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Fouling analysis of membrane bioreactor treating antibiotic production wastewater at different hydraulic retention times

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Abstract Membrane fouling, including foulants and factors, was investigated during hydraulic retention time (HRT) optimization of a membrane bioreactor (MBR) that treated wastewater from the production of antibiotics. The results showed that HRT played an important role in membrane fouling. Trans-membrane pressure (TMP), membrane flux, and resistance were stable at −6 kPa, 76 L m^{-2} h^{-1} bar^{-1}, and 4.5×10^{12} m^{-1} when HRT was at 60, 48, and 36 h, respectively. Using Fourier transform infrared spectroscopy, foulants were identified as carbohydrates and proteins, which correlated with effluent organic matter and effluent chemical oxygen demand (COD) compounds. Therefore, membrane fouling trends would benefit from low supernatant COD (378 mg L^{-1}) and a low membrane removal rate (26 %) at a HRT of 36 h. Serious membrane fouling at 72 and 24 h was related to soluble microbial products and extracellular polymeric substances in mixed liquor, respectively. Based on the TMP decrease and flux recovery after physical and chemical cleaning, irremovable fouling aggravation was related to extracellular polymeric substances' increase and soluble microbial products' decrease. According to changes in the specific oxygen uptake rate (SOUR) and mixed liquor suspended solids (MLSSs) during HRT optimization in this study, antibiotic production wastewater largely inhibited MLSS growth, which only increased from 4.5 to 5.0 g L^{-1} when HRT was decreased from 72 to 24 h, but did not limit sludge activity. The results of a principal component analysis highlighted both proteins and carbohydrates in extracellular polymeric substances as the primary foulants. Membrane fouling associated with the first principal component was positively related to extracellular polymeric substances and negatively related to soluble microbial products. Principal component 2 was primarily related to proteins in the influent. Additional membrane fouling factors included biomass characteristics, operational conditions, and feed characteristics.

Keywords Fouling · Foulant · HRT · Effluent organic matter · Extracellular polymeric substances · Soluble microbial product · Correlation analysis · Principal component analysis

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Introduction

Pharmaceutical wastewater treatment challenges have become more apparent in the last decade due to the rapidly increasing scale of discharges and more stringent discharge standards in China. The discharged pharmaceutical wastewater increased from 360 to 540×10^6 m^3 (150 %) between 2003 and 2013 (National Bureau of Statistic China 2014), which corresponds to 2 % of total industrial wastewater produced in China. Pharmaceutical production wastewater has attracted additional attention because of its broad health implications (Berendonk et al. 2015). Along with increasing environmental protection requirements, the pharmaceutical industry is listed as one of 10 key industries that should be subject to more stringent wastewater discharge standards, according to the Action Plan for Water Pollution Prevention and Control issued in April 2015 (State Council of China 2015). Therefore, there is considerable impetus to develop strategies for upgrading activated sludge systems in existing biological pharmaceutical wastewater treatment facilities.

One effective way to update existing conventional activated sludge (CAS) systems in pharmaceutical wastewater treatment is to use membrane bioreactors (MBRs). MBRs have demonstrated higher removal rates of chemical oxygen demand (COD) and pharmaceuticals than CAS, but fouling is still a critical challenge (Lin et al. 2012). Pharmaceutical wastewater contains 10~40 types of recalcitrant chemical materials that are the result of the complex pharmaceutical production process (Ministry of Environmental Protection of the People's Republic of China 2009). The existing activated sludge system is often shocked by volatile wastewater quantities and quality because pharmaceutical wastewater is typically discharged in batches (Ministry of Environmental Protection of the People's Republic of China 2009). Treated wastewater often contains a significant amount of recalcitrant pollutants because conventional activated sludge systems lack the capacity to remove them (Xing and Sun 2009). With prolonged solid retention time (SRT) achieved in a compacted reactor, MBR is now seen as a cost-effective method to remove recalcitrant organics (Hai et al. 2010). MBR is an attractive choice because stringent discharge standards can be satisfied within a limited footprint (Cheng 2012). Recalcitrant pollutants can be retained and removed by MBR (Sundararaman and Saravanane 2010; Xing and Sun 2009). When SRT is long enough, the retained recalcitrant organics may be biodegraded by the biomass within the limits of the substrates (Hai et al. 2010; Raj et al. 2013). *Lin* summarized the performance of MBR in treating pharmaceutical wastewater, highlighting the removal of pharmaceutical pollutants (Lin et al. 2012). Furthermore, downstream treatments can benefit from the higher organic removal rate provided by the MBR. Our previous studies have shown that a combined MBR-nanofiltration (NF) process with recycling NF concentrate not only had high-quality permeate to meet the wastewater reuse requirement but also achieved a high water yield of 92 ±5.6 % (Wang et al. 2014b). MBR could biodegrade proteins, polysaccharides, and humic-like substances which are otherwise retained and recycled by the NF membrane without significant changes in the microbial community (Wang et al. 2014a, b, 2015).

