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Assessment of Fouling Potentials of Extracellular Polymeric Substances in a Membrane Bioreactor Using Modified Fouling Index (MFI)

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ABSTRACT

Fouling potentials of extracellular polymeric substances represented by MFI were assessed in a full scale membrane bioreactor (MBR) treating domestic wastewater. Total EPS were divided into soluble EPS (SEPS), loosely bound EPS (LBEPS) and tightly bound EPS (TBEPS) by a series of extraction methods. The components and properties of three types of EPS were examined by colorimetric methods and three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy. Fouling potentials of EPS were measured by batch filtration tests with MFI as an indicator. The results showed that the majority of total EPS was TBEPS, which accounted for nearly 90% of the total EPS. In addition, LBEPS with the lowest content demonstrated the highest membrane rejection rate during filtration. EEM spectra analysis confirmed the components measurement of EPS and further revealed that tryptophan protein-like substances could be of significant importance. MFI of total sludge rather than individual component was significantly related to real MBR fouling rate (dTMP/dt). TBEPS showed the highest potential to foul the membrane; however, LBEPS contributed most to membrane fouling since they had the highest specific MFI and similar filtration behavior as activated sludge.

Keywords: Full scale membrane bioreactor; membrane fouling; extracellular polymeric substances; modified fouling index; fouling potential

1. INTRODUCTION

In recent years, membrane bioreactors (MBRs) have been widely applied to wastewater treatment and reuse since MBRs have several superior advantages over conventional activated sludge processes (Hai et al., 2013). However, membrane fouling is a troublesome issue limiting more wide-spread application of MBRs. Membrane fouling is found to be directly caused by the interaction between membrane and mixed liquor. Mixed liquor comprises activated biomass and microbial products, including extracellular polymeric substances (EPS), soluble microbial products (SMP) and inert biomass (Laspidou et al., 2002).

EPS and/or SMP have been regarded as significant fouling-causing substances in MBRs (Drews et al., 2007; Jiang et al., 2008). They are a group of complex organic substances produced by mixed bacterial populations. Furthermore, EPS are devided into bound EPS (BEPS), which include tightly bound EPS (TBEPS) containing capsular polymer and loosely bound EPS (LBEPS) containing slime, and soluble EPS (SEPS), which are polymers suspended in mixed liquor

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(Nielsen et al., 1997). SMP are soluble cellular products that are secreted by bacteria during substrate utilization and cell decay. According to the unified theory proposed by Laspidou and Rittmann, SEPS are the same as SMP (Laspidou et al., 2002), so it can be simply assumed that total EPS are assemble of three types of EPS abovementioned. However, in MBRs three different types of EPS coexisted in the suspended mixed liquor may make quite different contributions to sludge characteristics and membrane fouling, although their exact roles are still uncertain and under controversial (Drews, 2010).

Through lab experiments on conventional activated sludge process, it was noted that only LBEPS, and not the total EPS, correlated with performance of flocculation and sedimentation processes of sludge (Li et al., 2007). Recently, it was also found that LBEPS was more positively correlated with sludge flocculation, settling and filtration properties in a hybrid MBR process as compared to TBEPS (Wang et al., 2010). Moreover, it was reported that filterability of sludge decreased with the increase of BEPS (Nagaoka et al., 1996). However, in another study, contrary to some literature, no impact of bound EPS on the filterability could be observed, instead SEPS or SMP was found to have great impact on the filterability of sludge (Lyko et al., 2007). So it should be pointed out that fouling behavior cannot be attributed solely to BEPS due to the complex nature of activated sludge, and attention also should be paid to the influence of SEPS on MBR fouling. Because different types of EPS, both possessing complex composition, had diverse properties and fouling behaviors in MBRs, though no consensus had been achieved yet.

Furthermore, attempts were also tried to separate different components from mixed liquor and study their individual fouling potential. To study the fouling potentials of different EPS in a lab-scale MBR, batch filtration tests were conducted and it was found that the tightly bound EPS has the highest potential to foul the membrane; however, the loosely bound EPS contribute most of the filtration resistances of the whole sludges (Ramesh et al., 2007). Wang et al. (2009) adopted a mini-MBR to periodically analyze the membrane fouling rates of mixed liquors and correlated them with LBEPS and TBEPS contents, and by statistical analysis it was demonstrated that compared to TBEPS, LBEPS showed more significant correlations with membrane fouling (Wang et al., 2009). However, little information was available that distinguished the proportions of the three types of EPS and their different fouling potentials in pilot or full-scale MBRs. So the primary objective of this study was to contribute towards a better understanding of the properties and fouling potential of three types of EPS in a full scale MBR. Main components of EPS were measured by colorimetric method, and EEM spectroscopy was used to detect fluorescence properties of EPS. MFI was chosen as a parameter to assess fouling potential of EPS.

