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New insight into fouling behavior and foulants accumulation property of cake sludge in a full-scale membrane bioreactor



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ABSTRACT

Cake sludge attached on membrane surfaces was collected and characterized in a full scale membrane bioreactor (MBR) compared with bulk sludge. The morphological, chemical and microbial properties were examined through microscopic observations, particle size distribution (PSD) analysis, chemical analysis, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy-diffusive X-ray (EDX) analysis, specific oxygen utilization rate (SOUR) measurements and Biolog assay. The results showed that fiber-like substances might have served as the skeleton of larger size aggregates in cake sludge. Moreover, much more polysaccharides and inorganic elements such as multivalent cations were accumulated in cake sludge than proteins and humics. Cake sludge showed lower microbial activity for aerobic degradation than bulk sludge, but higher metabolic activity for the degradation of refractory substances (aromatic proteins and humics) other than polysaccharides. Based on batch filtration experiments, it was found that cake sludge had much higher cake layer fouling potential but lower membrane pore blocking resistance, probably due to the heterogeneous structure of cake sludge resulting from accumulation and interaction of various inorganic and organic foulants. This investigation could assist in obtaining a better understanding of the fouling behavior and foulants accumulation properties of cake sludge in the full-scale MBRs.

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1. Introduction

Recently, significant progress has been achieved in both research and application of membrane bioreactors (MBRs), which exhibit several advantages over conventional activated sludge processes during wastewater treatment and reclamation by adopting microfiltration/ultrafiltration for solid/liquid separation. However, membrane fouling remains not well resolved, which contributes considerably to the operating and maintenance costs and limits the more widespread applications of MBRs [1,2]. Membrane fouling is caused by the complex interaction between the membrane and mixed liquor component, including suspended solids, colloids, biopolymers, and solutes, which is introduced from raw wastewater or produced during biomass growth and decay [3].

The contributions of different sludge fractions to membrane fouling have been extensively studied [4–8]. Among the complex foulants, biopolymers, including soluble microbial products (SMP)

and extracellular polymeric substances (EPS), are widely accepted as primary fouling-causing substances [4]. It was found that due to the membrane rejection effect, SMP was more easily accumulated in MBRs, resulting in the poor filterability of sludge suspension [5]. Additionally, in a lab-scale submerged anaerobic MBR, it was observed that fine particles and a higher level of EPS preferentially accumulated in the cake sludge compared with bulk sludge [6]. Moreover, inorganic elements, especially metal cations (Mg, Al, Fe and Ca), were found to accumulate in cake sludge and enhance cake layer formation [7,8]. The above analysis indicated the important effects of various foulants on cake layer formation.

Nevertheless, cake layer formation on the membrane surface was inevitable, and cake layer fouling accounted for over 80% of the total filtration resistance in most MBR studies [9–11]. During long-term filtration, there is a continuous increase in filtration resistance caused by the accumulation and compression of the cake layer. Thus, recent efforts have been devoted to characterizing the cake layer, including the physical morphology and structure [9,12], chemical composition [8] and microbial properties [13], theoretical analysis and modeling [12]. One study using a series of analytical methods showed that structures, EPS concentrations

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and microbial communities changed significantly along the cake depth, and the continuous accumulation and interaction of foulants on the membrane surface decreased cake layer porosity, which was closely related to the TMP increase [10]. It was also reported that cake sludge had a specific filtration resistance nearly three orders of magnitude higher than that of bulk sludge [11], which was mainly due to the accumulation of the biopolymer clusters (BPC) within the cake sludge pores. Thus, the cake layer can be defined as a porous and inhomogeneous media formed on membrane surfaces due to the adsorption, deposition and accumulation of various foulants [14]. The above lab-scale/pilot-scale studies indicated that cake sludge has quite different fouling behavior from that of bulk sludge due to foulant accumulation, especially in MBRs during long-term operation; thus, it is highly appropriate to comprehensively characterize cake sludge to facilitate cake layer fouling control in MBR applications.

Most of the studies involving cake sludge characterization were conducted in lab-scale/pilot-scale MBR systems mainly fed with synthetic wastewater. Therefore, the results obtained may be inappropriate to be extended directly to full-scale MBR applications because of the difference in fouling behavior between lab-scale/pilot-scale and full-scale MBRs, as confirmed by several researchers [4,15], and also limited work has been done in full-scale MBR until present. Thus, the objective of this study is to investigate the characteristics of cake sludge in terms of fouling behavior, foulant accumulation and microbial properties in a full-scale MBR treating domestic wastewater for approximately four years by various analytical methods. The results obtained in this study are expected to provide new insights into cake sludge properties and membrane fouling control in full-scale MBR applications.

2. Materials and methods

2.1. The full-scale MBR system and operation

The MBR system with a treating capability of 2000 m³/d for domestic wastewater treatment and reclamation is located in the university campus of Xi'an, China. The biological treatment unit was divided into four sequential zones, namely, the anaerobic tank (150 m³), the anoxic tank (420 m³), the oxic tank (480 m³) and the MBR tank (120 m³), in series (Fig. S1 of Supporting information). 216 submerged PVDF hollow fiber membrane modules (MUNC-620AII type, Asahi Kasei Chemicals Corp., Ltd., Japan) with a nominal pore size of 0.1 μm and total membrane area of 5400 m² were installed in the MBR tank. The effluent was extracted by suction pumps at a fixed flow rate (16 L/m² h) under an intermittent operation mode (9 min on/1 min off). Air was monitored by flow rate meters and continuously supplied through the air diffusers to the oxic tank (2000 m³/h) and MBR tank (2000 m³/h) to provide oxygen demanded by microorganisms and scour the membrane surface, respectively. Accordingly, the concentration of dissolved oxygen in the oxic tank and MBR tank was in the range of 2–5 mg/L and 4–7 mg/L, respectively. After pretreatment by screening (coarse screen and fine screen with bar clearances of 5 mm and 1 mm, respectively) and regulation, domestic wastewater collected from the university campus was supplied into the AAO-MBR system. The characteristics of the raw wastewater can be found elsewhere [16]. The system was equipped with a programmable logic controller (PLC) for automatic control of the operation, including equipment involved in the influent, aeration, sludge recirculation and effluent. TMP was monitored by an online pressure gauge. The hydraulic retention time (HRT) was approximately 12.5 h, and the sludge retention time (SRT) was set at 20–40 d.