A common operational counter measure for volatile and recalcitrant organic pollutants involves decreasing the organic loading rate by prolonging hydraulic retention time (HRT) (Sipma et al. 2010; Sundararaman and Saravanane 2010); however, a definite connection between HRT adjustment and membrane fouling trends is still missing in the treatment of pharmaceutical wastewater using a MBR. *Tay* investigated the effect of HRT on system performance in a submerged MBR treating simulated high-strength wastewater, and cues of the potential solution for the membrane fouling were studied during optimization of HRT from 6 days to 12 h (Tay et al. 2003). *Zhou* investigated the effects of MBR's operational parameters on membrane fouling in a submerged MBR designed to treat synthetic coke wastewater, and an optimum operation strategy to minimize membrane fouling required a HRT of 12 h (Zhou et al. 2009).

Therefore, the object of this paper was to further understand the relationship between HRT and membrane fouling in pharmaceutical production wastewater treatment using a MBR. This objective was achieved by investigating foulants in mixed liquor and MBR effluent, respectively. Meanwhile, an advanced statistical analysis was used to further analyze the relationship between membrane fouling and antibiotic production wastewater, biomass, and operational conditions.

Materials and methods

MBR configurations

A submerged MBR was used in this study (Table 1). A flat sheet membrane (PVDF, SINAP Ltd., Shanghai) was used as a solid and liquid separation and submerged in an aeration tank at 100 L of working volume. Permeate from the MBR membrane was intermittently obtained by using a peristaltic pump (BT600, Longer Pump Ltd., China). The membrane was chemically cleaned (0.5 % NaOCl) every 2 weeks. Antibiotic production wastewater was pumped automatically from a storage tank into the bioreactor using a feeding pump (BT300, Longer Pump Ltd., China).

Pharmaceutical wastewater

The feed wastewater was the effluent of an anaerobic digestion process from a wastewater treatment station in a pharmaceutical company producing spiramycin in Wuxi (China). The characteristics of this effluent are listed in Table 1.

MBR operational conditions

The MBR system was used to simulate upgrades to an existing activated sludge system in the wastewater treatment facilities (500 m^3 day^{-1}) of the Wuxi 2nd Pharmaceutical Company. The initial operational conditions were the same as those for the existing activated sludge system (Online Resource 1). Extra alkalinity for nitrification was supplied in the form of Na_2CO_3 instead of CaO in situ. One-hundred liters of

Table 1 Reactor configurations, operation conditions, and feed pharmaceutical wastewater characteristics in MBR at different HRTs

Time (days)	1~14	15~28	29~42	43~56	(57) 71~84
Configurations					
Volume of bioreactor (L)	100 L				
Type of aeration tank	Continuous stirred-tank reactor				
Membrane module	Flat sheet, PVDF, pore size 0.1 μm, membrane area 4×0.055=0.22 m^2				
Operation conditions					
HRT (h)	72	60	48	36	24
T (°C)	24.8±1.6	27.3±0.7	28.0±1.3	29.7±0.5	28.1±1.3
pH	6.79±0.14	6.54±0.27	6.41±0.27	6.56±0.20	6.25±0.90
DO (mg L^{-1})	4.52±0.29	3.67±0.65	3.60±0.80	3.61±1.00	4.35±2.11
SRT (days)	70				
MLSS (g L^{-1})	4.47±0.82	3.74±0.24	3.96±0.07	3.82±0.01	4.95±0.77
MLVSS (g L^{-1})	3.33±0.68	2.76±0.14	2.76±0.10	2.91±0.20	3.88±0.63
MLVSS/MLSS	0.74±0.02	0.73±0.01	0.69±0.01	0.76±0.05	0.78±0.01
Organic loading rate ($kg_{COD}kg_{VSS}^{-1}day^{-1}$)	0.08±0.04	0.13±0.02	0.18±0.01	0.19±0.01	0.38±0.04
NH_4^+-N loading rate ($g_{NH4±N}kg_{VSS}^{-1}day^{-1}$)	0.016±0.00	0.024±0.00	0.035±0.00	0.047±0.00	0.094±0.02
Feed pharmaceutical wastewater					
COD (mg L^{-1})	800~2000	Spiramycin (mg L^{-1})			10~20
NH_4^+-N (mg L^{-1})	150~300	Cl^- (mg L^{-1})			1600~2000
TN (mg L^{-1})	280~400	Ca^{2+} (mg L^{-1})			200~300
TP (mg L^{-1})	6~20	Na^+ (mg L^{-1})			700~1200
SS (mg L^{-1})	700~1000	–			

activated sludge at 4.47±0.82 g L^{-1} mixed liquor suspended solid (MLSS) from the existing activated sludge system of Wuxi 2nd Pharmaceutical Company was inoculated at a sludge loading rate of 0.08±0.04 $kg_{COD}kg_{VSS}^{-1}day^{-1}$. The feeding pump was controlled by a level sensor and an electric relay (JYB-714, Xinling Ltd., China). The membrane filtration was intermittently sucked in the interval mode of 6 min on and 2 min off. The membrane was operated in constant flux mode. The membrane's critical flux was 12.24 L m^{-2} h^{-1}, and a subcritical flux value of 6.5 L m^{-2} h was used in this work (Cheng et al. 2012). The trans-membrane pressure (TMP) of the MBR was kept constant below −25 kPa by a pressure sensor (HSCH6/C-HRT, Shi-he Ltd., Beijing). If the pressure was too high, intensive aeration was performed firstly, and then, chemical cleaning was applied. As no excess sludge was discharged in this MBR operation except for during sludge sampling, the MLSS barely increased at any stage after the initial growth, which was similar to results found in a previous work (Sun et al. 2006).