2 MATERIALS AND METHODS

2.1 Full-scale MBR process

The full-scale MBR with a capacity of about 2000 m^3/d was applied to treat domestic wastewater using anaerobic-anoxic-aerobic MBR combined process, which was located in Siyuan University WWTP of Xi'an, China. In MBR tank, submerged PVDF hollow fiber membrane modules (Asahi Kasei Chemicals Corp., Ltd., Japan) with a nominal pore size of 0.1 μm were installed, and the total membrane area was 5400 m^2. Submerged impellers were equipped in anaerobic tank and anoxic tank for mixed liquor blending, while in oxic tank and MBR tank air was continuously supplied through the air diffuser to meet the oxygen

demand of microorganisms and to scour membrane surface. After pretreatment, the raw wastewater was fed into the bioreactors. The full-scale MBR was running stably through a comprehensive automatic control system. It was operated under the constant flow filtration mode with a flow rate of 16 L/(m^2·h), and an intermittent operation mode (9 min suction/1 min relaxation) was applied. The hydraulic retention time (HRT) was about 12.5 h, and the sludge retention time (SRT) was set at 20-40 d during the study period. Detailed description of the full scale plant can be found anywhere else (Hu et al., 2013).

2.2 EPS extraction and analysis

For properties and fouling potential analysis, EPS was firstly extracted from the activated sludge in the MBR tank adopting the heating extraction method modified from reference (Wang et al., 2009). The collected sludge was primarily centrifuged (6000 g, 10 min, 4˚C) to obtain supernatant, which was further centrifuged (10000 g, 20 min, 4˚C) and the solution obtained was named as SEPS. Then the residues of above step were resuspended with phosphate buffer solution (PBS) (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCl). After 2 min of ultrasonic treatment (Ultrasonic cleaner KQ 500DE, Kunshan Ultrasonic Machine Company, China) and twice centrifugation, LBEPS solution was harvested. Lastly, the residues after LBEPS extraction were resuspended with PBS to be subject to heating extraction method (60˚C, 30 min), and after another twice centrifugations the solution collected was regarded as TBEPS. After extraction, EPS samples were immediately used for further analysis within 24 h.

The extracted SEPS, LBEPS and TBEPS were used for EPS properties analysis, including components and fluorescent property measurement. The analysis of EPS components was conducted for proteins using the modified Lowry method (Hartree, 1972) with bovine serum albumin (BSA) as the standard reference, and for polysaccharides using the phenol-sulfuric acid method (Dubois et al., 1956) with glucose as the standard reference. UV_{254} of EPS samples was measured according to standard methods (APHA-AWWA-WEF, 1998) and used to roughly represent the content of humic substances (Meng et al., 2009).

The three-dimensional excitation-emission matrix (3DEEM) fluorescence spectra of EPS samples were measured using a FP-6500 spectroflurometer (Jasco Corp., Japan). The 3DEEM spectra were collected with corresponding scanning emission spectra from 220 nm to 550 nm and excitation wavelengths from 220 nm to 450 nm, all at 5 nm intervals. During measurement TBEPS samples were diluted for 10 times due to the high concentrations of fluorescent substances, and no dilution was conducted for other EPS samples.

2.3 Determination of fouling potential

Batch filtration tests were conducted to measure MFI (Arabi et al., 2010; Schippers et al., 1980), which was used to indicate the fouling potentials of EPS samples in this study. MFI was conducted in a 300 mL stirred filtration cell (MSC300, Mosu Corp., shanghai, China) using a PVDF flat-sheet membrane (100 kDa) and operated at a constant pressure of 20 kPa by pressurized nitrogen from a gas cylinder. The production of filtrate under pressure was continuously recorded by an electric balance (Sartorus, BSA2202S). A plot of t/V versus V (t in seconds and V in liters) was then constructed to determine the MFI. The slope ($\tan\alpha$) of the straight part of the filtration curve was firstly obtained. Afterwards MFI was calculated from the following equation (Eq. (1)) and corrected for the pressure and temperature of 210 kPa and 20˚C.

$$MFI = \frac{\eta_{20}}{\eta}\frac{\Delta P}{210}\tan\alpha \quad (1)$$

Where η_{20} is the viscosity at 20˚C, η is the viscosity at the water temperature, and ΔP is the pressure applied in kPa.