2.2. Analytical methods

2.2.1. Cake sludge collection and fractionation

Cake sludge formed on the membrane surface was scraped off by using a plastic sheet as the membrane module was taken out for physical cleaning, which was operated in accordance with the reported methods [7,10]. The exact time period for sampling was between June and July 2015. All of the collectable cake sludge from 12 membrane modules was collected to address the observed problem of an uneven distribution of cake layers on the membrane surface when the modules were lifted for physical cleaning (once per year). Additionally, within the sampling period, cake sludge was sampled more than three times from different membrane modules. The collected sample was diluted with deionized water to achieve the same MLSS level as that of bulk sludge, and then was placed on a magnetic blender and gently mixed. However, it was noted that after a long period (several hours to days) of mixing, a certain amount of large particles remained in the mixed cake sludge, which disrupted the accurate analysis of cake sludge properties, due to the difficulty in sampling. Therefore, further fractionation of cake sludge was proposed and conducted by using a series of stainless steel sieves, with sieve pore sizes of 2 mm, 0.5 mm and 0.2 mm, respectively. In addition, the effect of the proposed fractionation method on cake sludge properties is discussed in a subsequent section.

2.2.2. EPS extraction and analysis

EPS extraction from bulk sludge and cake sludge samples was performed using the thermal treatment method [16]. The content of the extracted EPS samples was analyzed in terms of proteins and polysaccharides. Proteins were quantified using the modified Lowry method, in which bovine serum albumin was used as the standard [17]. The polysaccharide concentration in EPS was determined according to the anthrone method, in which glucose was used as the standard [18].

2.2.3. PSD analysis

PSD of the cake sludge and bulk sludge 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 detailed pretreatment for cake sludge before the PSD analysis is described in Section 2.2.1. Three measurements of each sample were taken and typical PSD curves were reported.

2.2.4. Batch filtration experiment

The filtration properties of cake sludge and bulk sludge were evaluated by batch filtration experiments using a stirred dead-end cell (MSC300, Mosu corp., Shanghai, China) [19,20]. To avoid the effect of MLSS on filterability of different sludge samples, the MLSS concentration of raw cake sludge was diluted to the same level as that of bulk sludge (MLSS=4 g/L). The membranes employed for filtration were hydrophilic polyvinylidene fluoride (PVDF) flat sheet membranes with a nominal pore size of 0.1 μm and an effective membrane area of 28 cm² (VVLP9050, Millipore Corp., USA), which were the same material and pore size as the membrane used in the full-scale MBR process. Nitrogen gas was adopted to allow for a constant pressure for filtration. The permeate flux data were continuously logged using a top-loading electronic balance (Sartorius, BSA2202S, Germany) connected to a personal computer. Prior to each filtration, each of the new membranes was soaked in ultrapure water for 24 h and further cleaned through filtering ultrapure water for 30 min at a pressure of 25 kPa to remove any impurities and to stabilize the permeate flux. The filtration pressure was maintained constant at 10 kPa and the stirring speed in the cell was set at 200 rpm throughout the experiments. All of the experiments were conducted at room

temperature (25 ± 2 °C). After filtration, the membrane surface was gently cleaned by using a sponge and followed by rinsing with ultrapure water to remove the cake layer. Then, the membrane was used again to filter ultrapure water. The filtration resistances, including R_t , R_m , R_c and R_p , were calculated according to the resistance-in-series model [21], where R_t is the total membrane resistance, R_m is the intrinsic membrane resistance, R_c is the cake resistance, and R_p is the fouling resistance due to pore blocking. Three replicates of the filtration experiment for each sample were performed and typical filtration curves were plotted.

2.2.5. Component analysis

After the cake sludge was collected using the method described in Section 2.2.1, it was oven-dried at 60 °C for 24 h. The organic composition of the cake sludge was analyzed using a FTIR spectrometer (IR Prestige-21, Shimadzu Corporation, Japan). Powders of the sample were mixed with KBr powders at a mass ratio of 1:100 and pressurized into a pellet. The wave number spectrum was then determined over the range of 4000–400 cm^{-1} . The FTIR spectrum of bulk sludge was also obtained according to the same method. The inorganic elements in the cake sludge and bulk sludge were measured by employing the SEM-EDX analyzer (Oxford INCA Energy 350, UK). Each sample was detected three times for FTIR and EDX analysis.

2.2.6. 3D-EEM analysis

The 3D-EEM fluorescence spectra of the EPS samples were measured using an FP-6500 spectrofluorometer (Jasco Corporation, Japan). Emission spectra of EEM were subsequently scanned from 220 to 550 nm in 5 nm increments by varying the excitation wavelength from 220 to 450 nm in 5 nm steps. The software Origin Pro 8.0 (Origin Lab Corporation, USA) was used to assess the EEM spectra as the elliptical shape of contours were plotted and presented.

2.2.7. Microbial activity and community

The specific oxygen utilization rates (SOUR) of heterotrophic bacteria, ammonium oxidizers and nitrite oxidizers, namely, $(\text{SOUR})_{\text{H}}$, $(\text{SOUR})_{\text{NH}_4}$ and $(\text{SOUR})_{\text{NO}_2}$, were determined to indicate the microbial activity [22,23].

To determine the microbial community of sludge samples related to the community metabolic diversity (CMD), Biolog assay was adopted according to previously reported methods, with some modification [24,25]. Firstly, sludge samples were washed twice with sterile 0.85% NaCl solution and then diluted to a cell density with an optical density at 420 nm (OD_{420}) of 0.30 ± 0.05 . Next, the suspension was horizontally shaken at 250 rpm for 20 min, followed by settling for 30 min. Lastly, 150 μL of the supernatant was inoculated into each well of an EcoPlate (Biolog), which contains 31 individual carbon sources in triplicate and 3 negative controls (without carbon source) in a 96-well plate format. The plates were incubated at 28 °C for 120 h, during which time, the OD_{590} values were measured every 24 h using a plate reader (Biolog). The patterns of sole carbon source utilization were then expressed as an index of the net OD_{590} value in each well by subtracting the control reading from the sample reading. Microbial metabolic activity in the Biolog EcoPlate was expressed as the average well color development (AWCD), which was calculated according to the formula described in the reference [24].

2.2.8. Additional analysis

Photographs of the membrane modules were taken by an SLR camera (EPM2, Olympus Corporation, Japan). Microscopy observations of sludge samples were captured by a digital camera (N90i, Nikon Corporation, Japan) attached to a microscope. Mixed liquor suspended solids (MLSS) and mixed liquor volatile

suspended solids (MLVSS) were measured according to the standard methods [26].

