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Effective control of membrane fouling by filamentous bacteria in a submerged membrane bioreactor

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ABSTRACT

Two identical submerged membrane bioreactors (MBRs) for synthetic wastewater treatment were operated in parallel under different dissolved oxygen (DO) levels for over 3 months in this study. The digital biological microscopy, particle size distribution (PSD) analysis, gel filtration chromatography (GFC), three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy, and column chromatographic method, etc. were used to identify the difference between bulking sludge (BS) caused by filamentous bacteria (low DO operation, about 0.4 mg/L) and normal sludge (NS) (high DO operation, about 4.0 mg/L) and to obtain a comprehensive insight into the behaviours of filamentous bacteria in MBRs. Test results showed that the MBR with bulking sludge (BS-MBR) exhibited a better filtration performance and a reduced membrane fouling compared to the MBR with normal sludge (NS-MBR). It was found that the mitigation of membrane fouling by the abundant filamentous bacteria in the BS-MBR could be attributed to the larger PSD, lower hydrophobic contents in SMP, and the retention effects of a special fouling layer induced by filamentous bacteria.

1. Introduction

The conventional activated sludge (CAS) process is the most commonly used technology for wastewater treatment, which is comprised of a biochemical stage (aeration tank) for the degradation of contaminants by activated sludge and a physical settling stage (secondary clarifier) for solid/liquid separation and biomass recirculation. However, bulking sludge (BS), as a common problem in CAS systems, leads to biomass loss and poor effluent quality [1]. The overgrowth of filamentous bacteria has been often identified as the main reason causing sludge bulking [2].

In recent years, membrane bioreactors (MBRs), being an efficient technology for municipal and industrial wastewater treatment, have gained increasing popularity. MBRs, in which solid/liquid separation is performed by membranes, successfully solve the biomass separation problem occurring in CAS systems; however, a major obstacle for the applications of MBRs is the rapid decline of membrane flux as a result of membrane fouling [3–5]. In submerged MBRs, membrane fouling is influenced by a variety of factors, such as operational parameters (flux value, operational modes, etc.), membrane materials, sludge properties and so on [3]. Sludge properties are closely related to the physiological behaviours of microorganisms. If the species and/or dominant colony are changed, it will lead to different physiochemical characteristics and abnormal conditions of mixed liquor which could accordingly influence membrane fouling behaviours. Some recent studies reported that bulking sludge had significant negative effects on membrane fouling and might reduce the sustainable operation time of MBRs [3,6,7]. It was reported by Choi et al. [8] that severe membrane fouling occurred under a sludge bulking condition. Chang et al. investigated the ultrafiltration performance of various kinds of activated sludges and found that the order of the fouling potential was normal sludge (NS) < pinpoint sludge < bulking sludge [9]. With regard to the fouling mechanism of bulking sludge in MBRs, Meng et al. [6,7] insisted that severe membrane fouling under the excess growth of filamentous bacteria was caused by the change of physical and chemical properties of the activated sludge, and other researchers argued that the floc morphology of the bulking sludge had more important influences on membrane fouling [9]. However, according to the study of Li et al. [10], the effect of filamentous bacteria density on the membrane fouling rate was negligible even though filamentous bacteria could change the floc morphology. In our study, however, we interestingly found that the submerged MBR with excess growth of filamentous bacteria achieved better membrane permeability compared to a parallel submerged MBR with negligible filamentous bacteria, which is controversial to the above researches. It is very essential to investigate the membrane fouling mechanisms caused by

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the growth of filamentous bacteria and to analyze the difference between our study and other researchers' findings.

In this study, two identical submerged MBRs were operated in parallel under different dissolved oxygen (DO) levels for over 3 months. The digital biological microscopy, particle size distribution (PSD) analysis, gel filtration chromatography (GFC), three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy, and column chromatographic method, etc. were used to identify the difference between bulking sludge caused by filamentous bacteria and normal sludge and to obtain a comprehensive insight into the behaviours of filamentous bacteria in MBRs. The positive effects induced by the growth of filamentous bacteria on membrane fouling were discussed and the mechanisms for enhancing membrane filtration operation were proposed. The results obtained in this study are expected to provide a sound and all-round understanding of the role of filamentous bacteria in membrane fouling of MBRs.

2. Materials and methods

2.1. Experimental setup

As shown in Fig. 1, the experimental system consisted of two identical MBRs that were operated in parallel. Each reactor had an effective volume of 68.4 L, in which three membrane modules were mounted vertically between two baffle plates located above the air diffuser. The membranes were made of polyvinylidene fluoride (PVDF) membrane with a mean pore size of 0.20 μm and an effective filtration area 0.175 m^2 for each module. Air was monitored by a rotameter and supplied through the air diffuser which was below the membrane modules in order to supply oxygen demanded by the microorganisms and to induce a cross-flow velocity (CFV) along membrane surfaces. The characteristics and constituents of the influent wastewater are shown in Table 1. It is obvious that the influent wastewater was rich in COD, but short of nutrients, i.e., nitrogen and phosphorus, which was used to simulate some wastewater with poor nutrients (for instance, some water polluted by alcohol-distillery wastewater). The influent pump was controlled by a water level sensor to maintain a constant water level in the bioreactor over the experimental system. The membrane-filtered effluent was then obtained by suction using a pump connected to the modules. The effluent flow rate and transmembrane pressure (TMP) were monitored by a water meter and a pressure gauge, respectively.