3. RESULTS AND DISCUSSION

3.1 Concentration and membrane rejection rate of EPS

In this work sludge samples were collected nine times for further analysis during 4-months steady state operating time. The averaged MLSS and MLVSS concentrations were 4627 and 3490 mg/L, respectively. The MLVSS/ MLSS ratio was about 75%, which was similar with the previous report conducting a pilot-scale MBR in a WWTP for real municipal wastewater treatment (Wang et al., 2010). With respect to other parameters, such as diluted SV, viscosity and supernatant turbidity, they were quite similar as reported in the literature (Meng et al., 2006).

Three types of EPS (SEPS, LBEPS and TBEPS) extraction using the method described in Section 2.2. In this study, total EPS content was calculated as the sum of SEPS, LBEPS and TBEPS. The main components (proteins, polysaccharides and humics) of various EPS were measured and showed in Table 1. By comparison of three major components, it could be easily found that the content of TBEPS was always the highest and LBEPS content was lowest among three types of EPS. Moreover, TBEPS amounted to more than 90% of the total EPS, with SEPS and LBEPS one order magnitude lower than TBEPS. With respect to proteins and polysaccharides, there were more proteins than polysaccharides in all EPS samples.

During the batch filtration tests as mentioned in Section 2.3, both the extracted EPS for filtration and the filtrate were subject to component measurement, after that the membrane rejection rate was calculated and listed in Table 1. Although membrane with the same pore size and material was used during the filtration experiments, it was found that with regard to different components, three types of EPS showed diverse membrane rejection properties. For proteins, the rejection rate of distinct EPS was in the following order, LBEPS > SEPS > TBEPS; for polysaccharides and humics, those were LBEPS > TBEPS > SEPS and LBEPS > SEPS > TBEPS, respectively. It should be note that LBEPS always showed highest rejection rate than others regardless of the type of components, and SEPS revealed moderate rejection rate. It should be kept in mind that in real MBR process TBEPS were tightly incorporated into sludge flocs with little chance to foul the membrane despite its high concentration (Ramesh et al., 2007), in contrast, SEPS and LBEPS would cause more impacts on membrane fouling due to their suspending properties, to whom more attention should be paid, especially for LBEPS.

Table 1 Concentration and membrane rejection rates of EPS components

Type of EPS	Proteins (mg/L)	Rejection rate	Polysaccharides (mg/L)	Rejection rate	UV_{254} (cm^{-1})	Rejection rate
SEPS	6.8±1.0	48%±9%	1.7±0.4	25%±13%	0.08±0.008	38%±4%
LBEPS	3.3±0.6	78%±8%	0.7±0.5	61%±16%	0.04±0.005	45%±8%
TBEPS	114.0±7.8	25%±5%	12.8±3.0	59%±9%	1.37±0.147	30%±6%

Note: Values are given as mean ± standard deviation, and number of measurement: n=9