3. Results and discussion

3.1. Long-term operation performance of the full-scale MBR

The full-scale MBR system was operated for approximately four years at the local university campus of Xi'an, China, which is located in a water deficient district, showing excellent performance in terms of pollutant removal and membrane filtration performance. Detailed information about the treatment performance has been reported previously [16,27]. The treated effluent suitable for various non-potable purposes, such as toilet flushing, landscaping and irrigation, was totally reused in the campus. In addition, membrane filtration performance represented by the evolution of TMP with operational time was recorded and is presented in Fig. S2 of Supporting information. Chemical cleaning in-place (CIP) was conducted once after 900 days of operation. It was noted that the range of TMP variation was between 10 kPa and 30 kPa, and did not show the tendency of a 'TMP jump', as reported in previous studies [28,29]. A similar phenomenon was detected by other researchers [15,30], who claimed that the TMP jump was more frequently observed in small-scale experiments, and fouling rates measured at the lab-scale were inappropriate to describe long-term full-scale operation due to the distinct and inherent differences between them.

Moreover, many studies confirmed the important effect of cake layer formation on filtration performance, as cake layer fouling commonly account for more than 80% of the total filtration resistance [9–11]. Further observation of foulants (mainly the cake layer) on membrane modules was conducted. As shown in Fig. S3 of Supporting information, the uneven distribution of various foulants was observed, which was consistent with one previous study stating that the cake layer was not uniformly distributed on the entire surface of all of the membrane fibers [31]. The middle part of the modules was covered with a cake layer that could not be easily seen by the naked eye, which could be attributed to a thin gel layer. However, the top of the modules was obviously twined by fiber-like substances (including hair, paper scraps, plastic debris, etc.) with the length of several centimeters. These fiber-like substances were very light and could float on water surfaces, which were mainly derived from raw wastewater during long-term operation. The bottom of the modules was covered with an evident cake layer, which was attributed to the uneven distribution of aeration in the MBR tank and to the removal of foulants from the top and middle part of the modules when they were removed from the membrane tank. It should be noted that cake sludge samples could be collected only when the modules were lifted for cleaning, but unfortunately due to hydraulic disturbance, it was impossible to maintain the distribution of cake layers in the same status as the membrane was immersed in the MBR tank. Therefore, all of the collectable cake sludge was collected, mixed and used as representative sludge samples for further characterization.

3.2. Morphology and fouling behavior of cake sludge

3.2.1. Morphology of cake sludge

The morphology of sludge samples was analyzed by means of PSD analysis and microscopic observation. Fig. 1 shows the typical PSD of bulk sludge and cake sludge. Multimodal curves were observed both for bulk sludge and cake sludge. However, the majority of the flocs in the bulk sludge were distributed in the range of 0.4–100 μm , and some had sizes larger than 100 μm . Although

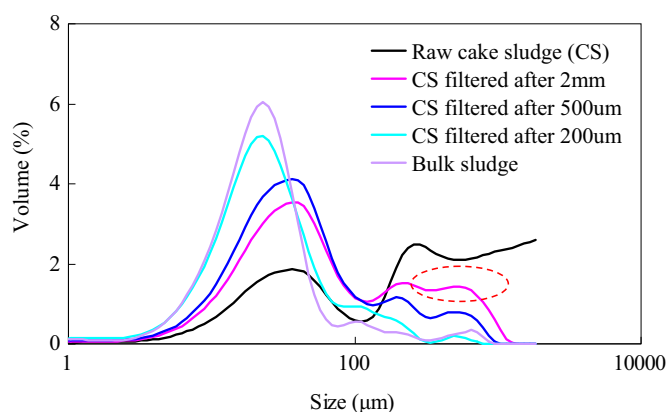


Fig. 1. PSD of bulk sludge and cake sludge.

the raw cake sludge had a widespread PSD with small flocs (0.4–100 μm), large flocs (more than 100 μm), and even large aggregates (more than 2 mm) existed. As described in Section 2.2.2, large aggregates (more than 2 mm) affected the characterization of cake sludge due to sampling problems. Thus, a fractionation method using a series of stainless steel sieves (2 mm, 500 μm , 200 μm) was proposed and applied for cake sludge sieving. As shown in Fig. 1, after fractionation, the cake sludge samples showed similar PSD curves to those of bulk sludge, although the PSD curves of cake sludge shifted to larger sizes and more large-sized flocs (more than 100 μm) were detected. It was observed by several researchers that smaller particles had a higher tendency to accumulate on the membrane surface in the initial stage of membrane fouling [1,6,7]. However, the aggregation of particles and other foulants occurred later in the cake layer, so larger aggregates were detected in the current study.

The obtained results could be clearly verified by microscopy observations of sludge samples shown in Fig. S4 of Supporting information. From Fig. S4 (a) and (b), bulk sludge was found to be porous and loose in structure, whereas raw cake sludge showed a dense and compact structure with a large amount of fiber-like substances existed. The fiber-like substances might have served as a skeleton and contributed to the cake layer formation. However, after fractionation by different pore size sieves, the cake sludge showed similar morphology to that of bulk sludge. The results emphasized the accumulation phenomenon of fiber-like substances and their important effects on the formation of large aggregates in cake sludge. It was unexpected that until present, little attention has been paid to this phenomenon. The fiber-like substances were further identified, which could be found in Supporting information (Fig. S5). Based on the SEM-EDX analysis, fiber-like substances in the cake sludge included abundant filamentous bacteria with relatively short lengths and small diameters, and also a certain amount of large size fiber-like substances, which could be both organic and inorganic in nature.

Apparently, fractionation of cake sludge using the 2 mm sieve could exclude most of the oversized aggregates and avoid their impact on the sampling problem in cake sludge characterization from full-scale MBR process. From the point of practical application, pretreatment of raw wastewater using the fine screen (pore size < 2 mm) is recommended to prevent the fiber-like substances from entering the biological treatment unit (especially for MBR tank) because the fiber-like substances promote the formation of a compact cake layer and hinder membrane fouling control. Moreover, as adopted in the investigated full-scale MBR system, currently in most MBR plants, super-fine screens (with pore size of 1 mm) were used, which could be effective in excluding large size fiber-like substances; however, some small size fiber-like

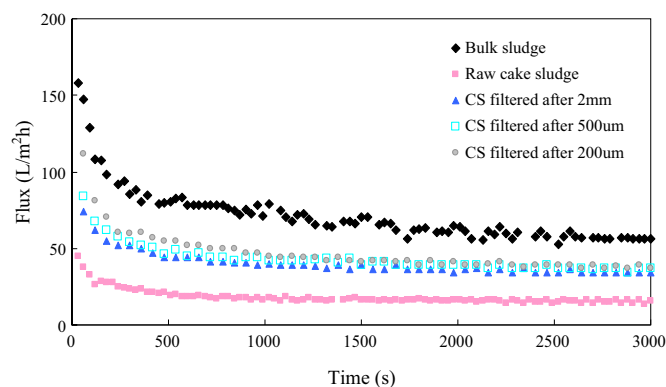


Fig. 2. Filtration curves (flux versus time) of bulk sludge and cake sludge samples.

substances with the diameters smaller than 1 mm still passed through the fine screen, so more attention still should be given to these fiber-like substances.