The MBR had been running for 76 days before this study began. The 205 days of the MBR's operation during this study were divided into two periods (Online Resource 1). Period 1 (0~121 days) operated steadily for 84 days at 72 h following the MBR's start-up in the first 37 days. Period 2 (122~207 days) operated for 85 days to allow for HRT adjustments, in which HRT was gradually decreased following a stepwise method. The HRT was reduced by 12 h once every 2 weeks; this was considered a steady state for over three HRTs (Viero and Sant'Anna 2008). Five stages were carried out over those 85 days (Table 1). No sludge was discharged except for sludge sampling, resulting in prolonged SRT over 70 days. In all HRT adjustment stages, effluent COD and MLSS achieved a stable state before the next adjustment.

Membrane foulants

Extracellular polymeric substances (EPSs) and soluble microbial products (SMPs) were extracted using a thermal treatment method (Chang and Lee 1998) twice a week approximately. The mixed liquor was firstly separated as supernatant and sludge using a centrifuge (4000 rpm, 30 min). The first supernatant was a SMP solution, while the remaining sludge was re-suspended with saline water (0.9 % NaCl solution) to its original volume. The re-suspended liquor was then subjected to a heat treatment (100 °C, 1 h) and centrifuged (4000 rpm, 30 min) again. The second supernatant was an EPS solution. Both supernatants were filtered through a 0.45-μm cellulose acetate membrane before proteins and carbohydrates were quantified (Malamis and Andreadakis 2009). Proteins and carbohydrates of both supernatants were determined using Lowry (Lowry et al. 1951) and Dubois's methods (DuBois et al. 1956). The reference standard was the bovine serum albumin and Dglucose (Sinopharm Chemical Reagent Co., Ltd). Effluent organic matter (EfOM) was analyzed by following the same procedure as outlined for proteins and carbohydrates. To compare these data with the measured COD effluent according to Jang's research (Janga et al. 2007), the following equation was used:

$$\text{Calculated COD} = 1.25 \ g_{COD}g^{-1} \times \text{Carbohydrate} + 1.80 \ g_{COD}g^{-1} \times \text{Protein} \qquad (1)$$

Membrane foulants were sampled for Fourier transform infrared spectroscopy (FTIR) analysis at the end of each HRT stage. Membrane foulants were first scratched from the membrane using a plastic board and then flushed with ultrapure water; next, they were dissolved into 500 mL deionized water using a magnetic stirrer. A 200 mL sample was dried at 70 °C for 48 h and then smashed as powder. The powder was mixed and compacted with KBr at a mass ratio of 2:100 for FTIR analysis by a Fourier transform infrared spectroscope (Nicolet 750, American).

Analysis

Samples of influent, mixed liquor and effluent were taken for analysis twice a week. The mixed liquor was firstly centrifuged at 8000 rpm for 10 min at 4 °C using a TGL-16 M (Xiangyi, China), and then, it was filtered through a 0.45-μm membrane (Membrana, Germany) to obtain the supernatant. The COD concentrations were determined following the standard method (Water and Wastewater Monitoring Method Editorial Committee 2002) via a spectrophotometer (DR2800, HACH Inc., USA). NH_4^+-N, TN, and TP were also determined following the standard method (Water and Wastewater Monitoring Method Editorial Committee 2002) using SAN^{++} (Skalar/SmartChem, USA). Total organic carbon (TOC) was measured with a LiquiTOC (Elementar, Germany). Ca^{2+} and Mg^{2+} were measured using an inductively coupled plasma with optical emission spectroscopy (Optima 2000DV, PE, USA). Cl^- was measured by an ion chromatograph (ICS-1000, Waters, USA). The concentrations of total suspended solids (TSSs) and volatile suspended solids (VSSs) were determined at 104 °C (4 h) and 600 °C (2 h), respectively. Specific oxygen uptake rate (SOUR) values were tested using a Winkler bottle and the oxygen consumption method (Jubany et al. 2005; Ng et al. 2006). Particle size distribution was analyzed using size exclusion chromatography (Malvern Mastersizer 2000, Malvern Instruments Ltd., England).

Statistical analyses, including a principal component analysis (PCA) and a Pearson's correlation analysis, were carried out using SPSS software (20.0, SPSS Inc., USA). The PCA is an advanced regression technique and a dimension reduction statistical method generally used to arrange a large set of variables into several major components (Naessens et al. 2012). PCA was carried out in this study to identify principal components (PCs) that would explain and illustrate the data in terms of the following four classic categories (Meng et al. 2009; Zuriaga-Agustí et al. 2014): feed characteristics (e.g., protein, carbohydrate, TOC, and ammonia), biomass characteristics (e.g., viscosity, specific viscosity, capillary suction time (CST), TOC, EPS, and SMP, including proteins and carbohydrates), membrane characteristics, and operational conditions (e.g., organic loading rate (OLR), mixed liquor volatile suspended solid (MLVSS), SOUR, *f*, resistance, and effluent). Our previous studies have shown that membrane characteristics are important in the membrane fouling of MBR treating pharmaceutical wastewater (Cheng et al. 2012), so these characteristics were not included in this investigation. In this study, three classic categories, including feed wastewater, biomass, and operational parameters, were used in the PCA to analyze the factors affecting membrane fouling.

