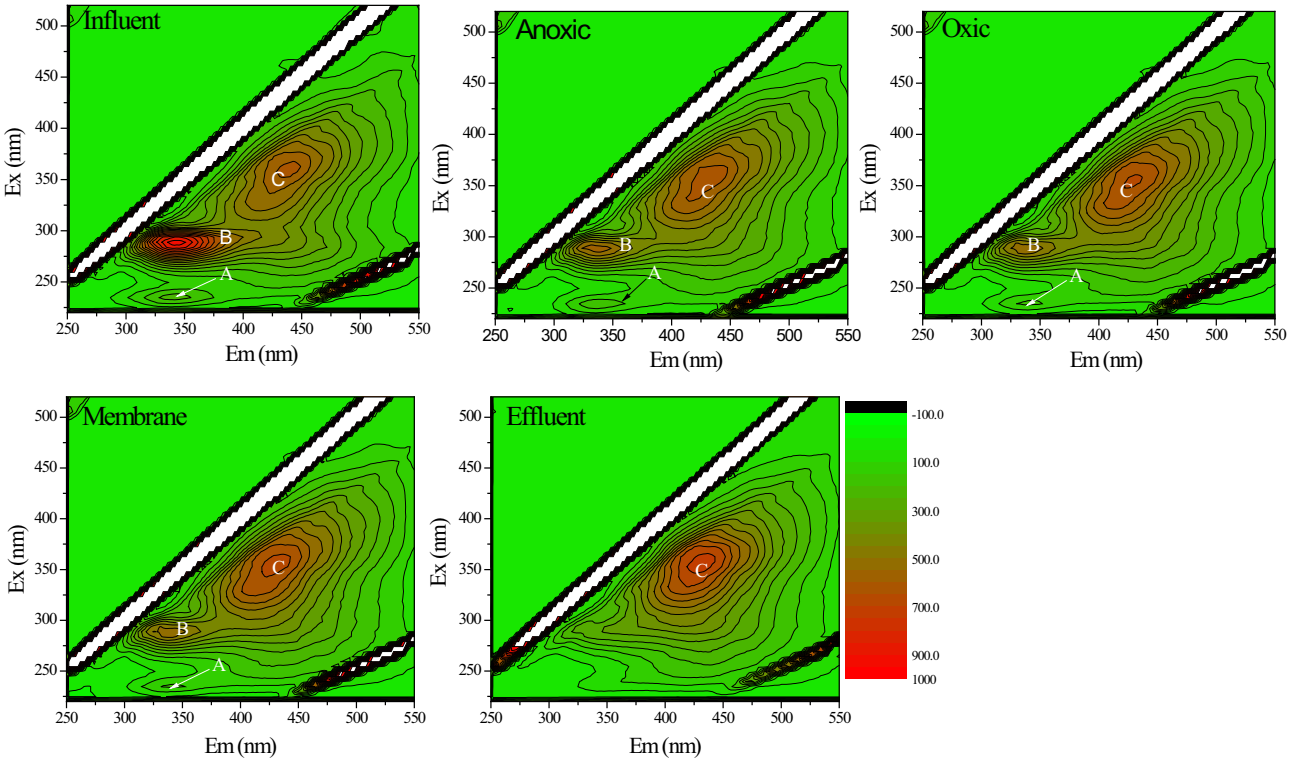

Fig. 6. 3D-EEM fluorescence spectra of DOM along the A/O-MBR array.

3.4. Perspective of using FLFW as external carbon source for MBR systems

The effects of using FLFW as external carbon source for enhancing denitrification in wastewater treatment has been reported in many studies but there have been different views on the feasibility for its application in MBR systems because membrane fouling is always a factor restricting the addition of organic substances of unknown composition which may either directly increase membrane foulants to MBRs or bring about the generation of additional foulants during the biological process. For this issue, the current study provided clear evidence that the FLFW addition might not aggravate membrane fouling because of the low concentration of typical foulants such as EPSs and DOMs in the membrane tank, accumulation of foulants in the cake sludge, and easiness of foulants removal from the membrane surface by chemical cleaning to restore TMP. As the A/O-MBR pilot system was operated continuously for 150 days with an obvious enhancement in nitrogen removal, the experimental work could simulate the practical condition of a full-scale MBR to a great extent. Furthermore, from the result of microbial metabolic activity characterization (Fig. 2) it was identified that there was no obvious difference between the organic components in the wastewater and the added FLFW so that FLFW addition only enriched the available carbon source for the denitrifying bacteria but not result in significant change in their 'food type'. The reason was, at least in this study, that the organic substances in the wastewater and the raw materials for producing the FLFW were from similar origin (in this study the food materials consumed by people in the university campus).

According to statistic data, in China 6.0×10^7 tons of food wastes were generated annually but only 20% were properly treated and reused. Arbitrary disposal of large amount of food wastes poses a threat to food safety and human health (Zhang et al., 2010). If these food wastes can be utilized to produce FLFW, they are good alternative carbon sources to meet the national needs for enhancing nitrogen removal in wastewater treatment (Zhang et al., 2016b). Thus, the cost to use commercial chemicals for supplementing carbon source can be greatly reduced as reported by Frison et al. (2013) who predicted that the specific cost for nitrogen removal could be reduced by 22% by using fermentation products to replace methanol as external carbon source. It can thus be concluded that FLFW is a perspective carbon source not only for conventional bioreactor but also for MBRs.

4. Conclusions

FLFW could effectively enhance denitrification in the pilot-scale A/O-MBR, with improvements in the nitrogen removal efficiency from 20% to 67%. After adding the FLFW, microbial metabolic activities were significantly enhanced. The change of microbial communities not only benefited nitrogen removal, but also contributed to mitigating membrane fouling. Based on the analysis of membrane foulants (EPS and DOM) and cake layer, it was clarified that FLFW addition did not induce significant increase in fouling rate and membrane foulants or severe irreversible membrane fouling. Therefore, FLFW is a promising alternative carbon source to enhance nitrogen removal for MBR systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.03.186.

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