3.2.2. Fouling behavior of cake sludge

The filtration properties of bulk and cake sludge were studied using batch filtration experiments. Fig. 2 indicates the typical filtration curves of sludge samples reflected by time-course filtration flux variation. By comparison, it was found that different initial fluxes and stable fluxes occurred. For filtering the bulk sludge, the initial flux was as high as 160 $\text{L}/\text{m}^2 \text{h}$, but for the raw cake sludge, the value was much lower (50 $\text{L}/\text{m}^2 \text{h}$). After a rapid flux decline for 2–4 min, the flux decreasing rate tended to decrease for the following 5–10 min. Then, the fluxes leveled off at relatively constant values of 65.6 $\text{L}/\text{m}^2 \text{h}$ and 16 $\text{L}/\text{m}^2 \text{h}$ for bulk sludge and raw cake sludge, respectively. After fractionation by a series of sieves, the filtration fluxes of the fractionated cake sludge were quite analogous and were distributed between those of bulk sludge and raw cake sludge. Thus, the filtration tests further verified the fractionation method and confirmed the important effect of large aggregates on fouling behavior of cake sludge.

To illustrate the filtration resistance distribution of sludge samples, bulk sludge and cake sludge filtered through the 2 mm sieve were used for specific filtration tests, as shown in Fig. 3. It was noted that two sludge samples showed different filtration properties in terms of fouling behavior and flux recovery. Obviously, bulk sludge showed better filterability than cake sludge, as demonstrated by the higher stabilized flux (65.6 $\text{L}/\text{m}^2 \text{h}$ vs 34.3 $\text{L}/\text{m}^2 \text{h}$). However, after physical cleaning, the flux recovery efficiencies of the two sludge samples were quite similar. Using a resistance-in-series model, different filtration resistances could be calculated. From Table 1, it was noted that cake sludge had much higher cake layer fouling potential than bulk sludge, as the cake layer fouling resistance of the former was approximately 7 times as high as that of the latter. Also the two sludge samples had nearly the same pore blocking resistance. Therefore, it was concluded that although the larger PSD and more heterogeneous structure of the cake sludge resulted in higher cake fouling resistance, the formed cake layer could effectively prevent membrane pore blocking by retention of potential foulants, such as fine particles and biopolymers. The cake layer formation on the membrane was beneficial to the filterability of the MBR process, which has been recognized by previous researchers [21,32]. It was shown that the cake layer acted as a secondary or dynamic membrane that screened the primary membrane from the more strongly fouling species, e.g., smaller sized particles [21]. A previous study reported that a thin cake layer on the membrane could improve MBR performance, as the cake could prevent the membrane from soluble microbial product (SMP)-related fouling and

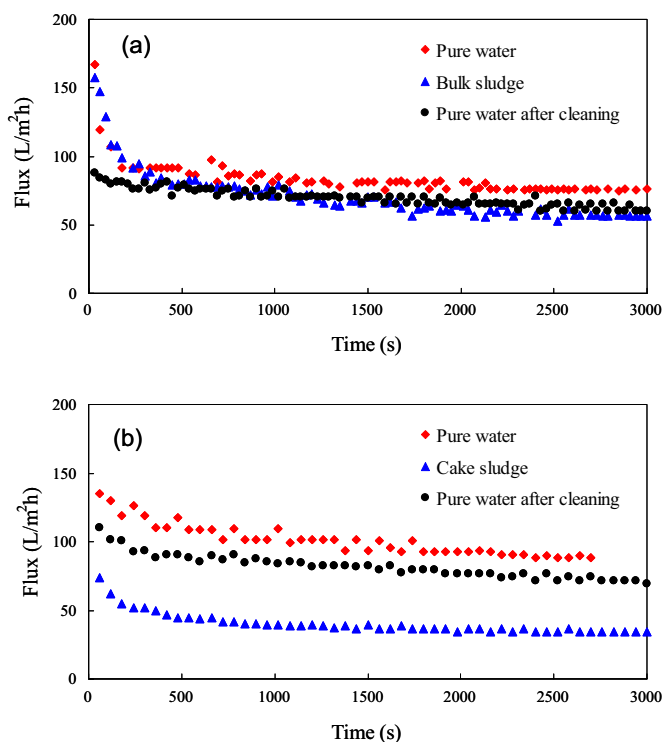


Fig. 3. Batch filtration curves for filtration resistance determination: (a) bulk sludge and (b) cake sludge filtered after 2 mm sieve.

Table 1
Filtration resistance distribution of bulk sludge and cake sludge.

Sludge samples	R_m	R_p	R_c	R_t
Bulk sludge	4.15 ± 0.10	0.79 ± 0.10	0.78 ± 0.08	6.32 ± 0.28
Cake sludge	4.09 ± 0.08	0.96 ± 0.10	5.48 ± 0.20	10.5 ± 0.38

Note: The unit is 10^{11} m^{-1} ; the data presented are the averaged values \pm standard deviation based on $n=3$.

protect against long-term fouling, and thus adopted a controlled aeration rate to facilitate the formation of a thin cake layer [33].