Results and discussion

Membrane fouling

TMP and flux values throughout the study are shown in Fig. 1a. TMP was stable when HRT ranged from 36 to 64 h and fluctuated beyond that range. A sudden increase in TMP was observed when HRT was at 72 h. Five days after the first increase in TMP, 24-h aeration without effluent operation was performed to physically clean the membrane; however, while TMP decreased for 1 day, it rose above −45 kPa on the seventh day again. Then, a cleaning in place (CIP) chemical cleaning and another aeration without effluent operation were executed, suggesting that high fouling rates occurred with the removable and irremovable foulants at a 72-h HRT. Then, the TMP fluctuated less at 60-h HRT. Chemical cleaning and intensive aeration were performed only twice, once on days 20 and 23, respectively. The TMP was stable around −6.3 kPa when HRT was at 36 and 48 h, which indicated that the fouling occurred slowly and was reversible in these two stages. However, the TMP linearly increased despite intensive aeration performed when HRT was at 24 h. These results clearly show that membrane fouling under −15 kPa in the early stages of treatment could be recovered by non-effluent aeration but could not be recovered from day 66 to day 73. Operating flux decreased on day 7 and day 21. The operating flux was stable but slightly lower than the subcritical flux when HRT was at 48 h; however, this value fluctuated when HRT was 24 h.

Figure 1b shows permeability flux and membrane resistance at these five HRT stages. Permeability flux could recover by non-effluent aeration and remain stable during the stages of HRT at 60, 48, and 36 h; however, these values dropped rapidly and failed to recover during the stage of HRT at 24 h. The CIP chemical cleaning recovered permeability flux

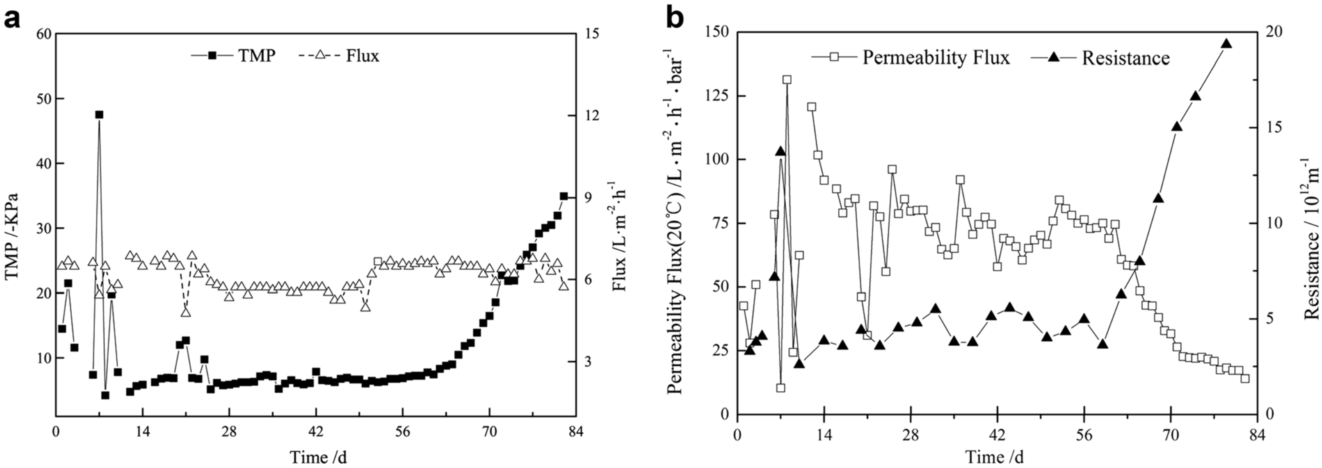

Fig. 1 Effects of HRT on membrane fouling processes (**a** TMP and flux and **b** permeability flux and resistance)

greatly, except when HRT was at 72 h. Irremovable fouling trends became more serious as HRT decreased to 24 h.

Based on the TMP decrease and flux recovery after the physical and chemical cleaning, fouling was determined to occur in the following three ways: removable fouling, irremovable fouling, and irreversible fouling between HRT adjustments. If the TMP decrease and flux recovery occurred as the result of a physical cleaning, it was classified as removable fouling. Irremovable fouling occurred when foulant remained after a physical cleaning but could be reversed by chemical cleaning. Irreversible fouling occurred when a foulant residue remained even after chemical cleaning (Meng et al. 2009). In the first two HRT stages, fouling was primarily removable and became irremovable after a few days. When HRT was decreased to 48 and 36 h, both removable and irremovable fouling were mitigated. The likelihood of irremovable fouling occurring was mitigated when the HRT was decreased from 72 to 36 h. A further decrease in HRT led to irremovable fouling increasing rapidly. Its TMP rapidly increased, and permeability flux sharply decreased when the HRT was decreased to 24 h. An optimized HRT that took into account membrane fouling mitigation was therefore set from 48 to 36 h to treat wastewater from antibiotics production using a MBR in this study. Obviously, HRT plays an important role in the fouling trends of MBR.