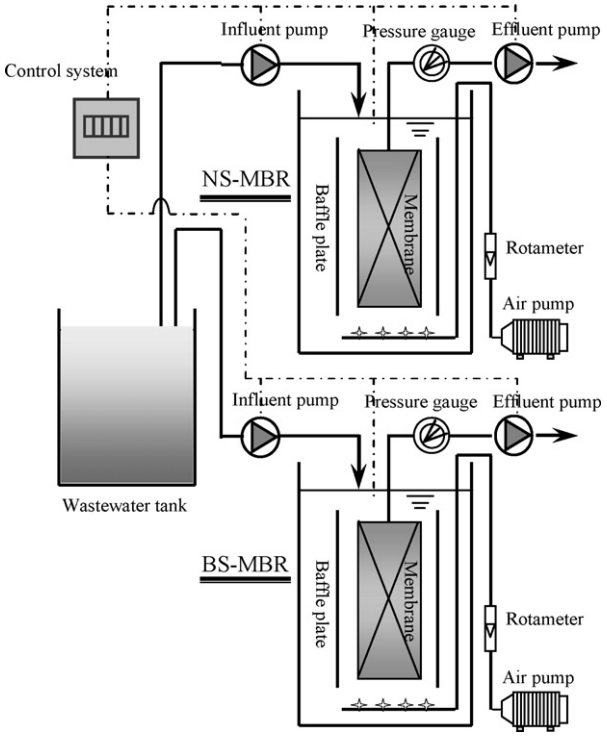

Fig. 1. Flow diagram of two parallel submerged MBRs.

Table 1
Components and characteristics of influent water.

Components	Concentration (mg/L)	Influent water parameters	Concentration (mg/L)
Glucose	450	COD	432–480
NH_4Cl	15	NH_4^+–N	3.7–3.9
KH_2PO_4	5	TP	1.0–1.1
$NaHCO_3$	200	pH	6.7–7.2
$MnSO_4$	5		
$FeCl_3$	2		
$MgSO_4$	2		

2.2. Operating conditions

The membrane flux of the two MBRs was kept constant at about 20 L/(m^2 h), which is lower than critical flux value as determined by step-wise method, during the experiment. A suction cycle of 10 min followed by 2 min relaxation (no suction) was employed. The suction mode was adopted based on our previous research and proven to be effective for controlling membrane fouling. The hydraulic retention time (HRT) and sludge retention time (SRT) were maintained at 7.8 h and 20 d, respectively. The only difference in the two MBRs was dissolved oxygen (DO) concentration in the mixed liquors by varying the supplied air flow rate. DO concentrations of 4.0 ± 0.4 and 0.6 ± 0.4 mg/L were maintained in the two MBRs, respectively. The two MBRs were seeded with activated sludge of a municipal wastewater treatment plant (WWTP) of Shanghai and acclimated for about 1 month before the filtration was begun (the TMP profiles were recorded in the following 2 months). Afterwards, the filtration experiment and a series of measurements were carried out. The steady-state mixed liquor suspended solids (MLSS) concentrations in the two MBRs were 8.5 ± 0.5 g/L (high DO operation) and 8.3 ± 0.4 g/L (low DO operation), respectively. The MBRs were operated with temperature in the range of 14–24 °C. Chemical cleaning-in-place procedure (0.5% (v/w) NaClO solution, 2 h duration) was carried out in order to recover membrane permeability if the TMP reached about 30 kPa during the operation.

2.3. Analytical methods

2.3.1. Digital biological microscope analysis

A mixed liquor sample was taken from one of the submerged MBRs by a pipette, and a little drop of the mixed liquors was placed on the center of a clean glass slide and then covered with a cover glass. It should be ensured that there were no gas bubbles trapped between the glass slide and the cover glass. The prepared sample was examined by a phase contrast digital microscope (Leica DMRME, Leica Microsystems, Germany). The Leica Qwin QG2-32 software was employed to process and analyze the images, which could create JPEG image format files of the microscopic examination samples. The microscopic examination of a mixed liquor sample was conducted in triplicate. The microorganisms in the two submerged MBRs were examined by the microscope at the same operational period (on the day 60) and the differences between them were compared.

2.3.2. PSD analysis

PSD of the mixed liquors in the two submerged MBRs was carried out by a focused beam reflectance measurement (EyeTech 60292, Ankersmid Ltd., Netherlands) in order to verify the differences between them. The EyeTech software (Version 1.1.2.0, Ankersmid Ltd., Netherlands) was used to handle the collected data and to obtain the PSD distribution.