3.3. Foulant accumulation properties of cake sludge

3.3.1. EPS analysis

EPS in bulk sludge and cake sludge after fractionation was extracted and the main components were determined through a series of measurements. Fig. 4 compares the EPS content of the two sludge samples. Proteins were found to be the main

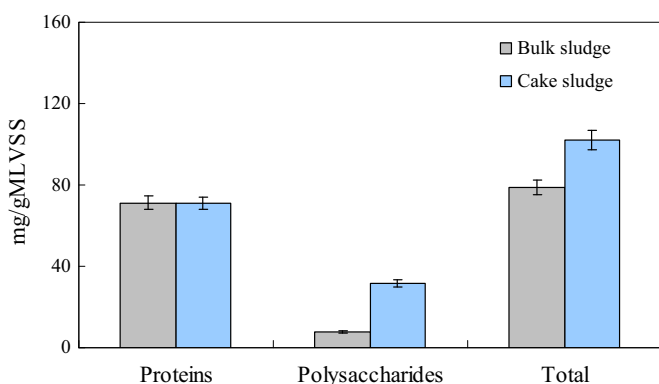


Fig. 4. EPS content in bulk sludge and cake sludge (the number of sample measurement: $n=3$).

components in both samples, the concentrations of which were nearly the same, i.e., 71 mg/gMLVSS. The polysaccharide concentrations were much lower than those of proteins, i.e., approximately 7.5 mg/g MLVSS and 31.5 mg/g MLSS in bulk sludge and cake sludge, respectively. Obviously, unlike proteins, more polysaccharides accumulated in the cake sludge. A previous study attributed the higher EPS in cake sludge to the following two points: (1) the rejection of dissolved organic matter (DOM) in sludge supernatant revealed by a consistently higher organics concentration in bulk sludge supernatant than in effluent; (2) the presence and accumulation of numerous fine particles in cake sludge, which have higher EPS than that of large flocs and bulk sludge [7]. The results were helpful for determining the important factors affecting EPS accumulation in cake sludge. However, this analysis did not consider the metabolism activity of microorganisms in cake sludge because microorganism could utilize certain parts of the accumulated organics for growth and other activities. The above analysis indicated that EPS concentration in cake sludge was controlled by at least three factors, although the exact contributions of each were not clear. Overall, EPS in cake sludge was higher than that in bulk sludge, and it is reasonable to hypothesize that the retention of DOM in bulk sludge contributed significantly to the EPS in cake sludge, as continuous sludge filtration constantly moves organics in bulk sludge towards the membrane surface.

Humic substances were also important components of EPS, as demonstrated by the EEM spectra of the two sludge samples in Fig. S5 of Supporting information. It was detected that the two spectra both demonstrated three fluorescent peaks, namely, Peak A, Peak B and Peak C. Peak A was detected at the excitation/emission wavelengths (Ex/Em) of 225–230/355–370 nm, whereas Peak B was located at the Ex/Em of 285–290/350–365 nm. The two peaks have been reported as protein-like fluorescent peaks, in which the fluorescence is associated with aromatic protein-like substances (Peak A) and tryptophan protein-like substances (Peak B) [34–36]. Another peak (Peak C) with Ex/Em at 315–325/410–420 nm was also observed, which has been reported to be associated with humic acid-like substances [36]. The fluorescent properties in terms of peak location and fluorescent intensity (FI) of the two samples were quite similar, indicating no significant accumulation of protein-like substances and humic acid-like substances, which partially confirmed that the EPS in bulk sludge and cake sludge was closely related.

3.3.2. Organic and inorganic component analysis

The FTIR spectra of bulk sludge and cake sludge are shown in Fig. 5, and similar profiles can be observed. The peaks near 3430, 2929 and 1045 cm^{-1} are attributed to the presence of polysaccharides or polysaccharide-like substances in MBRs [36]. Furthermore, the relatively higher peak intensities indicated that the cake sludge contained more polysaccharides than the bulk sludge, which is consistent with the component analysis discussed previously. The characteristic peaks for protein, included those near 1656, 1558 and 1454 cm^{-1} [37], so the nearly identical peak intensities of the two samples indicated the presence of similar amounts of proteins in sludge samples. The other organic substances identified also included aliphatic substances (the peaks near 2929 and 2857 cm^{-1}), fat and/or cellulose (the peaks at 1411 and 1239 cm^{-1}) [38,39]. Based on the FTIR spectra analysis, the major organic components in sludge samples were identified as proteins and polysaccharides.

EDX analysis was further performed for both bulk sludge and cake layer to identify the inorganic content. Compared with bulk sludge (Fig. 6(a)), the elements of Fe, Mg, Al, Si, Ca, P, S, K and Na were obviously detected in cake sludge (Fig. 6(b)), especially for multivalent cations. Similar results can be found in the literature

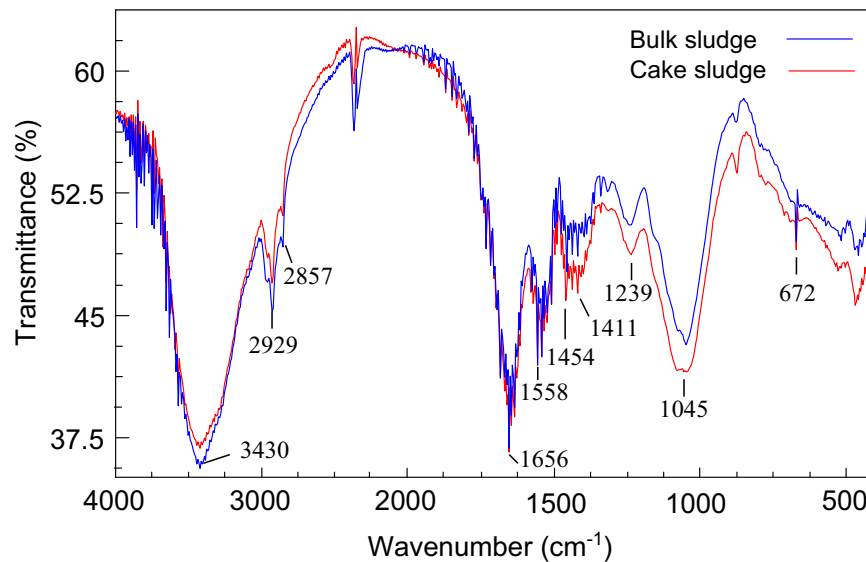


Fig. 5. FTIR spectra of bulk sludge and cake sludge.

