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Role of EPS in membrane fouling of a submerged anaerobic-anoxic-oxic (A-A-O) membrane bioreactor for municipal wastewater treatment

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ABSTRACT

An anaerobic-anoxic-oxic membrane bioreactor for real municipal wastewater treatment was operated under various conditions in order to investigate extracellular polymeric substances (EPS) properties and their role in membrane fouling. Test results showed that EPS, proteins in EPS (EPS_p) and carbohydrates in EPS (EPS_c) had correlations with membrane fouling. EPS_p were found to have more significant correlations with membrane fouling while EPS and EPS_c had loose correlations with membrane fouling, indicating that EPS_p were an important factor in membrane fouling. Three-dimensional excitation-emission matrix fluorescence spectra analysis revealed that Peak C had potential correlations with the membrane fouling. Peak C, which was associated with fulvic acid-like substances, should be mainly originated from microbial metabolism. Gel filtration chromatograph (GFC) demonstrated that the EPS had narrower molecular weight (MW) distribution compared to the influent wastewater. It was also found that the number-average MW (M_n) and the weight-average MW (M_w) of EPS increased with the increase of operation time.

Keywords: Anaerobic-anoxic-oxic (A-A-O) process; Biological nutrient removal; Extracellular polymeric substances (EPS); Membrane bioreactor (MBR); Membrane fouling; Wastewater treatment

1. Introduction

Nitrogen and phosphorus are known as significant nutrients to induce eutrophication of waterbodies. In recent years, considerable research has been performed on biological nutrient removal (BNR) systems which remove the problematic nutrients that cause eutrophication. The BNR systems developed include the sequencing batch reactor (SBR), the University of Cape Town (UCT) system, the Bardenpho process, and the anaerobic-anoxic-oxic (A-A-O) multiple reactor system, etc. [1].

With the rapid development of membrane technology, the integration of membrane filtration into multi-stage conventional BNR systems has attracted much attention. Compared to conventional BNR systems, the hybrid membrane process for BNR offers a series of advantages including a smaller footprint, less sludge production and better effluent quality [2–4]. A number of researchers have dedicated their efforts to the hybrid BNR system for wastewater treatment. Geng et al. [5] employed an A-A-O MBR for enhanced removal of phosphorus and analyzed the fouling mechanisms in this process. It was also reported by Banu et al. [6] that 68–75% of nitrogen and 74–84% of phosphorus were removed in the A-A-O MBR system at a flux of 17 l/(m^2h) for domestic wastewater treatment. Zhao et al. [7] used an A-A-O MBR to treat coke plant wastewater and found that 89.8%, 99.9%, 99.5%, 71.5% and 98.3% of chemical oxygen demand (COD), phenol, ammonium, total nitrogen and acute toxicity could be successfully removed. Those intensive

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efforts are very helpful to understand the behaviours and performance of hybrid BNR systems.

However, the information on fouling mechanisms of A-A-O MBR system is still insufficient. As the A-A-O MBR process consists of three compartments, namely anaerobic, anoxic and aerobic reaction zones in series and always requires mixed liquor recirculation, it is more complex than common MBRs that comprise only one compartment (either aerobic or anaerobic). Extracellular polymeric substances (EPS), which are excreted by bacteria and comprised of a variety of organic substances [8,9], are reported as an important factor resulting in membrane fouling in MBRs by a group of researchers [10–12]. However, the role of EPS in membrane fouling of A-A-O MBR system is still limited. Due to the differences of process configuration between common MBR and the hybrid A-A-O MBR system, the EPS behaviours and fouling mechanisms in the process might be different accordingly. Therefore, it is very essential to clarify the role of EPS in membrane fouling in this process and to gain a sound understanding of the system's performance.

In this study, an A-A-O MBR at an existing wastewater treatment plant (WWTP) was operated under a series of operating conditions in order to investigate EPS properties and their correlations with membrane fouling. The results obtained in this study are expected to provide a sound understanding of EPS behaviours in the A-A-O MBR system and to facilitate the application of this technology in nutrient removal from wastewaters.

