Original Article

Polarity-Molecular Weight Profile of Extracellular Polymeric Substances in a Membrane Bioreactor: Comparison between Bulk Sludge and Cake Layers

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ABSTRACT
Extracellular polymeric substances (EPS) are reported to be the major foulant in membrane bioreactor (MBR) processes. It is important to understand the EPS fractions which cause irreversible fouling to reduce operation cost and energy consumption in MBR. In this study, we developed polarity-molecular weight profiling, in which EPS components were plotted on two-dimensional matrix of its polarity and molecular weight, and applied it to investigate EPS of bulk sludge and cake layers from a lab-scale MBR. The EPS components were also characterized via three-dimensional excitation-emission matrix (3D-EEM) spectroscopy. The result showed that hydrophilic substances as large as 100 – 670 kDa was found only in loosely-bound EPS (LB-EPS) of bulk sludge but not in that of cake layers nor in permeate. Hydrophobic substances smaller than 20 kDa were mainly found in soluble microbial products (SMP) in bulk sludge. Hydrophilic substances larger than 670 kDa was mainly found in tightly-bound EPS (TB-EPS) of bulk sludge and in LB- and TB-EPS of cake layers. These findings suggest that, after conditioning of micropores of virgin membrane by hydrophobic substances smaller than 20 kDa in SMP, hydrophilic biopolymers as large as 100 – 670 kDa in bulk sludge clog the narrowed micropores inside membrane, causing irreversible fouling.

Keywords: extracellular polymeric substances (EPS), polarity-molecular weight profile, membrane bioreactor (MBR), membrane fouling, excitation-emission matrix (EEM)

INTRODUCTION
Extracellular polymeric substances (EPS) are defined as a complex mixture of high-molecular weight (MW) polymers, found both outside of microbial cells and in the interior of microbial aggregates [1]. Main components of EPS reportedly consist of polysaccharides, proteins, and humic acids [2]. EPS are usually fractionated into three forms: soluble EPS or soluble microbial products (SMP); loosely bound-EPS (LB-EPS), which are present outer layer of EPS matrix; and tightly bound-EPS (TB-EPS) which are present in the inner layers of the EPS matrix [3]. Several review papers have highlighted roles of EPS in treatment performance of biological wastewater treatments such as conventional wastewater treatment, membrane bioreactor (MBR) [2], and forward osmosis membrane processes [4]. They have proved the fact that EPS considerably affect properties of activated sludge, directly determining charge, hydrophobicity/hydrophilicity and adhesion ability of sludge surface [5].

Extracellular polymeric substances in bulk sludge and SMP in sludge supernatant reportedly play pivotal roles in membrane clogging and cake layer formation – two important mechanisms of biofouling development [6]. For instance, SMP can permeate microfiltration (MF) and/or ultrafiltration (UF) membrane pores or adsorb in the pores then cause membrane plugging while membrane rejected microbial flocs and their EPS matrix accumulate on membrane surface forming a biofouling layer [7]. Such complicated mechanisms have been increasingly investigated. Recently, it has reported that concentrations and characteristics of EPS are two important factors determining the extent and
severity of fouling condition [8]. Poorer floculability and lower settleability of bulk sludge as the result of a higher level of LB-EPS reportedly cause an increase in cake layer resistance, as well as total membrane resistance [9]. SMP are reported to encourage a several-time increase in filtration resistance of cake layers [10]. EPS characteristic understandings will help to comprehend MBR fouling mechanisms: pore clogging, gel layer formation, cake layer formation [5]. Among of EPS characteristics, polarity (hydrophobicity/ hydrophilicity) and molecular size of EPS from cake layers and bulk sludge are the most vital indicator of MBR fouling since they are directly related to the interaction of EPS (potential foulants) with the applied membrane.