The HRT range of fouling changes is broad for pharmaceutical wastewater when compared to MBRs used to treat municipal wastewater, but the relative value was narrow. *Meng* reported that fouling trends greatly changed when HRT decreased from 10–12 to 6–8 h, but further decreasing the HRT to 4–5 h resulted in limited changes to fouling trends of municipal wastewater. The effects of the OLR on membrane fouling were emphasized in municipal wastewater treated by MBR. A high OLR (1.5 g TOC L^{-1} day^{-1}) revealed a sudden increase in the TMP and a decrease in flux after 40 days in municipal wastewater treatment by MBR, but a OLR of 0.38± 0.04 $kg_{COD}kg_{VSS}{}^{-1}day^{-1}$ resulted in similar serious fouling trends (Meng et al. 2007; Nagaoka et al. 1998; Shariati et al. 2011). In this study, the OLR remained stable when HRT was at 60, 48, and 36 h, respectively, and sharply doubled when HRT was set to 24 h. Correspondingly, membrane fouling showed similar trends. The data obtained from this investigation, together with previous works in the literature related to municipal wastewater treatment, indicate that HRT is a very significant operating parameter that affects membrane fouling and performance in municipal and industrial MBR systems.

Membrane foulants

Determining membrane foulants using FTIR

To identify organic compounds that contributed to irreversible membrane fouling, an FTIR analysis of membrane foulants scratched from the surface of the membrane was carried out (Fig. 2). There were many characteristic peaks present within the detection range of 4000 and 400 cm^{-1}. The large peak recorded approximately 3427 cm^{-1} is related to the hydroxyl (O-H) band, and the peak at 2926 cm^{-1} is attributed to vibration of the hydrocarbon subsidiary(C-H) band (Croue et al. 2003). There are two characteristic protein peaks at 1655 and 1554 cm^{-1}, which are related to the amide I (carbonyl) and amide II (N-H) bands (Saha et al. 2007), respectively. These peaks indicated that foulants in this study contained proteins. The peak at 1384 cm^{-1} is linked to the C-O band. Polysaccharides typically have intense C-O stretching bands at 1043 cm^{-1} (Croue et al. 2003). These results indicate that the gel layer also contained polysaccharides (Pendashteh et al. 2011). FTIR results suggested that the membrane foulants in this study contained proteins and carbohydrates.

Membrane foulants quantified as bound EPS, bound SMP, and EfOM

EfOM is a complex mixture of SMP secreted by microorganisms during biological treatment processes and synthetic