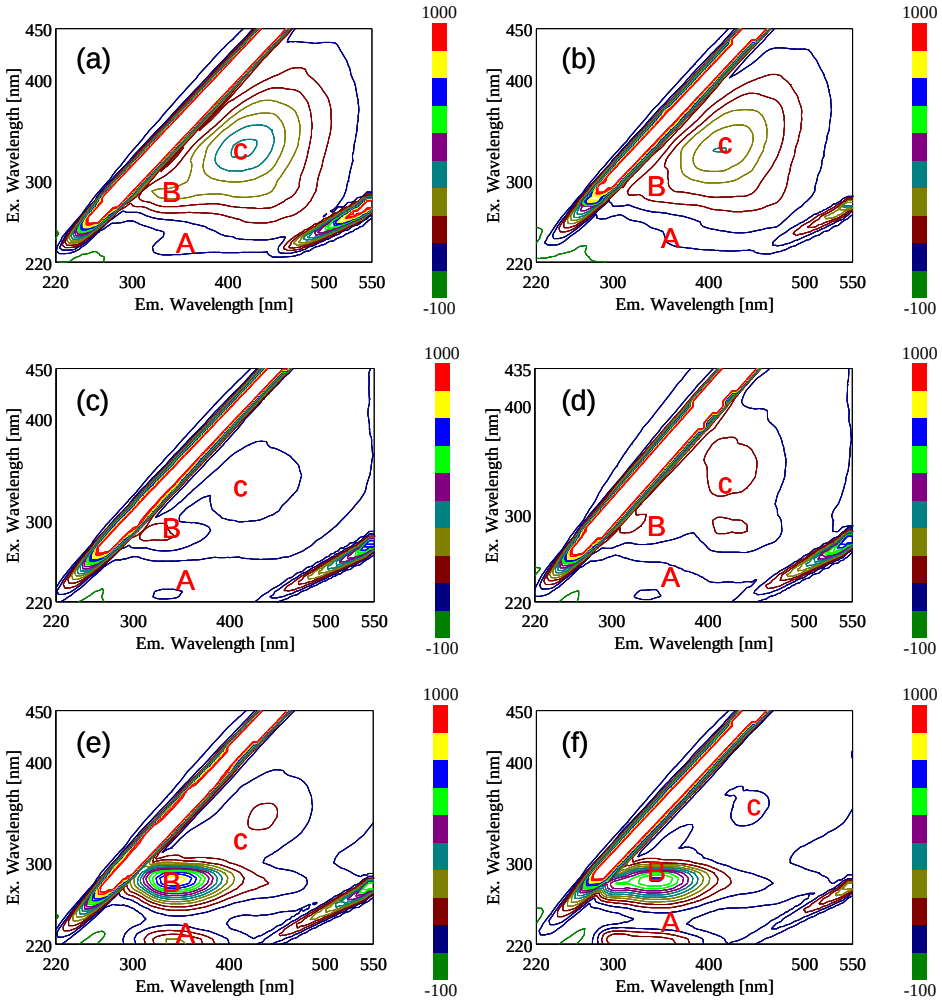

Figure 1 EEM spectra of EPS (a) SEPS; (b) SEPS after filtration; (c) LBEPS; (d) LBEPS after filtration; (e) TBEPS and (f) TBEPS after filtration

3.2 3DEEM fluorescence spectroscopy analysis

According to the fluorescence of different spectral regions, EEM fluorescence spectroscopy could be used to distinguish the fluorescence compounds, such as proteins and humic substances, except polysaccharides because of their non-fluorescence property. The EEM spectra of SEPS, LBEPS and TBEPS samples and those after filtration tests were presented in Fig. 1 (a)-(f). It was evident that three main peaks could be readily identified from all the EEM spectra. The first peak (Peak A) was detected at the excitation/emission wavelengths (Ex/Em) of 225~235 nm/ 310~360 nm, and the second peak (Peak B) was located at the excitation/emission wavelengths (Ex/Em) of 285~295 nm/ 320~350 nm, while the third peak (Peak C) was observed at the excitation/emission wavelengths (Ex/Em) of 330~355 nm/ 415~445 nm. According to the literature (Chen et al., 2003; Wang et al., 2010), Peak A and Peak B were reported as proteins-like peaks, in which the fluorescence was associated with the aromatic protein-like substances (Peak A) and tryptophan protein-like substances (Peak B). Peak C was

regarded as humic-acid like peak. Fluorescence parameters of the spectra included peak location (Ex/Em) and fluorescence intensity (FI), which were summarized and shown in Table 2.