The presence of charged groups and apolar groups in EPS take responsibility for amphoteric property of EPS [5]. EPS can be hydrophobic or hydrophilic depending on the relative contribution of hydrophobic groups (e.g., alkyl, hydrophobic amine, and benzyl) and hydrophilic groups (e.g., hydroxyl, carboxyl, etc.) in their components. Relative hydrophobicity in EPS is also expressed as the percentage of the reduction in aqueous phase concentration after extraction in n-hexane [11]. Surface hydrophilicity/hydrophobicity are assessed using the free energy of interaction ($\Delta G_{sw}$) between two identical surfaces immersed in water [5]. Hydrophilic and hydrophobic substances can be fractionated by using adsorbent resins (e.g., Amberlite DAX-8, Amberlite XAD-4 resins) [12]. The hydrophobicity of bulk sludge reportedly has different effluences in the dense of cake layer formation and the rate of TMP increase in MBRs with hydrophilic/ hydrophobic membranes [13]. Hydrophilicity/ hydrophobicity in the surface of membrane and foulant is an essential factor for the interaction between them when the two surfaces initially contact. An MBR possessing less relatively hydrophobic proteins and polysaccharides in EPS of bulk sludge has been reported to show a lower fouling rate than an MBR with high level of relative hydrophobicity in its EPS components [14]. Moreover, by fractionating hydrophilic and hydrophobic substances in organic matter and EPS, Yamamura et al. [12] and Kimura et al. [15, 16] have suggested that hydrophobic substances of humics initially condition MBR membrane, followed by the accumulation of hydrophilic substances of polysaccharides. It should be noted that hydrophobicity and hydrophilicity of EPS are important in the interactions of not only between foulant and membrane but also among foulants.

High pressure size exclusion chromatography (HPSEC) or gel filtration chromatography (GFC) coupled with of various detectors such as UV, diode array, refractive index (RI), organic carbon (OC) and static light scattering (SLS) can be applied to investigate MW distribution of EPS [17]. Besides, liquid chromatography – organic carbon detection (LC-OCD) is often employed to acquire MW profile of organic matters in EPS [12, 15], SDS-PAGE and molecular-weight-cut-off (MWCO) are also applied for molecular size separation [18]. MW distribution of SMP and EPS have been indicated to impact membrane fouling. Substances larger 100 kDa in SMP reportedly cause severe flux decline and pore plugging [19], while MW distribution of EPS affect the stability of sludge flocs, and then play a role in membrane fouling [5]. Substances larger than 100 kDa (e.g., polysaccharides), which are mostly rejected by the membranes, will accumulate and form cake layers on membrane surface [18]. Substances smaller than 100 kDa in SMP such as humic acid-like substances which are much smaller than the average size of microfiltration membrane pores, can be rejected by these membranes [20, 21]. It indicated that understanding in MW distribution of EPS-fraction components are necessary to control biofouling caused by EPS in bulk sludge.

Recently the question of which EPS fractions can be irreversible foulants is a serious concern in membrane fouling study since these foulants cause an increase of energy consumption during filtration and exponential fouling development. Besides characteristics of pore foulants and membrane foulants are need to be comprehensively explored. In fact, EPS of bulk sludge and cake layers of fouled MBRs have been fractionated and characterized in a few previous studies. For instance, fouling properties of EPS extracted from bulk sludge and cake were different [22], causing by the discrepancy in free energy for interaction [5]. In addition, small flocs, colloids, LB-EPS hydrophobicity and negative charge are vital in cake layer formation and sludge aggregation [23]. SMP and bound EPS also reportedly play important roles in the initial and late fouling stages, respectively [24], in which the initial fouling (conditioning of membrane) was caused by humics, whereas later fouling was mainly caused by polysaccharides [16]. Furthermore, SMP have a MW distribution of lower 200 kDa while EPS of cake layers obtained a larger MW one (488 – 3,397 kDa) [25]. However, the findings are still limited in information of polarity-molecular weight to trace potential source of foulant in MBR and understanding the development of membrane fouling. In this study, we developed polarity-molecular weight profiling, in which EPS components were plotted on two-dimensional matrix of its polarity and molecular weight, and applied it to investigate EPS of bulk sludge and cake layers from a lab-scale MBR. The EPS components were also characterized via three-dimensional excitation-emission matrix (3D-EEM) spectroscopy.
MATERIALS AND METHODS

Lab scale MBR operation

A lab-scale MBR was operated under aerobic condition to treat artificial municipal wastewater. Microfiltration flat sheet membrane of chlorinated polyethylene with the average pore size at 0.2 μm (Kubota, Tokyo, Japan) was immersed in aeration tank to separate biomass of activated sludge and treated water. Transmembrane pressure was operated at the constant level of 80 kPa. Fouling development was monitored by permeate flux, which ranged 5.6 – 18.2 L/(m²·h). Artificial sewage ingredient and operation condition are described in Table 1.