2.3.3. GFC analysis

The dissolved organic matters (DOM) in the mixed liquor supernatants and the MBR effluent, which were obtained by filtering 100 mL of the collected sample with a 0.45 μm-pore-size-filter paper, were fractionated by a GFC analyzer. The GFC system consisted of a TSK G4000SW type gel column (TOSOH Corporation, Japan) and a liquid chromatography spectrometer (LC-10ATVP, SHIMADZU, Japan). Polyethylene glycols (PEGs) with molecular weight (MW) of 1,215,000, 124,700, 11,840 and 620 Da (Merck Corporation, Germany) were used as standards for calibration. It is well known that solution environments have significant effects on MW fractionation of the samples [11]. Therefore, pure water was used as eluent. The elution at different time intervals was collected by an automatic fraction collector and automatically analyzed by using UV spectroscopy and dissolved organic carbon (DOC) analyzer to obtain a MW distribution curve.

2.3.4. Three-dimensional EEM spectroscopy

All the three-dimensional EEM spectra of DOM in the mixed liquor supernatants were measured using a luminescence spectrometry (F-4500 FL spectrophotometer, Hitachi, Japan). The EEM spectra are a collection of a series of emission spectra over a range of excitation wavelengths, which can be used to identify the fluorescent compounds present in complex mixtures. In this study, the EEM spectra were collected with corresponding scanning emission spectra from 200 to 500 nm at 5 nm increments by varying the excitation wavelength from 200 to 400 nm at 5 nm sampling intervals. The excitation and emission slits were maintained at 10 nm and the scanning speed was set at 1200 nm/min for this study. A 290 nm emission cutoff filter was used in scanning to eliminate the second order Raleigh light scattering. The spectrum of deionized (DI) water was recorded as the blank. The software Origin 7.0 (OriginLab Inc., USA) was employed to process the EEM data. The EEM spectra are plotted as the elliptical shape of contours. The *X*-axis represents the emission spectra from 200 to 500 nm while the *Y*-axis indicates the excitation wavelength from 200 to 400 nm, and the third dimension, i.e., the contour line, is shown to express the fluorescence intensity at an interval of 5.

2.3.5. Column chromatographic analysis

The hydrophobic/hydrophilic and charge properties of DOM in mixed liquor supernatants were investigated using column chromatographic method, which was also adopted by other researchers [12]. DOM can be fractionated into four components by using the fractionation procedure as shown in Fig. 2, i.e., strong hydrophobic (HoS), weak hydrophobic (HoW), charged hydrophilic (HiC), and neutral hydrophilic fraction (HiN). The fractionation was performed using solid phase extraction (SPE) chromatography columns (Supelclean, Supelco Company, PA, USA) with a series of resin adsorbents, including a non-ionic DAX-8 resin (Supelco Company, PA, USA), a macro-porous XAD-4 resin (Amberlite, Rohm & Hass Company, PA, USA) and an IRA-958 anion exchange resin (Amberlite, Rohm & Hass Company, PA, USA).

2.3.6. Other item analysis

Measurements of chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), ammonia (NH_3–N) and pH in the influent and membrane effluent, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) in the system were performed according to the Chinese NEPA standard methods [13]. SVI was determined every two or three days in the two reactors, and the average values were reported. The DO concentration in the reactor was measured by a dissolved oxygen meter (YSI 58, YSI Research Inc., OH, USA). Total organic carbon (TOC) was determined by a TOC analyzer (TOC-VcPN, SHIMADZU, Japan). The procedures for extracellular polymeric substances (EPS) and soluble microbial products (SMP) were according to the methods described in the literature [14]. The measurement of proteins (PN) was carried out by Lowry et al. methods [15]. BSA was used as a standard. The phenol–sulfuric acid method of Dubois et al. [16] was used for polysaccharides (PS) determination. Glucose was used as a standard.

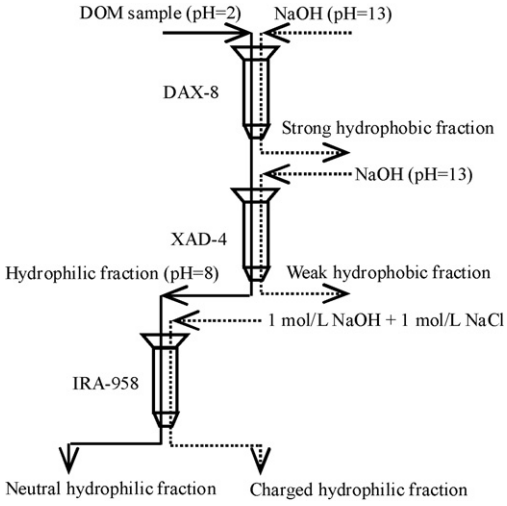

Fig. 2. DOM fractionation procedure using column chromatographic method.