From Fig. 1 (a), (c) and (e), it indicated that the EEM spectra of three EPS samples were quite different in terms of peak shape and FI. Comparing the FI of three peaks, it was found to be always in the following order: TBEPS > SEPS > LBEPS, which was consistent with the results achieved by EPS components analysis. Further examination of FI of the three fluorescence peaks was conducted (as shown in Table 2). With regard to Peak A (aromatic protein-like substances), the FI of SEPS and LBEPS was quite low (< 30), and it was about 240 for TBEPS. Moreover, after membrane filtration little amount of these substances was retained, so it was deduced that aromatic protein-like substances would not contribute much to membrane fouling due to their low concentration and membrane rejection rate. While for Peak B (tryptophan protein-like substances), low FI (100~300) was detected in SEPS and LBEPS, while quite high FI (> 800) was found in TBEPS. The FI was obviously higher as compared to that of Peak A, and it indicated tryptophan protein-like substances were the main components of proteins existed in EPS. The membrane rejection rate of these substances was in the range of 11%~44%, which meant that tryptophan protein-like substances could be a type of potential foulants. Additionally, Peak C (humic-acid like substances) had moderate FI in all EPS samples, but a low rejection rate (< 20%) was found, which indicated that humic-acid like substances with low molecular weight would not impact much on cake formation (Hu et al., 2013), but attention should be paid to their effects on pore blocking.

Table 2 Fluorescence spectral parameters of EPS samples

EPS samples	Peak A		Peak B		Peak C	
	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
SEPS	235/335	26	285/340	242	330/415	405
SEPS after filtration	235/360	20	295/330	145	330/415	342
LBEPS	230/340	17	290/325	152	335/430	145
LBEPS after filtration	225/340	16	295/320	136	335/430	108
TBEPS	220/345	239	285/345	813	345/435	129
TBEPS after filtration	225/310	220	285/350	674	355/445	74