2. Materials and methods

2.1. Experimental setup

The A-A-O MBR with an effective volume of 58.6 l was used in this study (Fig. 1), which consisted of four zones, i.e., anaerobic zone (8.0 l), anoxic zone (15.3 l), alterable zone (7.3 l) and MBR zone (28.0 l). The MBR zone was installed with two 0.2 μm polyvinylidene fluoride (PVDF) flat-sheet membrane modules (SHZZ-MF, Zizheng Environmental Inc., Shanghai, China). The effective filtration area of each module was 0.175 m^2. Aeration was provided at the bottom of the MBR zone in order to supply oxygen for microorganisms and to induce a cross-flow velocity along membrane surfaces for membrane fouling control. The dissolved oxygen (DO) concentration in the MBR zone and other zones were kept in the range of 1–3 mg/l and <0.2 mg/l, respectively. An aeration diffuser together with a stirrer was located at the alterable zone to achieve different operation modes. The first operation mode could be obtained by turning off the aeration and switching on the stirrer of the alterable zone, and the second operation mode was achieved when the aeration on and the stirrer off. Mixed liquors of the MBR zone and the alterable zone were recycled to the anoxic zone (recirculation I) and the anaerobic zone (recirculation II), respectively. Different operation runs were obtained by changing the sludge retention time (SRT), hydraulic retention time (HRT), aeration mode and recycle rate. The detailed operating conditions of the 8 runs based on orthogonal experiment design [13] are summarized in Table 1. A pump was used to withdraw the effluents through the membrane at a filtration-to-idle ratio of 8 min/2 min. The membrane filtration was operated under constant flux mode (membrane flux ranging from 19.2 to 23.5 l/(m^2h)), and the trans-membrane pressure (TMP) was monitored with a pressure gauge. Chemical cleaning-in-place procedure (0.5% (v/v) NaClO solution, 2 h duration) would be carried out if the TMP reached about 30 kPa during the operation. The influent to the experimental setup was municipal wastewater, the characteristics of which are listed in Table 2.

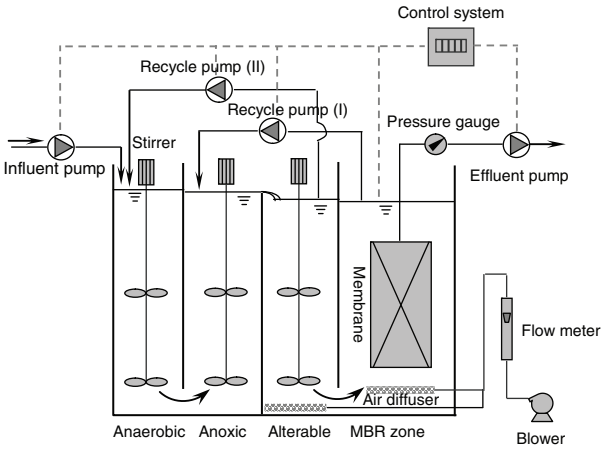

Fig. 1. Flow diagram of the MBR setup.

Table 1
Operation conditions of 8 MBR runs

Run	1	2	3	4	5	6	7	8
SRT (d)	60	60	40	40	20	20	10	10
HRT (h)	10.2	8.2	8.2	10.2	8.2	10.2	10.2	8.2
Rate of recirculation I	3	2	3	2	3	2	3	2
Rate of recirculation II	1	0.5	1	0.5	0.5	1	0.5	1
Aeration mode[a]	1	2	2	1	1	2	2	1
Operating time (d)	100	100	80	80	40	40	50	50

[a] 1 and 2 means that the alterable zone was operated under the first operation mode and the second operation mode, respectively.

Table 2
Influent and effluent water characteristics of 8 runs in the A-A-O MBR[a]