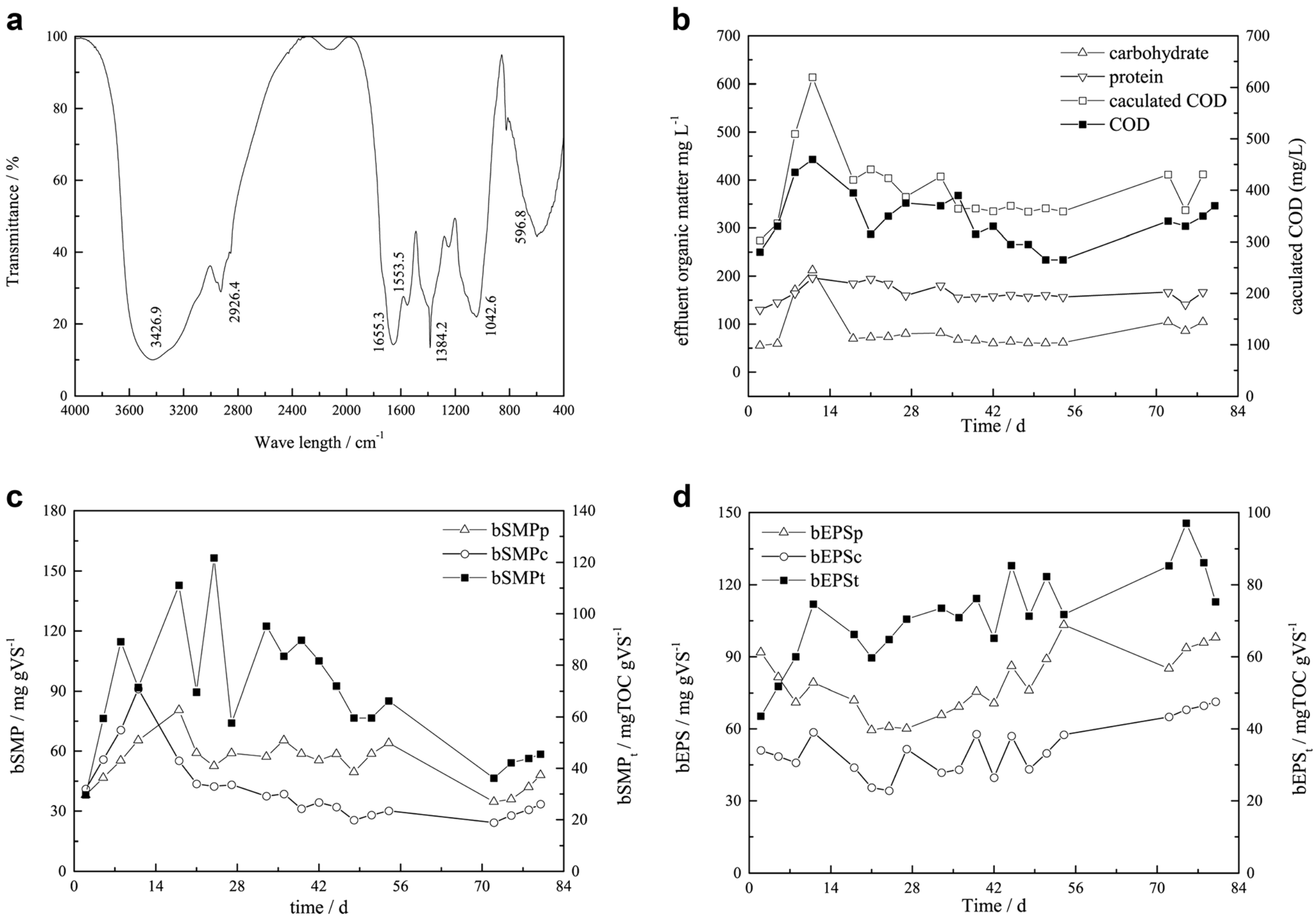

Fig. 2 Effects of different HRTs on foulant (**a** FTIR analyses of foulant, **b** protein and carbohydrate in permeate, **c** bSMP in mixed liquor, and **d** bEPS in mixed liquor)

organics due to human activities, which should be highlighted in pharmaceutical wastewater treatment and natural organic matter (NOM) presence in tap water (Shon et al. 2006). As shown in Fig. 2b, EfOM (particularly carbohydrates) suddenly increased to 213 mg L^{-1}, over twice the normal levels at the 72-h HRT stage. These values fluctuated less when the HRT equalled 60 h and were relatively stable when HRT equalled 48 and 36 h. EfOM (Fig. 2b) results showed concordant trends with TMP (Fig. 1a), which fluctuated at the beginning, stable in the middle stage, and rose near the end. In addition, calculated COD effluent exhibited similar patterns and approximate values with effluent COD. These results showed that proteins and carbohydrates were not only the major foulant components but were also the major compounds of the permeate COD.

As shown in Fig. 2c, d, bound EPS (bEPS), bound SMP (bSMP), and their compounds within the mixed liquor were quantified as proteins and carbohydrates according to the FTIR results. MLVSS remained stable throughout this study (Table 1). As HRT decreased, bSMP and bEPS values decreased and increased, respectively (Fig. 1b). In the first HRT stage (HRT = 72 h), $bSMP_t$ suddenly rose to 118 mg gVS^{-1} when permeability flux dropped (Fig. 1b). Another two peaks were observed in both $bSMP_t$ and permeability flux at a HRT of 60 h. Both $bSMP_t$ and permeability flux were stable at 48 and 36 h; however, $bSMP_t$ and permeability flux trends diverged when HRT equalled 24 h. The analysis, which was based on physical and chemical cleaning, showed that removable fouling and bSMP followed similar trends. The decreasing trends in permeability flux were associated with increasing trends in $bEPS_t$ (149 mg gVS^{-1}) when HRT equalled 24 h. bEPS (Fig. 1d) fluctuated initially and then rose, similar to irremovable fouling trends identified in this investigation. To be more specific, $bEPS_p$ (0.826, $p<0.01$) and $bEPS_c$ (0.797, $p<0.05$) were significantly related to fouling rate. According to the fouling and foulant results in this study, it can further be inferred that the dominant foulants transitioned from SMP to bEPS as HRT decreased from 72 to 24 h in the MBR treating pharmaceutical wastewater. Initial foulants were dominated by bSMP, which was secreted by microorganisms during prolonged HRT (72 h) and inhibited by man-made chemical agents. Under an optimized HRT (48 and 36 h), both bSMP and bEPS were limited. Final fouling was dominated by bEPS, which could not be removed using man-made chemical agents during the shortened HRT. However, further investigation is still needed to provide more direct evidence related to this assumption.

Pollutants removed by membrane and biomass

When mixed liquor permeates a membrane, total COD is separated into two parts by the membrane. The first part was retained by the cake layer and the membrane as foulant (Fig. 3a). The other parts permeate the membrane as EfOM (Fig. 2d). Both the supernatant- and membrane-removed COD concentrations were lowest at 36 h (Fig. 3a, b), which was consistent with fouling trends (Fig. 1). This also indicates that the highest biomass removal rate occurs when HRT is set at 36 h. The supernatant COD concentrations during the other four HRT stages were much higher than those recorded in the 36-h stage (Fig. 3b). The membrane's COD removal rate was also lowest (25.84±1.68 %) when HRT equalled 36 h (Fig. 3a). However, the situation changed for increased supernatant COD and membrane removal for the other four stages of HRT. Serious membrane fouling was observed when HRT equalled 72 or 24 h, but supernatant COD and membrane removal during these stages were not higher than during the stages of HRT at 60 and 48 h (Fig. 3a, b). COD membrane removal results suggest that membrane fouling trends can benefit from low supernatant COD (377.75±21.68 mg L^{-1}) and a low removal rate (25.84±1.68 %) when HRT is at 36 h, but the correlation was not significant at higher supernatant COD and membrane removal rates. Compared to the CAS system, effluent COD calculated in the MBR system decreased from 709.93±62.75 to 280±17.32 mg L^{-1} (Table S1). The improved COD removal rate was much higher than the common membrane removal rate of 60~80 %, which implies that the removal of recalcitrant organics was enhanced by both the membrane and biomass through bioabsorption and biodegradation due to the increased presence of biomass and low food to microorganism (F/M) rates caused by prolonged SRT (>70 days) (Hai et al. 2010; Estrada-Arriaga and Mijaylova 2011; Raj et al. 2013).