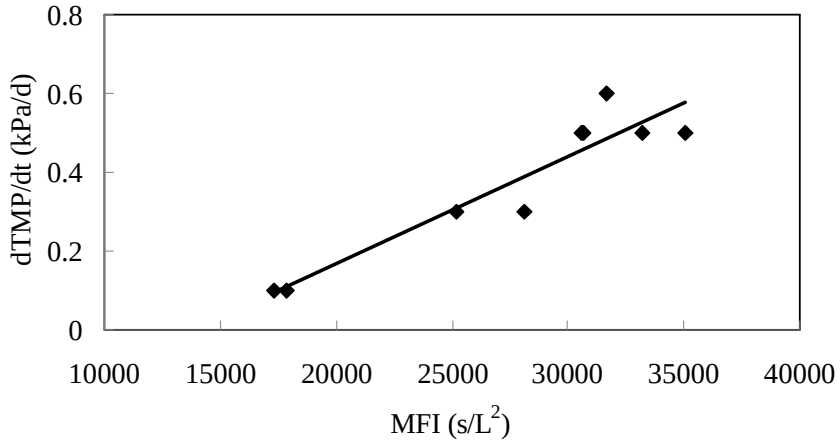

Figure 2 Correlation between activated sludge MFI and fouling rate in the MBR

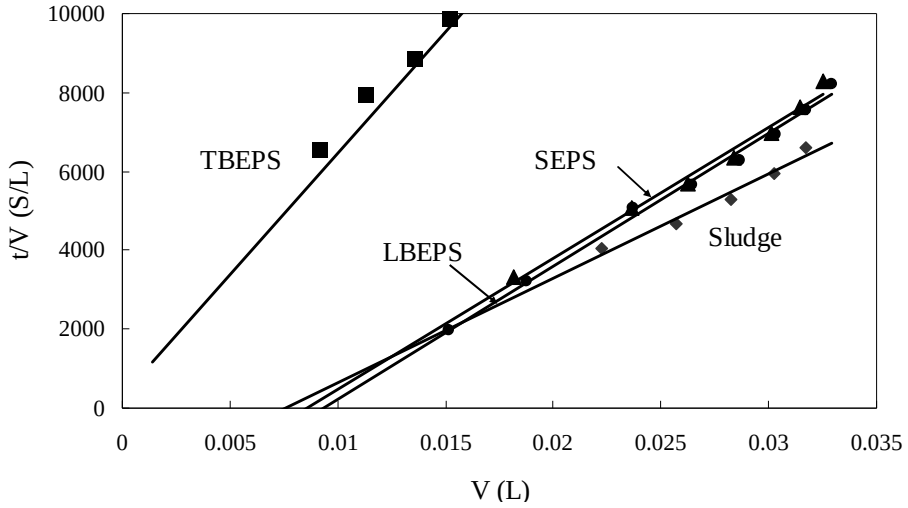

Figure 3 Filtration curves (t/V vs V) of samples

3.3 Relationship between total MFI and MBR fouling rate

Batch filtration tests were commonly conducted to measure the modified fouling index (MFI), which was widely used to characterize fouling potentials of filtration samples, such as model organics, natural organic matters (NOM), mixed liquor, SMP (sludge supernatant) and EPS (Arabi et al., 2010; Boerlage et al., 2003; Ramesh et al., 2007). In order to validate the relevance of MFI to real MBR fouling rate, an attempt was made to find the relationship between total MFI of activated sludge and real MBR fouling rate (dTMP/dt) as presented in Fig. 2. It was noted that total MFI was significantly related to membrane fouling rate with the correlation coefficient (R^2) of 0.89. Another study also illustrated that a positive linear correlation was discovered between the fouling rate and MFI with R^2 of 84% (Ramesh et al., 2007). However, no significant relationships between MFI of EPS and dTMP/dt were detected, which may be due to the fact that in real MBR process membrane modules contacted directly with mixed liquor rather than EPS, which would be discussed in the next section. However, the above analysis indicated that MFI test might have a certain drawback, but it could be still an effective and convenient tool to reflect fouling potentials of various membrane foulants.

3.4 Fouling potentials of EPS

Nine sets of batch filtration tests were conducted to study the fouling potential of different EPS samples, and similar results were found. So, in Fig. 3, typical filtration curves in t/V vs V (where t and V are filtration time and total filtrate volume) of sludge and EPS samples were showed. It was observed that the overall filtration resistances for all sludge samples followed: TBEPS > SEPS > LBEPS > whole sludge. It should be note that although the whole sludge contained SEPS, LBEPS, TBEPS and other solids and components, EPS samples all showed higher filtration resistances than activated sludge, especially for SEPS and LBEPS with low concentrations than others. With regard to TBEPS, it was reported the similar filtration behavior of TBEPS, and most of the hard-to-filter TBEPS was strongly associated with the sludge flocs to form part of the "particles" in the filter cake and had no chance to be in contact with the membrane during filtration (otherwise, the noted filtration resistance of the whole sludge should be

dominated by the TBEPS) (Ramesh et al., 2007). As shown in Fig. 3, it was found the filtration curves of activated sludge, LBEPS and SEPS were close to each other, which meant that the combination of LBEPS and SEPS controlled the filtration resistances of overall sludge.

Further calculation of MFI was conducted and shown in Table 3. Obviously, the fouling potentials of EPS samples were in the following order: TBEPS > LBEPS > SEPS. If MFI was standardized by EPS concentration, specific MFI was obtained, and it was unexpected to find that LBEPS had the highest specific MFI value, which was about one time more than SEPS and much more than TBEPS. So through the comparison of specific MFI, attention should be paid to the significant effect of LBEPS on membrane fouling and how to reduce LBEPS content in MBR.

Table 3 Fouling potential calculation of EPS samples

Parameters	**SEPS**	**LBEPS**	**TBEPS**
Concentration (mg/L)	8.5	4.0	126.8
Slope of fitting line (tanα)	330984	336106	615519
MFI (s/L^2)	31522	32010	58621
Specific MFI (s/mg • L)	3708.5	8002.5	462.3

CONCLUSIONS

Three types of EPS were separately extracted from mixed liquor in a full scale MBR to investigate their properties and fouling potentials. Components analysis showed that TBEPS occupied nearly 90% of total EPS, much more than SEPS and LBEPS; however, LBEPS showed the highest membrane rejection rate during filtration tests. EEM spectra analysis confirmed the results of EPS components analysis and further revealed that tryptophan protein-like substances could be important foulants. MBR fouling rate (dTMP/dt) was controlled by activated sludge but not directly related to various components. Additionally, LBEPS might contribute most to membrane fouling because of their highest specific MFI and similar filtration behavior as the whole sludge, so to which much attention should be paid during long term MBR operation.

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REFERENCES

APHA-AWWA-WEF (1998). *Standard Methods for the Examination of Water and Wastewater, 20th edition.* American Public Health Association/American Water Works Association/Water Environment Federation, Washington, D.C., USA.

Arabi, S. and Nakhla, G. (2010). Impact of molecular weight distribution of soluble microbial products on fouling in membrane bioreactors. *Separation and Purification Technology*, 73(3), 391-396.