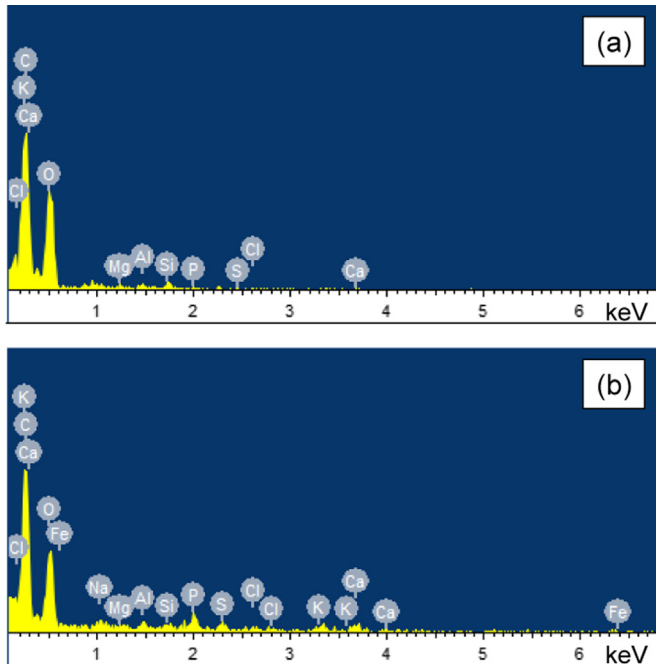


Fig. 6. EDX analysis of samples: (a) bulk sludge and (b) cake sludge.

either in aerobic or anaerobic MBRs. In a lab-scale MBR treating synthetic wastewater, the main inorganic elements in the cake layer were P, Si, and Ca, followed by S, K, Fe, Al and Mg [8]. The following elemental composition was detected in the cake layer from a lab-scale anaerobic MBR: 71.67% C, 9.03% O, 4.45% Ca, 4.39% S, 3.54% P, 1.94% Mg, 1.72% Al, 1.46% Si, 0.68% Cl, 0.60% Na, 0.30% K, and 0.15% Mn. Moreover, it was reported that inorganic elements played an important role in thick cake layer formation and high cake layer resistance [7]. Although the content of the accumulated elements (especially for Mg, Al, Fe and Ca) were less than that of other foulants (fine particles and biopolymers), they would enhance cake layer formation through charge neutralization and the bridging effect [7,8]. Specifically, the fine particles and colloids had negative surface charges, whereas the biopolymers contained anion groups, such as SO_4^{2-} , CO_3^{2-} , PO_4^{3-} and OH^- , which facilitated their interaction with multivalent cations through charge

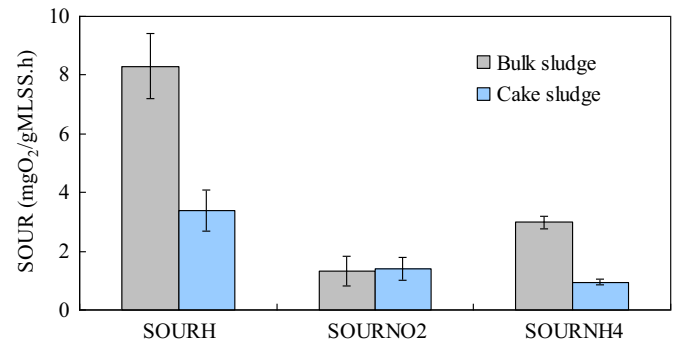


Fig. 7. Microbial activity reflected by SOUR of bulk sludge and cake sludge (the number of sample measurement: $n=3$).

neutralization. Moreover, because organic foulants (i.e., polysaccharides and proteins) had high molecular weights and complex structures, the multivalent cations could be easily captured by these negative ions by the bridging effect. The synergistic interaction among various foulants (fine particles, organics and inorganic substances) by the above-mentioned two effects would cause biological precipitation and enhance cake layer fouling [40], which might also contribute to the heterogeneous structure of cake sludge during long-term MBR operation, as discussed previously.

3.4. Microbial activity and community structure of cake sludge

Cake sludge had a relatively compact and less porous structure and always attached to membrane surfaces, although the movement of a few fine particles was observed [29]. The unique structure of the cake sludge resulted in a limitation effect of substrates and dissolved oxygen for microorganisms due to the limitation of mass transfer [7]. Thus, during the long-term MBR operation, the microbial activity and community structure in the cake sludge were expected to be different from those of bulk sludge.

To characterize microbial properties, the cake sludge was diluted to nearly the same sludge concentration as the bulk sludge. Firstly, the MLVSS/MLSS ratios of bulk sludge and cake sludge were measured, which were about 75% and 60%, respectively. The MLVSS/MLSS ratio was used to roughly assess biomass activity in

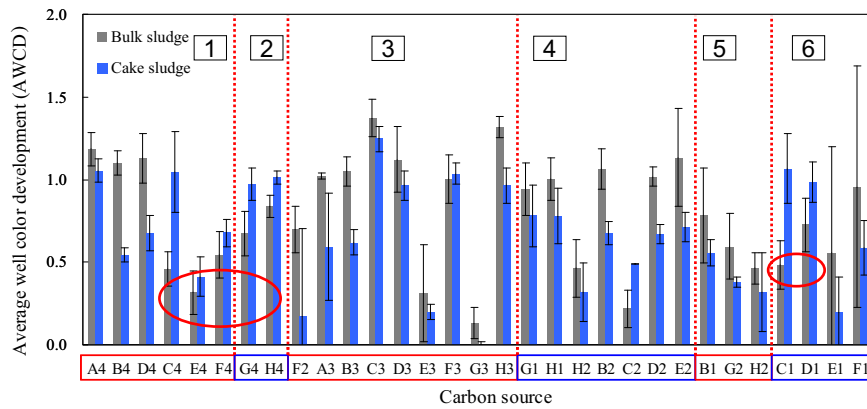


Fig. 8. Microbial community distribution determined by Biolog assay in bulk sludge and cake sludge. (six types of carbon source: 1-amino acid; 2-amines; 3-carboxylic acids; 4-carbohydrates; 5-complex; 6-polymers; the number of sample measurement: $n=3$).

mixed liquor. The results indicated that the biomass in the cake layer showed lower activity and obvious inorganic substance accumulation. SOUR was adopted to reflect the respirometric activities of aerobic microorganisms in the sludge samples. The respirometric activity of heterotrophic bacteria, ammonium oxidizers and nitrite oxidizers was described by $(SOUR)_H$, $(SOUR)_{NH_4}$ and $(SOUR)_{NO_2}$, respectively. From Fig. 7, it was found that $(SOUR)_H$ and $(SOUR)_{NH_4}$ in the bulk sludge were 8.2 and 3.7 $mgO_2 / gMLVSS\ h$, which were twice as high as those in the cake sludge. However, $(SOUR)_{NO_2}$ of the bulk sludge and cake sludge was almost the same, and thus the total SOUR of microorganisms in the cake sludge was much lower. The result was similar to one study conducted in a dynamic membrane bioreactor, which reported that the organic degradation activity and nitrification activity of the suspended biomass were higher than those of the biomass in the cake layer, whereas nitrosification activity of the two sludge samples was almost the same. Additionally, it was claimed that although the biomass in the dynamic membrane (DM) originated from the mixed liquid, its activity was decreased when it attached to the module. This might be due to the insufficient organic matter and dissolved oxygen supply in the DM [41]. From the above analysis, it was noted that other aspects, such as foulant accumulation and potential microbial community difference might also be factors affecting biomass activity.