Items	COD		TN		NH_3-N		TP	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Run 1	299 ± 297	24 ± 48	48.9 ± 25.6	13.3 ± 6.6	31.7 ± 17.1	1.3 ± 3.3	5.4 ± 1.7	2.6 ± 1.4
Run 2	277 ± 241	22 ± 16	39.5 ± 16.1	13.3 ± 9.9	26.7 ± 23.1	0.5 ± 1.8	3.9 ± 2.5	2.3 ± 1.1
Run 3	182 ± 45	15 ± 17	29.8 ± 16.7	11.1 ± 7.2	23.1 ± 6.4	0.5 ± 1.5	5.0 ± 1.5	2.1 ± 2.0
Run 4	304 ± 137	22 ± 34	41.2 ± 9.7	11.1 ± 6.4	28.7 ± 9.8	0.8 ± 1.6	5.1 ± 2.0	2.0 ± 1.1
Run 5	226 ± 202	21 ± 11	30.8 ± 20.3	6.4 ± 4.7	21.0 ± 20.0	1.0 ± 3.4	4.4 ± 2.8	2.1 ± 1.3
Run 6	184 ± 30	22 ± 19	33.1 ± 13.4	8.8 ± 3.1	23.9 ± 0.3	0.8 ± 0.8	3.9 ± 1.7	2.4 ± 1.0
Run 7	314 ± 147	31 ± 40	32.1 ± 18.3	12.6 ± 8.6	32.8 ± 16.3	4.5 ± 17.3	5.5 ± 2.7	1.4 ± 1.2
Run 8	293 ± 185	24 ± 27	33.9 ± 38.2	11.2 ± 7.5	31.8 ± 17.3	5.5 ± 13.8	4.6 ± 3.6	1.7 ± 1.8

[a] Values are given as average value ± standard deviation. Number of measurements: n = 30 (Run 1); n = 27 (Run 2); n = 13 (Run 3); n = 22 (Run 4); n = 8 (Run 5); n = 8 (Run 6); n = 11 (Run 7); n = 18 (Run 8).

2.2. Analytical methods

Measurements of chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and ammonia (NH_3-N) in the influents and membrane effluents, mixed liquor suspended solids (MLSS) in the system were performed according to the Chinese NEPA standard methods [14]. The DO concentration in the reactor was measured by a dissolved oxygen meter (Model YSI 58, YSI Research Inc., OH, USA). Total organic carbon was analyzed by a total organic carbon (TOC) analyzer (LiquiTOC trace, ELEMENTAR, Germany). Bound EPS were extracted from biomass in the MBR according to the thermal treatment method described by the reference [15]. Carbohydrates in EPS (EPS_c) were measured according to the phenol-sulfuric acid method [16] with glucose as the standard reference, whereas the modified Lowry method [17] was used for proteins in EPS (EPS_p) with bovine serum albumin (BSA) as the standard reference.

The three-dimensional excitation-emission matrix (EEM) fluorescence spectra were measured using a luminescence spectrometry (F-4500 FL spectrophotometer, HITACHI, Japan). The EEM spectra were collected with the scanning emission spectra from 200 nm to 500 nm at 5 nm increments by varying the excitation wavelengths from 200 nm 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. The EEM spectra are plotted as the elliptical shape of contours. The X-axis represents the emission spectra while the Y-axis indicates the excitation wavelength, and the third dimension, i.e., the contour line, is shown to express the fluorescence intensity at an interval of 5.

The molecular weight (MW) distribution of the DOM samples were measured by GFC analyzer, which consisted of a TSK G4000SW type gel column (TOSOH Corporation, Japan) and a liquid chromatography spectrometer (LC-10ATVP, SHIMADZU, Japan). Polyethylene glycols (PEGs) (CAS number: 25322-68-32, Merck Chemicals Inc., Germany) were used as standards for calibration. The elution at different time intervals was collected by an automatic fraction collector and automatically analyzed by using a UV spectroscope and a dissolved organic carbon (DOC) analyzer to obtain a MW distribution curve.

3. Results and discussion

3.1. MBR process performance

Table 2 summarizes the average effluent water characteristics of the 8 runs. Under different operating conditions, the organic removal efficiency was also varied. Lowest effluent TN concentration was achieved during Run 5, while TP was most successfully removed at Run 7.

The TMP variations in the MBR of Run 1 (TMP1) and Run 2 (TMP2) are illustrated in Fig. 2. The variations of TMP in the rest 6 runs are not shown here. The fouling rate was determined by the TMP increase rate per unit time. In order to compare the overall fouling behaviours among the 8 runs, the TMP increase rate in a run was averaged among all the cycles of the run. The order of fouling rate according to the TMP increase rate was Run 2 (0.54 kPa/d) < Run 4 (1.4 kPa/d) < Run 3 (1.6 kPa/d) < Run 6 (1.7 kPa/d) < Run 1 = Run 8 (2.2 kPa/d) < Run 7 (2.5 kPa/d) < Run 5 (4.1 kPa/d). Run 2 demonstrated best filtration performance while membrane fouling occurred most rapidly in Run 5.