3. Results and discussion

3.1. MBR performance

Due to the difference of DO concentration in the MBRs, the two MBRs exhibited different sludge characteristics, i.e., one MBR with normal sludge under high DO concentration operation (NS-MBR) and the other with bulking sludge under low DO concentration operation (BS-MBR) caused by filamentous bacteria. The BS-MBR and NS-MBR were defined according to the filamentous bacteria intensity in the reactors, i.e., the BS-MBR with abundant filamentous bacteria and NS-MBR with little even negligible filamentous bacteria. Fig. 3 illustrates the digital biological microscope images of the microorganisms in the NS-MBR and the BS-MBR. It could be observed that there was an excess growth of filamentous bacteria in the BS-MBR compared with the NS-MBR. SVI of the NS-MBR and the BS-MBR during the experiment was 77 ± 12 and 140 ± 40 mL/g, respectively, indicating that sludge settleability in the BS-MBR was significantly deteriorated due to the overgrowth of filamentous bacteria compared to that in the NS-MBR.

Table 2
COD and NH_3–N removal efficiencies of BS-MBR and NS-MBR.[a].

Items	BS-MBR	NS-MBR
COD removal (%)	93.0 ± 7.3	95.7 ± 3.7
NH_3–N removal (%)	94.1 ± 3.4	97.7 ± 2.0

[a] Values are given as mean ± standard deviation, number of measurements: $n = 10$ (BS-MBR); $n = 9$ (COD of NS-MBR), $n = 6$ (NH_3-N of NS-MBR).

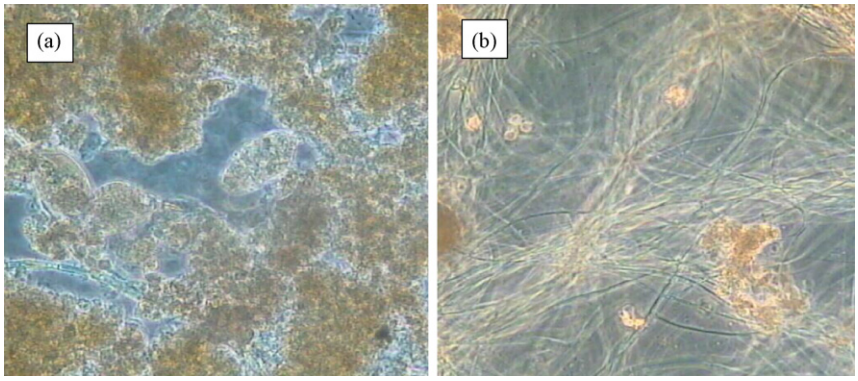

Fig. 3. Digital biological microscope images of the microorganisms in (a) NS-MBR, and (b) BS-MBR.

Table 2 summarizes the general performance of the BS-MBR and the NS-MBR in terms of COD and NH_3-N removal. The COD removal efficiencies were excellent and stable in both MBRs, with an average 93% in the BS-MBR and over 95% in the NS-MBR. Very good NH_3–N removal efficiencies were also achieved in the two MBRs mainly attributed to microorganism growth. It could also be observed that COD and NH_3–N removal efficiencies of the NS-MBR were relatively better than those of the BS-MBR, which might be due to the different dominant microbial species and their varied functions in consuming organic pollutants. The release of SMP induced by filamentous bacteria could contribute to the COD removal efficiency, i.e., lower removal efficiency in the BS-MBR compared to that in the NS-MBR.

It could be observed from Fig. 4 that TMP increased more rapidly in the NS-NBR compared with the BS-MBR. The membrane modules were cleaned three times during the experiment in the NS-MBR, i.e., on day 16, day 39 and on day 54, whereas only one time cleaning of membranes in the BS-MBR was conducted on day 40. The fouling in the BS-MBR was more reversible while it was more irreversible in the NS-MBR. The reversible fouling in the BS-MBR should be due to the blocking effects of filamentous bacteria and large size flocs formed on membrane surfaces, which acted as a secondary membrane and could prevent irreversible fouling from forming onto membrane surfaces and/or into membrane pores. It indicated that the mitigation of membrane fouling in the BS-MBR was achieved by the superfluous growth of filamentous bacteria in this study. The results are inconsistent with and even contradictory to other researchers' reports [6,7,9,10], and we will systematically analyze and discuss the mechanisms of the effects of filamentous bacteria on membrane fouling based on a series of experiments in the following sections.

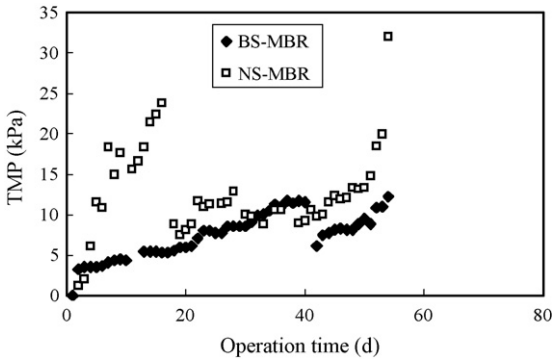

Fig. 4. TMP variations in BS-MBR and NS-MBR.