Generally, MLSS concentrations will raise when HRT is decreased (Mutamim et al. 2012). However, in this study, MLSS increased from 4.47±0.82 to 4.95±0.77 g L^{-1} when HRT was decreased from 72 to 24 h. Considering that SRT> 70 days in this study, the MLSS was unusually lower than that

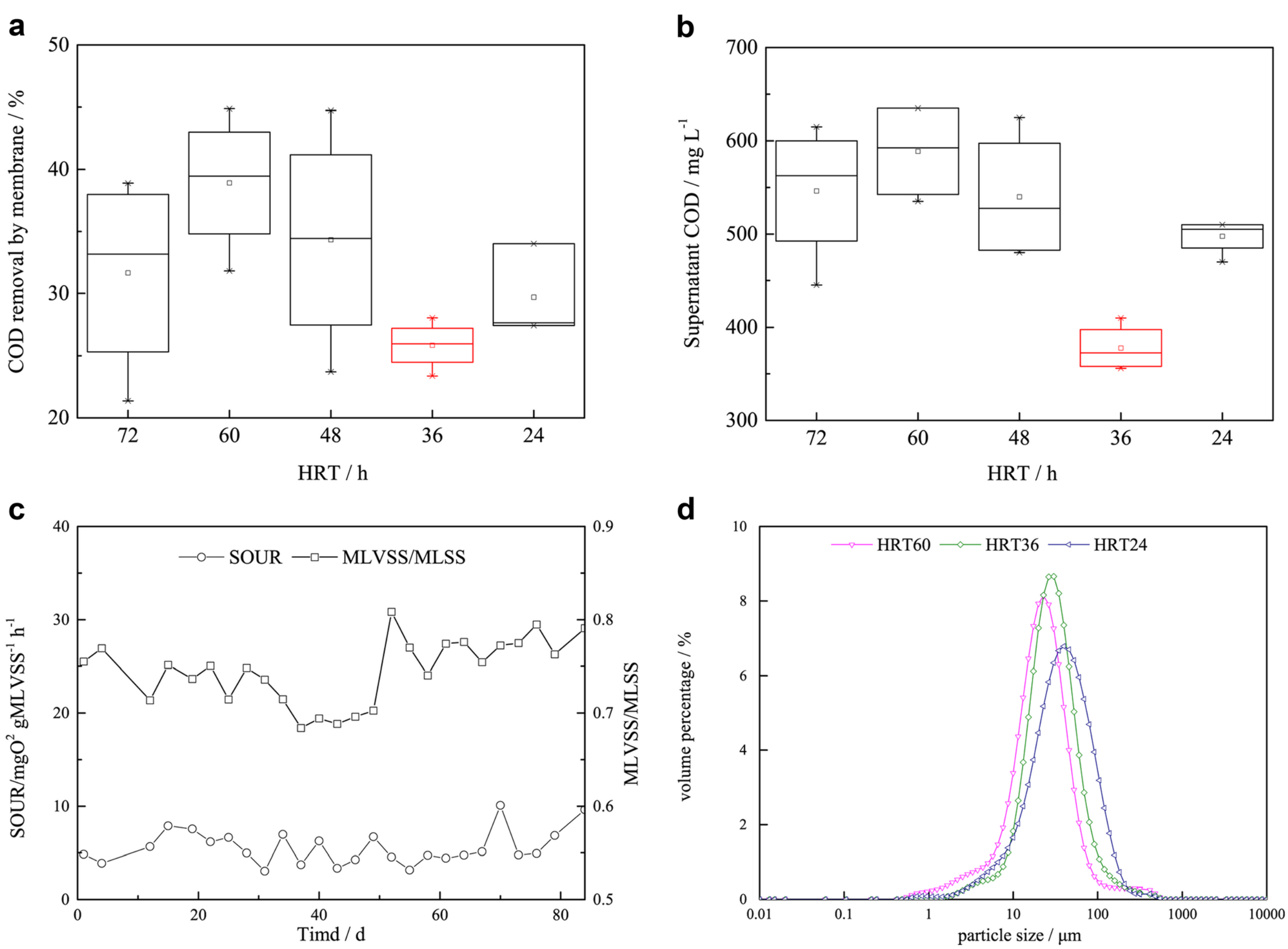

Fig. 3 Effects of different HRTs on mixed liquor (**a** COD removed by membrane, **b** supernatant COD, **c** biomass activity, and **d** particle size distribution)

in the MBR system used to treat municipal wastewater (Meng et al. 2007). The same low MLSS concentration was observed at the pharmaceutical wastewater treatment station located at the Wuxi 2nd Pharmaceutical Factory. The station was operated at a volume loading rate (VLR) of 0.18–1.3 kg COD m^{-3} day^{-1}, and dissolved oxygen (DO) was measured at approximately 3 mg L^{-1}, which fell within the typical range for the CAS process. Given that the limited organic loading rate (F/M) and sludge discharged might inhibit MLSS increases, the organic loading rate was increased gradually and maintained sufficient DO and ALK levels (Table 1). However, compared with the 72-h measurements, MLSS dropped when the OLR doubled at 48 h. When HRT decreased, MLSS exhibited limited growth, which varied largely from the linear growth measured in a municipal MBR system (Tay et al. 2003). The possible reasons for this quite low MLSS in the treatment of pharmaceutical wastewater are thought to be the low F/M rate and endogenous decay under prolonged SRT (Hai et al. 2010).