Biolog assay has been a common method to characterize microbial metabolic diversity in various environments, such as surface water, ground water, soil and wastewater treatment plants [24,25]. However, the microbial community functional diversity of bulk sludge/cake sludge in MBRs is poorly understood. Thus, Biolog assay was chosen to examine the microbial community structure of two sludge samples. In this study, the Biolog EcoPlate was used, which contained 31 different carbon sources as follows: two amines, six amino acids, seven carbohydrates, nine carboxylic acids, three complexes and four polymers [25].

Fig. 8 demonstrates the distribution of carbon source utilization activity of the bacterial community in the sludge samples. By comparison, it was noted that for four types of carbon sources (i.e., amino acids, carbohydrates, carboxylic acids and complexes), the bulk sludge showed higher metabolic activity than the cake sludge. However, for the other two types of carbon source (amines and polymers), the cake sludge unexpectedly showed higher metabolic activity than bulk sludge. Careful analysis showed that the four types of carbon sources that could be more easily utilized by bulk sludge belonged to readily biodegradable substances, whereas the other two types of carbon sources were slow biodegradation or non-biodegradation substances. Thus, it was observed that the accumulated organics in cake sludge, especially for the non-biodegradation substances, such as aromatic proteins,

humic acids and esters had a very long retention times. During the long-term MBR operation, under the condition of substrate limitation, microorganisms in cake sludge gradually change their metabolic patterns to adapt to the new environment, which eventually changes the microbial community, as verified by various studies [7,13]. Therefore, the microbial community structure in cake sludge was found to be different to that in bulk sludge, which could apparently explain the accumulation phenomenon of organics foulants in cake sludge (Section 3.3.1). However, it is not clear why the cake sludge shows higher metabolic activity for proteins and humic and lower metabolic activity for carbohydrates. More research is needed to clarify the underlying mechanism.

4. Conclusions

The characteristics of cake sludge were investigated in comparison with bulk sludge in a full-scale MBR during long-term operation. Morphological analysis showed that fiber-like substances might have served as the skeleton for the formation of large aggregates and compact cake sludge structures, resulting in higher cake layer fouling potential, but effectively prevented pore blocking, as verified by batch filtration using cake sludge and bulk sludge. It was also found that considerably polysaccharides and inorganic elements such as multivalent cations were accumulated in the cake sludge than other foulants. As a result of the SOUR analysis and Biolog assay, it was further recognized that although the cake sludge had lower respirometric activity for aerobic degradation, it showed higher metabolic activity in the degradation of refractory substances, such as aromatic proteins and humics. The heterogeneous structure, fouling behavior and microbial properties might be due to a series of complicated interactions among the accumulated inorganic and organic substances in the cake sludge.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.memsci.2016.02.058>.