3.2. Sludge characteristics in the BS-MBR and the NS-MBR

3.2.1. PSD analysis of mixed liquors

It is well known that mixed liquors consist of suspended particles, colloids and DOM and all fractions contribute to membrane fouling in MBRs [17,18]. It is very essential to discern the physiochemical characteristics of the sludge components by using a variety of instruments.

The MLSS concentration in the BS-MBR and in the NS-MBR during the experiment was 8.3 and 8.5 g/L, respectively. Thus, the concentration difference between them was not considered as the major reason for the different membrane fouling behaviours, whereas importance should be attached to other factors. Fig. 5 shows the PSD of the mixed liquors in the two MBRs. From this number histogram of PSD, it could be observed that the particle size of mixed liquors in the BS-MBR (mean particle size 59.4 μm)

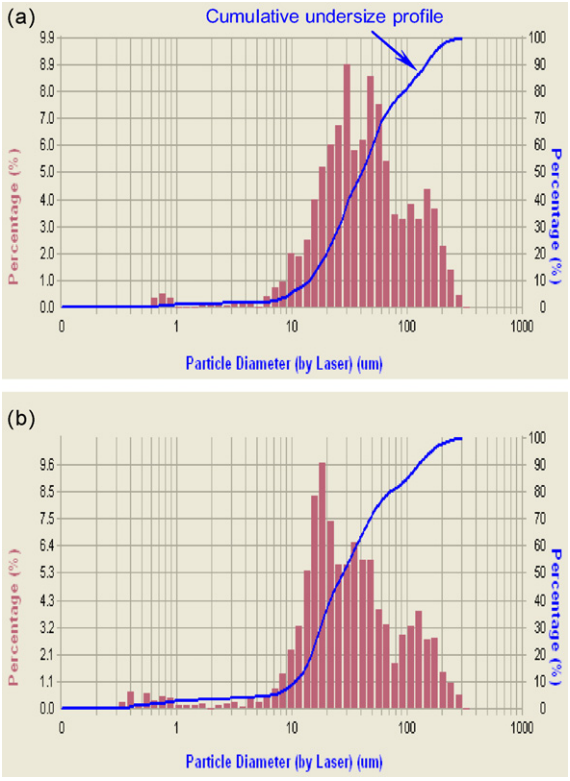

Fig. 5. PSD (number distribution) analysis of mixed liquors in (a) BS-MBR and (b) NS-MBR.

Table 3
Concentration of SMP and EPS of the mixed liquors in BS-MBR and NS-MBR.

Items	BS-MBR	NS-MBR
SMP (mg/L)	190.7 ± 19.1 (124.3 ± 13.5)[a]	44.6 ± 4.6 (27.5 ± 3.2)[a]
Colloids (mgCOD/L)	8.1 ± 0.9	8.1 ± 1.0
EPS (mg/g SS)	139.2 ± 19.0	96.7 ± 9.0
PS (mg/g SS)	52.2 ± 3.9	38.5 ± 0.7
PN (mg/g SS)	87.1 ± 15.1	58.3 ± 9.7

[a] COD (TOC), number of measurements: $n = 4$.

was larger than that in the NS-MBR (mean particle size 48.4 μm). Many researchers reported that a larger size of sludge particles could be conducive to mitigate membrane fouling caused by sludge suspended particles [14,19,20]. The larger PSD of the BS-MBR was a positive factor resulting in a better filtration performance in the BS-MBR compared to the NS-MBR. The larger size of mixed liquors in the BS-MBR could be due to the enlacing effects of filamentous bacteria. The different DO concentration induced by aeration in the two reactors might be another influential factor, and low aeration intensity could facilitate to forming large biomass flocs.

3.2.2. SMP and EPS components in supernatants

SMP and colloids concentrations in supernatants, EPS, PS and PN concentration of biomass were determined and listed in Table 3. It could be seen that the two MBRs had almost identical colloids in supernatants while SMP and EPS of the BS-MBR were higher than those of the NS-MBR. It was reported by many researchers that SMP/EPS had significant correlations with membrane fouling, and the accumulation of SMP/EPS in MBRs could cause severe membrane fouling [17,21]; however, in our study, the BS-MBR with higher concentration of SMP and EPS demonstrated lower fouling rate. It indicated that membrane fouling was not only associated with SMP or EPS concentration but also other factors, such as the SMP components, SMP chemical characteristics, etc., which will be discussed in Section 3.2.4 in order to clarify why the BS-MBR with higher SMP concentration had lower fouling rate. The higher SMP concentration in the BS-MBR should be due to the bulking sludge conditions. The low DO operation might be another important reason inducing a high SMP production.