Boerlage, S.F.E., Kennedy, M., Aniye, M.P. and Schippers, J.C. (2003). Applications of the MFI-UF to measure and predict particulate fouling in RO systems. *Journal of Membrane*

Science, 220(1/2), 97-116.

Chen, W., Westerhoff, P., Leenheer, J.A. and Booksh, K. (2003). Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science & Technology*, 37(24), 5701-5710.

Drews, A. (2010). Membrane fouling in membrane bioreactors- Characterisation, contradictions, cause and cures. *Journal of Membrane Science*, 363(1/2), 1-28.

Drews, A., Mante, J., Iversen, V., Vocks, M., Lesjean, B. and Kraume, M. (2007). Impact of ambient conditions on SMP elimination and rejection in MBRs. *Water Research*, 41(17), 3850-3858.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356.

Hai, F.I., Yamamoto, K. and Lee, C.H. (2013). *Membrane Biological Reactors: Theory, Modeling, Design, Management and Applications to Wastewater Reuse*. IWA Publishing, United Kingdom.

Hartree, E.F. (1972). Determination of protein: a modification of the Lowry method that gives linear photometric response. *Analytical Biochemistry*, 48(2), 422-427.

Hu, Y., Wang, X.C., Zhang, Y., Li, Y., Chen, H. and Jin, P. (2013). Characteristics of an A^2O-MBR system for reclaimed water production under constant flux at low TMP. *Journal of Membrane Science*, 431, 156-162.

Jiang, T., Myngheer, S., DePauw, D.J.W., Spanjers, H., Nopens, I., Kennedy, M.D., Amy, G. and Vanrolleghem, P.A. (2008). Modeling the production and degradation of soluble microbial products (SMP) in membrane bioreactors (MBR). *Water Research*, 42(20), 4955-4964.

Laspidou, C.S. and Rittmann, B.E. (2002). A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research*, 36(11), 2711-2720.

Li, X.Y. and Yang, S.F. (2007). Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research*, 41(5), 1022-1030.

Lyko, S., Al-Halbouni, D., Wintgens, T., Janot, A., Hollender, J., Dott, W. and Melin, T. (2007). Polymeric compounds in activated sludge supernatant-characterisation and retention mechanisms at a full-scale municipal membrane bioreactor. *Water Research*, 41(17), 3894-3902.

Meng, F., Drews, A., Mehrez, R., Iversen, V., Ernst, M., Yang, F., Jekel, M. and Kraume, M. (2009). Occurrence, source and fate of dissolved organic matter (DOM) in a pilot-scale membrane bioreactor. *Environmental Science & Technology*, 43(23), 8821-8826.

Meng, F., Zhang, H., Yang, F., Zhang, S., Li, Y. and Zhang, X. (2006). Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors. *Separation and Purification Technology*, 51(1), 95-103.

Nagaoka, H., Ueda, S. and Miya, A. (1996). Influence of bacterial extracellular polymers on the membrane separation activated sludge process. *Water Science & Technology*, 34(9), 165-172.

Nielsen, P.H., Jahn, A. and Palmgren, R. (1997). Conceptual model for production and comparison of exopolymers in biofilm. *Water Science & Technology*, 36(1), 11-19.

Ramesh, A., Lee, D.J. and Lai, J.Y. (2007). Membrane biofouling by extracellular polymeric substances or soluble mcirobial products from membrane bioreactor sludge. *Applied Microbiology and Biotechnology*, 74(3), 699-707.

Schippers, J.C. and Verdouw, J. (1980). The modified fouling index, a method of determining the fouling characteristics of water. *Desalination*, 32, 137-148.

Wang, X.C., Liu, Q. and Liu, Y.J. (2010). Membrane fouling control of hybrid membrane bioreactor: effect of extracellular polymeric substances. *Separation Science and Technology*, 45(7), 928-934.

Wang, Z.W., Tang, S.J., Zhu, Y.F., Wu, Z.C., Zhou, Q. and Yang, D.H. (2010). Fluorescent dissolved organic matter variations in a submerged membrane bioreactor under different sludge retention times. *Journal of Membrane Science*, 355(1/2), 151-157.

Wang, Z.W., Wu, Z.C. and Tang, S.J. (2009). Extracellular polymeric substances (EPS) properties and their effects on membrane fouling in a submerged membrane bioreactor. *Water Research*, 43(9), 2504-2512.