References

- [1] Z. Wang, Z. Wu, X. Yin, L. Tian, Membrane fouling in a submerged membrane bioreactor (MBR) under sub-critical flux operation: membrane foulant and gel layer characterization, *J. Membr. Sci.* 325 (1) (2008) 238–244.
- [2] S.I. Patsios, A.J. Karabelas, An investigation of the long-term filtration performance of a membrane bioreactor (MBR): the role of specific organic fractions, *J. Membr. Sci.* 372 (1–2) (2011) 102–115.
- [3] C.S. Laspidou, B.E. Rittmann, A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass, *Water Res.* 36 (11) (2002) 2711–2720.
- [4] F.G. Meng, S.-R. Chae, A. Drews, M. Kraume, H.-S. Shin, F.L. Yang, Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material, *Water Res.* 43 (6) (2009) 1489–1512.
- [5] I.T. Yeom, Y.M. Nah, K.H. Ahn, Treatment of household wastewater using an intermittently aerated membrane bioreactor, *Desalination* 124 (1–3) (1999) 193–204.
- [6] H.J. Lin, K. Xie, B. Mahendran, D.M. Bagley, K.T. Leung, S.N. Liss, B.Q. Liao, Factors affecting sludge cake formation in a submerged anaerobic membrane bioreactor, *J. Membr. Sci.* 361 (1–2) (2010) 126–134.
- [7] H.J. Lin, B.-Q. Liao, J.R. Chen, W.J. Gao, L.M. Wang, F.Y. Wang, X.F. Lu, New insights into membrane fouling in a submerged anaerobic membrane bioreactor based on characterization of cake sludge and bulk sludge, *Bioresour. Technol.* 102 (3) (2011) 2373–2379.
- [8] F. Meng, H. Zhang, F. Yang, L. Liu, Characterization of cake layer in submerged membrane bioreactor, *Environ. Sci. Technol.* 41 (11) (2007) 4065–4070.
- [9] Y. Miura, Y. Watanabe, S. Okabe, Membrane biofouling in pilot-scale membrane bioreactors (MBRs) treating municipal wastewater: impact of biofilm formation, *Environ. Sci. Technol.* 41 (2) (2007) 632–638.
- [10] W.J. Gao, H.J. Lin, K.T. Leung, H. Schraft, B.Q. Liao, Structure of cake layer in a submerged anaerobic membrane bioreactor, *J. Membr. Sci.* 374 (1–2) (2011) 110–120.
- [11] X.-M. Wang, X.-Y. Li, X. Huang, Membrane fouling in a submerged membrane bioreactor (SMBR): characterisation of the sludge cake and its high filtration resistance, *Sep. Purif. Technol.* 52 (3) (2007) 439–445.
- [12] X. Su, Y. Tian, H. Li, C. Wang, New insights into membrane fouling based on characterization of cake sludge and bulk sludge: an especial attention to sludge aggregation, *Bioresour. Technol.* 128 (2013) 586–592.
- [13] X. Zhang, Z. Wang, Z. Wu, T. Wei, F. Lu, J. Tong, S. Mai, Membrane fouling in an anaerobic dynamic membrane bioreactor (AnDMBR) for municipal wastewater treatment: characteristics of membrane foulants and bulk sludge, *Process. Biochem.* 46 (8) (2011) 1538–1544.
- [14] C.H. Lee, P.K. Park, W.N. Lee, B.K. Hwang, S.H. Hong, K.M. Yeon, H.S. Oh, I. S. Chang, Correlation of biofouling with the bio-cake architecture in an MBR, *Desalination* 231 (1–3) (2008) 115–123.
- [15] M. Kraume, D. Wedi, J. Schaller, V. Iversen, A. Drews, Fouling in MBR – what use are lab investigations for full scale operation? *Desalination* 236 (1–3) (2009) 94–103.
- [16] Y. Hu, X.C. Wang, Y. Zhang, Y. Li, H. Chen, P. Jin, Characteristics of an A²O-MBR system for reclaimed water production under constant flux at low TMP, *J. Membr. Sci.* 431 (2013) 156–162.
- [17] E.F. Hartree, Determination of protein: a modification of the Lowry method that gives linear photometric response, *Anal. Biochem.* 48 (2) (1972) 422–427.
- [18] B. Frølund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, *Water Res.* 30 (8) (1996) 1749–1758.
- [19] Y. Shen, W. Zhao, K. Xiao, X. Huang, A systematic insight into fouling propensity of soluble microbial products in membrane bioreactors based on hydrophobic interaction and size exclusion, *J. Membr. Sci.* 346 (1) (2010) 187–193.
- [20] L. Wang, R. Miao, X. Wang, Y. Lv, X. Meng, Y. Yang, D. Huang, L. Feng, Z. Liu, K. Ju., Fouling behavior of typical organic foulants in polyvinylidene fluoride ultrafiltration membranes: characterization from microforces [J], *Environ. Sci. Technol.* 47 (8) (2013) 3708–3714.
- [21] J. Lee, W.-Y. Ahn, C.-H. Lee, Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor, *Water Res.* 35 (10) (2001) 2435–2445.
- [22] Y. Liu, S.F. Yang, J.H. Tay, Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria, *J. Biotechnol.* 108 (2) (2004) 161–169.
- [23] J. Wang, L. Wu, X. Qi, Y. Qian, Characterization of nitrification activity of activated sludge by oxygen uptake rate (OUR), *Acta Sci. Circumstantiae* 19 (3) (1999) 225–229, In Chinese.
- [24] H. Zhang, T. Huang, S. Chen, L. Guo, T. Liu, X. Yang, Microbial community functional diversity and enzymatic activity in the sediments of drinking water reservoirs, Northwest China, *Desalin. Water Treat.* 52 (7–9) (2014) 1608–1614.
- [25] C. Yang, W. Zhang, R. Liu, Q. Li, B. Li, S. Wang, C. Song, C. Qiao, A. Mulchandani, Phylogenetic diversity and metabolic potential of activated sludge microbial communities in full-scale wastewater treatment plants, *Environ. Sci. Technol.* 45 (17) (2011) 7408–7415.
- [26] Chinese NEPA, *Water and Wastewater Monitoring Methods*, 4th ed., Chinese Environmental Science Publishing House, Beijing, China, 2002.
- [27] R. Chen, X.C. Wang, K. Wu, Y. Zhang, Y. Hu, Wastewater reclamation for various non-potable supplies and water quality control in a minimum-emission hydrologic cycle, *Desalin. Water Treat.* 52 (25–27) (2014) 5015–5020.
- [28] B.D. Cho, A.G. Fane, Fouling transients in nominally sub-critical flux operation of a membrane bioreactor, *J. Membr. Sci.* 209 (2) (2002) 391–403.
- [29] J. Zhang, H.C. Chua, J. Zhou, A.G. Fane, Factors affecting the membrane performance in submerged membrane bioreactors, *J. Membr. Sci.* 284 (1–2) (2006) 54–66.
- [30] A. Pollice, A. Brookes, B. Jefferson, S. Judd, Sub-critical flux fouling in membrane bioreactors – a review of recent literature, *Desalination* 174 (3) (2005) 221–230.
- [31] H.P. Chu, X.Y. Li, Membrane fouling in a membrane bioreactor (MBR): sludge cake formation and fouling characteristics, *Biotechnol. Bioeng.* 90 (3) (2005) 323–331.
- [32] Z. Wang, Z. Wu, A review of membrane fouling in MBRs: characteristics and role of sludge cake formed on membrane surfaces, *Sep. Sci. Technol.* 44 (15) (2009) 3571–3596.
- [33] E. Giraldo, M. LeChevallier, Let them wear cake, *Water Environ. Technol.* 19 (3) (2007) 47–51.
- [34] Z. Wang, Z. Wu, S. Tang, Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy, *Water Res.* 43 (6) (2009) 1533–1540.
- [35] W. Chen, P. Westerhoff, J.A. Leenheer, K. Booksh, Fluorescence Excitation-emission matrix regional integration to quantify spectra for dissolved organic matter, *Environ. Sci. Technol.* 37 (24) (2003) 5701–5710.
- [36] Z.W. Wang, S.J. Tang, Y.F. Zhu, Z.C. Wu, Q. Zhou, D.H. Yang, Fluorescent dissolved organic matter variations in a submerged membrane bioreactor under different sludge retention times, *J. Membr. Sci.* 355 (1–2) (2010) 151–157.
- [37] Y. Hu, X.C. Wang, W. Tian, H.H. Ngo, R. Chen, Towards stable operation of a dynamic membrane bioreactor (DMBR): operational process, behavior and retention effect of dynamic membrane, *J. Membr. Sci.* 498 (2016) 20–29.
- [38] T. Maruyama, S. Katoh, M. Nakajima, H. Nabetani, T.P. Abbott, A. Shono, K. Satoh, FT-IR analysis of BSA fouled on ultrafiltration and microfiltration membranes, *J. Membr. Sci.* 192 (1–2) (2001) 201–207.
- [39] J.P. Crou, M.F. Benedetti, D. Violleau, J.A. Leenheer, Characterization and copper binding of humic and nonhumic organic matter isolated from the South Platte River: evidence for the presence of nitrogenous binding site, *Environ. Sci. Technol.* 37 (2) (2003) 328–336.
- [40] A. Seidel, M. Elimelech, Coupling between chemical and physical interactions in natural organic matter (NOM) fouling of nanofiltration membranes: implications for fouling control, *J. Membr. Sci.* 203 (1) (2002) 245–255.
- [41] Y. Wu, X. Huang, X. Wen, F. Chen, Function of dynamic membrane in self-formation dynamic membrane coupled bioreactor, *Water Sci. Technol.* 51 (6–7) (2005) 107–114.