3.2.3. GFC analysis

GFC analysis results of SMP and effluent DOM in the BS-MBR and the NS-MBR are summarized in Table 4. In order to better understand MW distributions of the two systems, number-average molecular weight (Mn), weight-average molecular weight (Mw) and the coefficient of MW distribution in terms of Mw/Mn were used in this study. A low coefficient of Mw/Mn indicates that organic substances have a narrow distribution of MW [22]. From Table 4, it could be observed that the MW distribution in the BS-MBR and the NS-MBR varied significantly. In the BS-MBR, SMP had a broad distribution of MW while the MW distribution of the effluent DOM became narrow. It suggested that part of high MW organic molecules were removed by membranes and by the deposited layer on membrane surfaces in the BS-MBR. The abundant filamentous bacteria in the BS-MBR could form a fouling layer,

Table 4
MW distribution of SMP and effluent DOM in BS-MBR and NS-MBR.

Items		BS-MBR	NS-MBR
SMP	Mw (kDa)	951.3	341.5
	Mn (kDa)	31.1	26.6
	Mw/Mn	30.6	12.9
Effluent DOM	Mw (kDa)	169.8	334.9
	Mn (kDa)	23.9	199.1
	Mw/Mn	7.1	1.7

Table 5
Column chromatographic analysis results of SMP in NS-MBR and BS-MBR.

Items	BS-MBR (%)	NS-MBR (%)
HoS	4.98	18.69
HoW	2.40	11.46
HiC	8.10	28.19
HiN	89.20	17.61

which served as a secondary dynamic membrane to filter SMP and to prevent high MW organic molecules from adsorbing onto the surface and inside membrane pores [23]. This might be another reason for the mitigation of membrane fouling in the BS-MBR. The MW distribution of the effluent DOM in the NS-MBR showed different properties from that in the BS-MBR, i.e., a much narrower distribution (Mw/Mn = 1.7) in the NS-MBR compared with that (Mw/Mn = 7.1) in the BS-MBR. The MW distribution of the effluent DOM should be influenced by the interactions of organic molecules with fouling layer and membranes, and it might be inferred that the fouling layer formed on membrane surfaces in the NS-MBR was structurally different from the fouling layer of the BS-MBR.

3.2.4. Hydrophobic/hydrophilic and charge properties of SMP in the MBRs

Table 5 presents column chromatographic analysis results of the SMP. It could be observed that the components of SMP in the BS-MBR and in the NS-MBR were different. In the BS-MBR, HiN was the most abundant fraction of SMP while hydrophobic fractions, i.e., HoS and HoW, only accounted for 4.98% and 2.40%, respectively. However, in the NS-MBR, the proportion of HoS and HoW components was larger compared to that in the BS-MBR. Hydrophobic components, probably humic and fulvic acids, were reported as major fouling factors in membrane filtration systems [24,25]. Higher concentration of hydrophobic substances could induce more severe membrane fouling. This might be an explanation for the different fouling behaviours in the two MBRs. HiN, the most abundant fraction of SMP, in the BS-MBR was not a major contributor to the membrane fouling, which could be attributed to its neutral characteristics. The HiC fraction, due to its charge properties, might have interactions with mixed liquors and membranes, and its role in membrane fouling is worth further investigating.

3.2.5. Three-dimensional EEM spectroscopy

The three-dimensional EEM fluorescence spectra of SMP and effluent DOM samples in the two MBRs are illustrated in Fig. 6. Each EEM image gave spectral information about the chemical compositions of the samples. Two main peaks could be readily identified from the fluorescence spectra of SMP and effluent DOM samples in the two MBRs. The first main peak was located at the excitation/emission wavelengths (Ex/Em) of 235/340–345 nm (Peak A), while the second main peak was observed at the Ex/Em of 280–285/320 nm (Peak B). The two peaks have been reported as protein-like peaks, in which the fluorescence is associated with the aromatic protein-like substances (Peak A) and tryptophan protein-like substances (Peak B), respectively [4,26].

The fluorescence parameters of the spectra including peak locations and fluorescence intensity are summarized in Table 6, which could be used for quantitative analysis. Peak locations of the SMP samples in the two MBRs showed slight difference. The location of Peak A in the NS-MBR was red-shifted by 5 nm along the emission axis while Peak B of the NS-MBR demonstrated a blue shift of 5 nm along the excitation axis and a blue shift of 10 nm along the emission axis compared to the locations of Peak A and Peak B in the BS-MBR. A red shift is related to the presence of carbonyl-containing substituents, hydroxyl, alkoxyl, amino groups and carboxyl constituents [4], while a blue shift is associated with