The sludge concentrations together with MLVSS/MLSS (*f*) ratios of Runs 1 & 2 are plotted in Fig. 3. The MLSS of Run 1 was in the range of 4.0–7.2 g/l and stabilized at about 7.0 g/l, while it ranged from 8.0 g/l to 10.0 g/l in Run 2 and averaged at 9.0 g/l. The variation figures of sludge concentrations in the rest 6 runs are not listed here. Their stabilized sludge concentration during

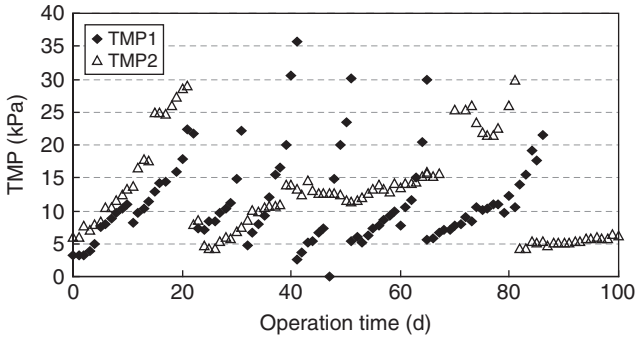

Fig. 2. TMP variations during the operation of Run 1 and Run 2.

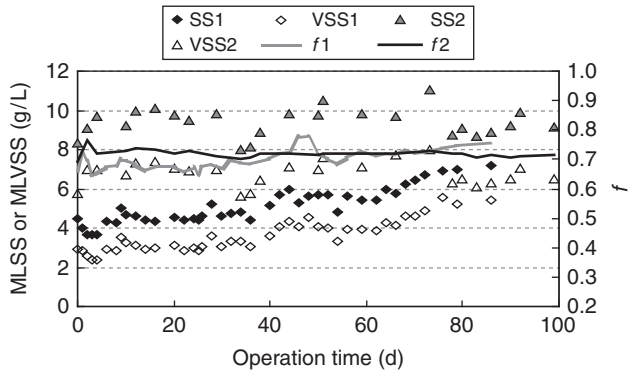

Fig. 3. Sludge concentration variations during Run 1 and Run 2.

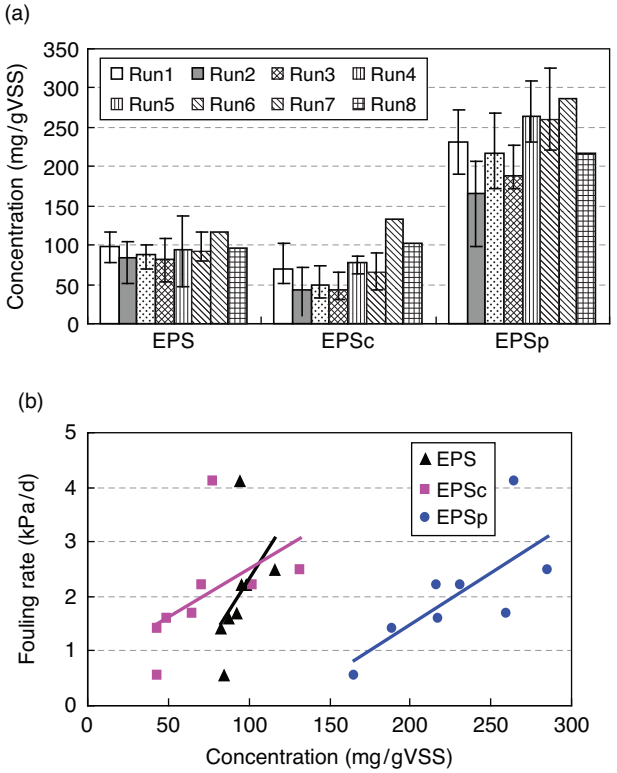

Fig. 4. (a) Variations of EPS, EPS_c and EPS_p in 8 runs; (b) correlations of EPS, EPS_c and EPS_p with membrane fouling rate.

the experiments in each run was as follows: 4.2 g/l (Run 3), 6.0 g/l (Run 4), 3.5 g/l (Run 5), 3.2 g/l (Run 6), 3.6 g/l (Run 7), and 3.9 g/l (Run 8). In this study, it could be found that the sludge concentrations had no obvious correlations with membrane fouling rate. Therefore, in the following sections, we will analyze and discuss the possible relations of EPS to membrane fouling in the 8 runs.