As sludge growth was inhibited, another concern raised was sludge activity. The results of the MLVSS/MLSS ratio and SOUR data are shown in Fig. 3c. The MLVSS/MLSS ratio was 0.76±0.47. The floc size distribution increased with decreasing HRT (Fig. 3d). According to the changes in SOUR and MLSS values during HRT optimization in this study, pharmaceutical wastewater seemed to inhibit MLSS growth but did not limit sludge activity in the SOUR.

Factor analysis of membrane fouling

Figure 4 shows the results of the principal components' (PCs) loading rates. Two PCs were extracted at loading values of 53.2 and 9.6 %, respectively. PC 1 was not only positively correlated to the biomass characteristics EPS_p and EPS_c and operating conditions OLR and MLVSS but was also negatively correlated to the biomass characteristics specific viscosity, CST, TOC_m, and SMP, and the operating condition TOC_e. The

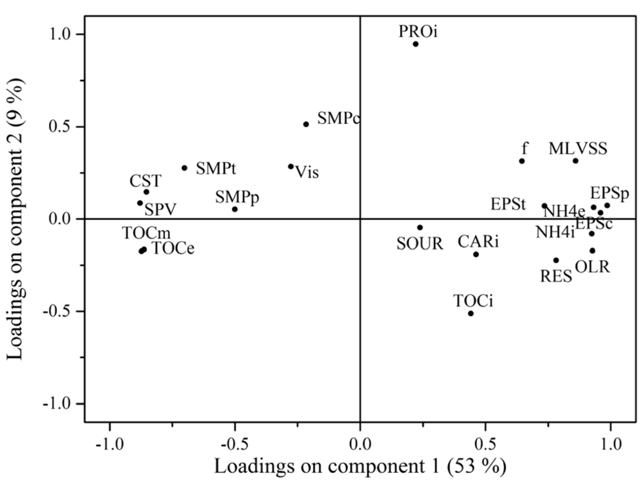

Fig. 4 Principal component analysis loading plot of PC 1 and PC 2

analysis identified EPS_p and EPS_c as major foulants in the MBR treatment of pharmaceutical wastewater. Membrane resistance was clustered with EPSc and OLR. PC 2 was positively correlated with protein in feed wastewater and negatively correlated with TOC in feed wastewater. Influent proteins played a critical role in PC 2 and may be linked to the production of antibiotics. In a summary, biomass characteristics, operational conditions, and feed characteristics were ranked in order according to their contributions to fouling trends in the MBR treatment of pharmaceutical wastewater.

Decreasing both EPS and SMP mitigates fouling rates in municipal MBR, which means that fouling rates are positively correlated with EPS and SMP (Chae et al. 2006; Trussell et al. 2006). Increased bound EPS leads to increasing membrane resistance and fouling in municipal MBR systems (Chae et al. 2006; Lesjean et al. 2005). These results were similar to those found in the present investigation. However, the effects of SMP on fouling were reversed in pharmaceutical wastewater. PCA results were shown to negatively correlate in this investigation (Fig. 4), and fouling (Fig. 1a) and bSMP (Fig. 2c) revealed opposite trends. Another notable difference is that influent proteins were an important component of PC 2. Additional investigation is needed to identify the mechanism behind these opposite trends.

Conclusions

(a) HRT was a significant operating parameter that affected membrane fouling and performance in a pharmaceutical MBR system. When HRT was decreased from 72 to 36 h, irremovable fouling was maintained, and a further decrease to 24 h of HRT resulted in an increase of the irremovable fouling.
(b) Membrane foulants mainly contained proteins and carbohydrates; they were composed of bSMP, bEPS, and EfOM. Initial foulants were dominated by the bSMP in the 72-h HRT stage, and both bSMP and bEPS were major foulants when HRT equalled 60, 48, and 36 h; the final fouling was dominated by bEPS. Decreasing HRT resulted in decreasing bSMP and EfOM, as well as increasing bEPS. This was related to the organic matter removal pathways via biodegradation or membrane retention.
(c) Statistic analyses have shown that $bEPS_p$ and $bEPS_c$ were significantly related to the membrane fouling rate. Other critical categories affecting membrane fouling were ranked in order as biomass characteristics, operational conditions, and feed characteristics.

ALK alkalinity, *bEPS* bound EPS, *bSMP* bound SMP, *CAR* carbohydrate, *CAS* conventional activated sludge, *CST* capillary suction time, *CIP* cleaning in place, *DO* dissolved oxygen, *EPS* extracellular polymeric substance, *f* MLVSS/MLSS, *F/M* food to microorganism, *MLVSS* mixed liquor volatile suspended solid, *NF* nanofiltration, *PRO* protein, *RES* membrane resistance, *SEC* size exclusion chromatography, *SOUR* specific oxygen uptake rate, *SPV* specific viscosity of mixed liquor, *SMP* soluble microbial product, *SRT* solid retention time, *TOC* total organic carbon, *Vis* viscosity of mixed liquor, *i* influent, *m* mixed liquor of membrane bioreactor, *e* effluent, *p* protein, *c* carbohydrate, and *t* sum of related proteins and carbohydrates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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