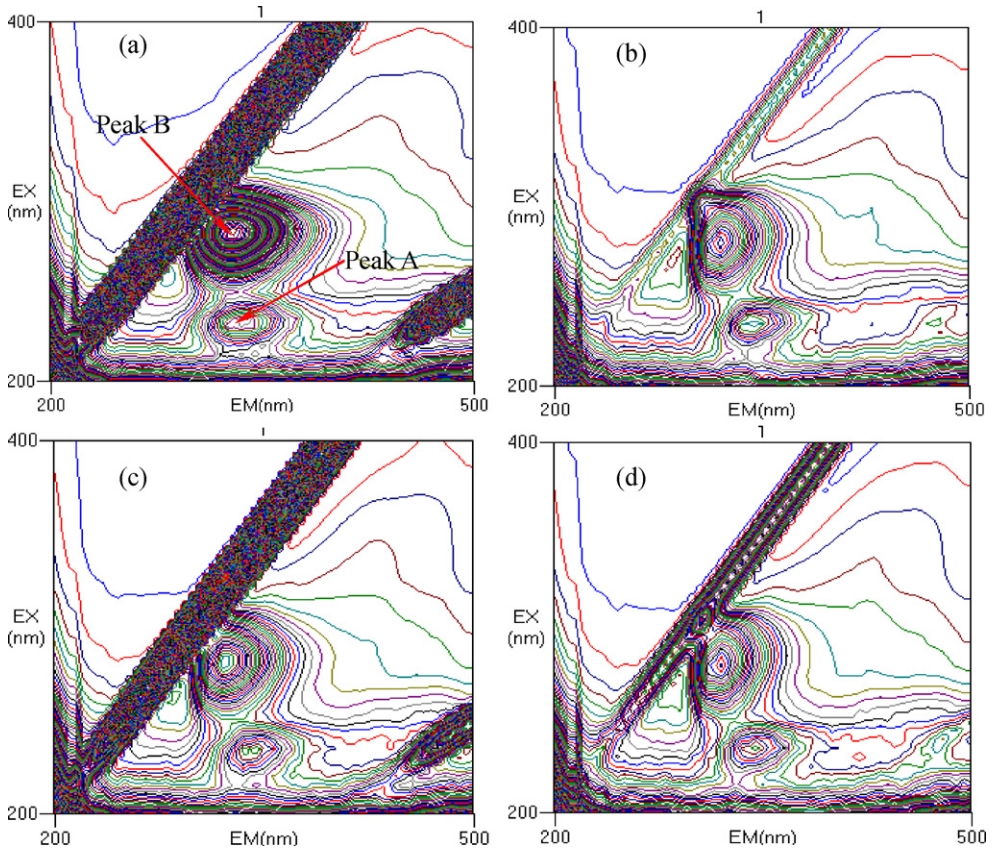

Fig. 6. EEM fluorescence spectra of SMP and effluent DOM in BS-MBR and NS-MBR. (a) SMP in BS-MBR; (b) Effluent DOM of BS-MBR; (c) SMP in NS-MBR; (d) effluent DOM of NS-MBR.

a decomposition of condensed aromatic moieties and the break-up of the large molecules into smaller fragments, such as a reduction in the degree of the π-electron system, a decrease in the number of aromatic rings, a reduction of conjugated bonds in a chain structure, a conversion of a linear ring system to a non-linear system or an elimination of particular functional groups including carbonyl, hydroxyl and amine [27,28].

It could also be observed that the peak locations of SMP and effluent DOM samples in the NS-MBR were the same while in the BS-MBR the peak locations of SMP and effluent DOM samples were varied, i.e., Peak A of the effluent DOM was red-shifted by 5 nm along the emission axis and Peak B showed a blue shift of 5 nm along the excitation axis. It indicated that the SMP in the BS-MBR was interacted with the fouling layer and membranes as they passed through them to enter the effluent. The intensity reduction rate, as listed in Table 6, could be employed to represent for the removal efficiency of fluorescent substances by the retention of fouling layer and membranes. It is obvious that the BS-MBR achieved better removal efficiency compared to the NS-MBR. This might attributed to the fact that in the BS-MBR the plenty filamentous bacterial formed a special fouling layer that could prevent fluorescent substances, to some extent, from entering the effluent [23], which also played an important role in suppressing membrane fouling in the BS-MBR.

3.3. Discussion

In our study, the bulking sludge in the BS-MBR showed better filtration performance compared to the normal sludge in the NS-MBR. This is inconsistent with other researchers' findings. Meng et al. [6] reported that the sludge flocs with filamentous bacteria created the formation of a non-porous cake layer on the membrane surface in a submerged MBR for the treatment of synthetic wastewater. However, in our study, as shown in Fig. 7, a loose and thin cake layer (Fig. 7(b)) was formed on the membrane surface in the BS-MBR, and a slime gel layer (Fig. 7(c)) was observed on the membrane surface in the NS-MBR. The excess growth of filamentous bacteria favored the formation of bigger flocs with a loose structure in the BS-MBR [10], which was also supported by the PSD analysis (see Fig. 5). Those bigger flocs with a loose structure were conducive to form the special layer. This unique layer in the BS-MBR might play an important role in preventing the EPS, SMP, etc. from adsorbing onto

Table 6
Peak location and fluorescence intensity of SMP and effluent DOM in the systems.