3.2. EPS variations and their relations to membrane fouling

In the 8 runs, the bound EPS concentration, EPS_c, and EPS_p were determined, respectively, and are listed in Fig. 4 (a). In order to investigate the correlations of EPS with membrane fouling, Fig. 4 (b) is plotted based on the fouling rate and EPS concentrations in the 8 runs. Through Fig. 4 (b), it could be seen that the EPS, EPS_c, and EPS_p had correlations with membrane fouling. This is consistent with the previous study on an aerobic MBR [15]. Based on single variable linear regression analysis, it showed that EPS_p had more significant correlations with membrane fouling than EPS and EPS_c, The coefficient of single variable linear regression of EPS_p with membrane fouling was $r = 0.7344 > r_\alpha = 0.707$ (at $\alpha = 0.05$ level, $n = 8$), while the coefficients of EPS and EPS_c were 0.4836 and 0.5208, respectively. It indicated that EPS_p might be an important factor in membrane fouling of the MBR while EPS and EPS_c had loose correlations with membrane fouling. This might be due to the fact that EPS_p have a generally a hydrophobic tendency while EPS_c is more hydrophilic [18]. The hydrophobic organic substances might be prone to inducing membrane fouling.

3.3. EEM analysis of EPS properties

Fig. 5 shows the EEM spectra of EPS in Run 1. In the rest 7 runs, the EEM spectra were similar to those of Run 1 except for the fluorescence intensity of peaks. It could be readily identified from Fig. 5 that there were three major peaks in the spectra. Peak B was located at excitation/emission wavelengths (Ex/Em) of 290/350 nm, which was associated with tryptophan protein-like substances [19]. Peaks C and D were located at the Ex/Em of 350/440 nm, and 415/475 nm, which have been reported as fulvic acid-like substances (Peak C) and humic acid-like substances (Peak D), respectively [19,20]. In the EEM spectra of influent wastewater (data not shown), it was found that three main peaks, i.e., Peaks A, B & C, could be identified. Peak A was located at the Ex/Em of 235/345 nm, and the locations of Peaks

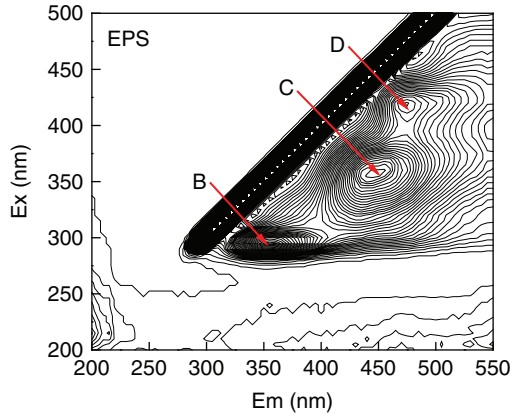

Fig. 5. Fluorescent EEM spectra of EPS in Run 1.

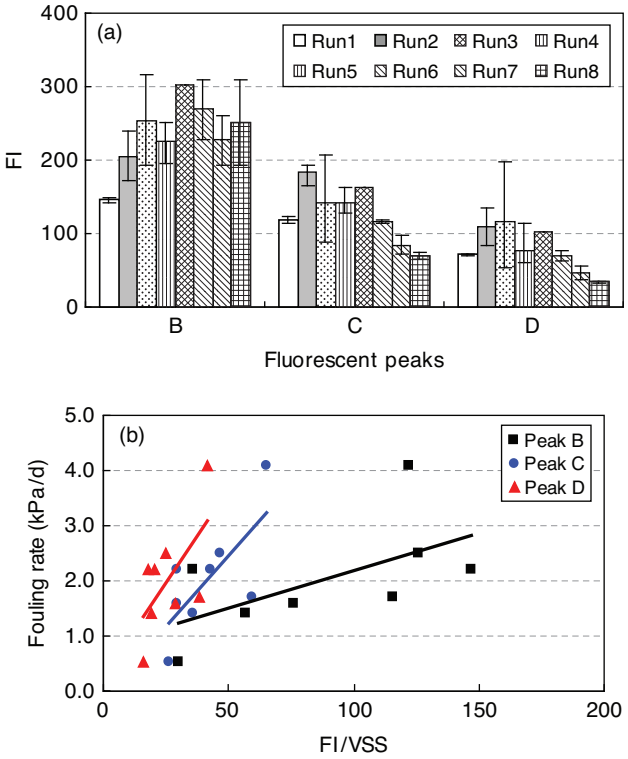

Fig. 6. (a) FI of Peaks B, C and D in the 8 runs; (b) correlations of FI/VSS with membrane fouling rate.

B & C were similar to those of spectra of EPS. These differences might suggest that Peak D of EPS's spectra should be mainly excreted by microorganisms during the degradation of organic pollutants.

In order to analyze the correlations of EPS with membrane fouling, the fluorescence intensities (FI) of main peaks are illustrated in Fig. 6(a). Fig. 6(b) shows the relations of normalized FI index (FI/VSS) to membrane fouling rate. It could be observed that the FI of Peaks B, C & D had obvious correlations with membrane fouling rate. The coefficient of single variable linear regression of Peak C's FI with membrane fouling was $r = 0.7208 > r_{\alpha} = 0.707$ (at $\alpha = 0.05$ level, n = 8), indicating that the fulvic acid-like substances also had potential correlations with membrane fouling in the A-A-O MBR. However, the coefficients of Peaks B & D's FI were 0.5937 (significant at 0.15 level) and 0.6263 (significant at 0.10 level), respectively, suggesting a poor correlations with membrane fouling.

3.4. GFC analysis of EPS properties

The MW distributions of EPS and influent wastewater are shown in Fig. 7 (a). It demonstrates that the influent wastewater had broader MW distribution than EPS. The peak value of EPS was located at 7.7 kDa, while the influent wastewater showed three main peaks, i.e., at 0.34 kDa, 38.0 kDa and 297.5 kDa, respectively. For better understanding of MW distribution, number-average molecular weight (M_n) and weight-average molecular weight (M_w) were used in this study. Fig. 7 (b) shows the variations of M_n and M_w of EPS in Run 2 during the operation. It indicated that M_n and M_w increased gradually with the increase of operation time. The MW increase of EPS might also contribute to membrane fouling during the experiment, which was due to the retention of large MW organic substances by membrane pores [4].

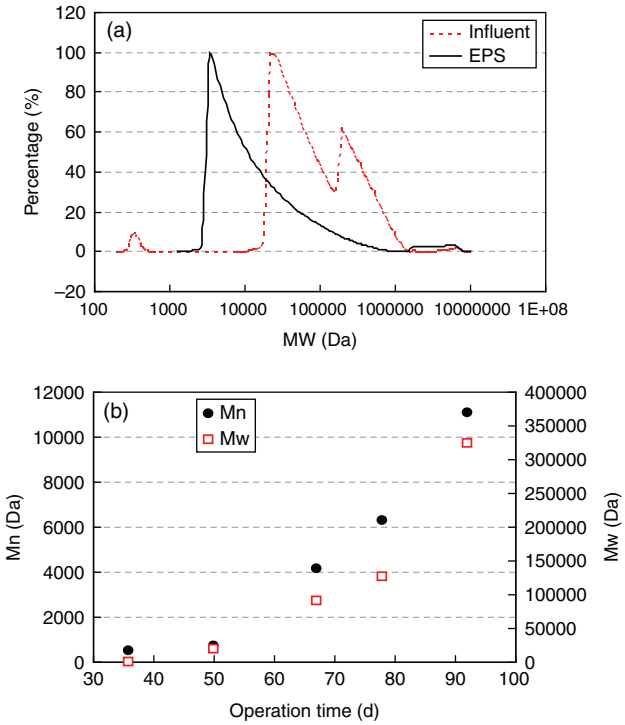

Fig. 7. (a) MW distribution of EPS and influent wastewater; (b) variations of M_n and M_w during the operation in Run 2.

4. Conclusions

EPS properties and their relations to membrane fouling were systematically investigated in the A-A-O MBR, and the following conclusions could be drawn.

1. EPS_p were found to have more significant correlations with membrane fouling compared to EPS and EPS_c, indicating that proteins might play an important role in membrane fouling.
2. Through three-dimensional EEM spectra analysis, it showed that Peak C had certain relations to the membrane fouling. Peak C, which was associated with fulvic acid-like substances, was mainly originated from microbial metabolism.
3. GFC demonstrated that the EPS had narrower MW distribution compared to the influent wastewater. It was also found that the M_n and M_w of EPS increased with the increase of operation time.

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