Items	BS-MBR				NS-MBR			
	Peak A		Peak B		Peak A		Peak B	
	Location (Ex/Em)	Intensity	Location (Ex/Em)	Intensity	Location (Ex/Em)	Intensity	Location (Ex/Em)	Intensity
SMP	235/340	151.2	285/330	324.4	235/345	129.8	280/320	184.7
Effluent DOM	235/345	133.1	280/320	169.1	235/345	128.4	280/320	166.3
Intensity reduction rate (%)	–	12.0	–	47.9	–	1.1	–	10.0

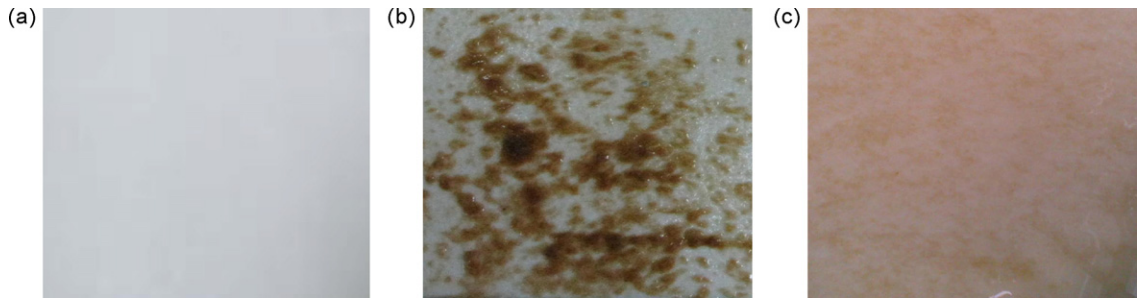

Fig. 7. Images of (a) clean membrane, (b) fouled membrane in BS-MBR, and (c) fouled membrane in the NS-MBR.

membrane surfaces or blocking membrane pores [23]. The mechanism was also supported by the research of Giraldo and LeChevallier [29], in which they observed that a thin cake layer on the membrane surface could improve MBR performance. It should be the main reason for the mitigation of membrane fouling in the BS-MBR. The differences of species and density of filamentous bacteria in our study and other researches might contribute to the varied role of filamentous bacteria in the filtration performance. In addition, the length of filamentous bacteria could also play an important role in the filtration operation. In our study, it was observed that the length was more than 200 μm. If the species of the filamentous bacteria is changed, it could result in different and even contrary filtration performance. The parameters to cultivation of filamentous bacteria at a specific influent wastewater should be studied, such as the proper DO, organic loading rate and so on if the pathway of using filamentous bacteria to control membrane fouling is employed.

It was also observed in our study that the SMP and EPS concentrations were elevated due to the overgrowth of filamentous bacteria, which is in agreement with the findings of Meng et al. [6,7]. However, the analysis on the hydrophobic/hydrophilic and charge properties of SMP in the two MBRs indicated that HiN was the most abundant fraction in the BS-MBR while HoS and HoW quantity in the NS-MBR was higher. It has been reported that hydrophobic components, probably humic and fulvic acids, were the major fouling factors in membrane filtration systems [24,25], while HiN might have weak fouling propensity due to its neutral properties. This might be another positive factor for the better filtration performance in the BS-MBR.

It should be pointed out that different permeability of bulking sludge between our study and other researchers' might be mainly associated with the various influent wastewater used. In our study, the influent wastewater, which was rich in carbon source but short of nitrogen and phosphorus, was different from the synthetic wastewater employed by others [6,7]. This difference might lead to different characteristics of bulking sludge and even diverse fouling behaviours. Some other factors might also influence the membrane fouling behaviour, such as various operation modes (constant flux mode or constant TMP mode), flux values, and membrane modules (flat-sheet, hollow fiber, etc.) between our study and other researches [6,7,10]. The detailed properties of bulking sludge, such as filamentous bacteria species, their physiology, etc. are worth further studying in order to obtain much deeper understanding on the behaviours and role of them in MBRs. In particular, the identification of filamentous bacteria species would, to a great extent, facilitate the understanding the differences between our study and other reports.

4. Conclusions

Two submerged MBRs with the same configuration and same influent wastewater were operated in parallel under different DO levels for over 3 months. The digital biological microscopy, PSD analysis, GFC technique, three-dimensional EEM analysis, and column chromatographic method, etc. were used to identify the differences between the two MBRs. Based on this study, the following conclusions could be drawn.

(1) The two MBRs showed different sludge characteristics due to the difference of DO concentration in the MBRs, i.e., one MBR with normal sludge under high DO concentration operation (NS-MBR) and the other with bulking sludge under low DO concentration operation (BS-MBR) caused by the excess growth of filamentous bacteria.
(2) The BS-MBR exhibited a better filtration condition and a reduced membrane fouling status compared to the NS-MBR. If similar wastewater is treated, it could be used to control membrane fouling by facilitating filamentous bacteria growth using proper operational parameters.
(3) Based on the PSD, GFC, EEM and column chromatographic analysis, the mitigation of membrane fouling by the abundant filamentous bacteria in the BS-MBR could be attributed to the larger PSD, lower hydrophobic contents in SMP, and to the retention effects of a special fouling layer induced by filamentous bacteria.

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