Membrane was physically cleaned every 7 days. Membrane modules were taken out of aeration tank and cleaned by brushing and backwash. The foulant removed by brushing was considered as a cake layer sample. The physically cleaned membrane was rinsed several times with pure water, then was backwashed with 50 mL of pure water at the rate of 5 mL/min. Chemical cleaning was conducted once to observe change in components of membrane permeate in the initial stage of filtration. In chemical cleaning, NaClO (0.1% of active chlorine) was added into membrane module and soaked for 2 hours.

EPS fraction and extraction

EPS extraction was based on our previous study [26]. A supernatant fraction of centrifuge (13,000 × g, 10 min) from 50 mL bulk sludge was collected as SMP. The remaining sediment after SMP extraction was re-suspended with pure water to initial volume to extract LB-EPS. The re-suspended sample was treated with ultra-sonication with amplitude of 3.5 W/ml at 5 cm depth for 2 min. The sonified liquor was centrifuged at 13,000 × g for 15 min and the supernatant was collected as LB-EPS. The residual solids of LB-EPS extraction was re-suspended with pure water to extract TB-EPS by alkaline-formaldehyde method. After 0.3 mL of 36 – 38% formaldehyde solution was added into 30 mL of LB-EPS extraction residue and stirred at approximately 600 rpm at 4°C for 1 h, 20 mL of 1 mol/L sodium hydroxide solution was subsequently added and stirred at approximately 600 rpm at 4°C for 1 h. The residue of formaldehyde and sodium hydroxide was removed with dialysis tubing membrane - Biotech CE Tubing, MWCO: 100 – 500 Da (SpectrumLabs.com, Rancho Dominguez, USA). A 50 mL sample of TB-EPS extract was treated with the dialysis tubing membrane via submerged in pure water for 24 h. Pure water was changed 3 times at 2 – 4 h, 6 – 8 h and 10 – 14 h. Cake layers were collected from membrane surface every week, re-suspended with 150 mL pure water. LB- and TB-EPS of cake layers were extracted under ultra-sonication and alkaline-formaldehyde methods, respectively, in the same manner as extraction from bulk sludge.

Relative hydrophobicity

Relative hydrophobicity of protein and polysaccharide in SMP and EPS were conducted based on the study of Arabi and Nakhla [11]. The procedure is as follows: a 20-mL sample was agitated for 20 min, with 20 mL n-hexane. After 30 min, when the phases were separated completely, the aqueous solution was collected prior to protein and carbohydrate analysis. The relative hydrophobicity was expressed as the reduction ratio of protein/ polysaccharide concentration before and after extraction with n-hexane as indicated in the
following equation (1):

$$\text{RH} (\%) = \frac{(C_b - C_a) \times 100}{C_b} \quad (1)$$

Where RH is relative hydrophobicity, $C_a$ is the concentration of proteins or polysaccharides in the aqueous phase after extraction, $C_b$ is the original concentration of proteins or polysaccharides.

The polysaccharide content was analysed via phenol-sulphuric acid method [27] with glucose standards. Modified Lowry method [28] was applied to analyze protein and humic substances, using bovine albumin serum as standard.

**Polarity-molecular weight profile**

First, EPS components of bulk sludge and cake layers were separated by its polarity under a preparative high-performance liquid chromatography (HPLC)-Model 1260 (Agilent, Waldbronn, Germany) equipped with a reversed-phase column - Shodex C18 M 4D (Showa Denko, Tokyo, Japan) and an evaporative light scattering detector (ELSD). The detected peaks were collected by its fraction collector-Model 1260 (Agilent, Waldbronn, Germany). Methanol: Water (70:30) was used as mobile phase for the separation. One-hundred μL a sample was injected after filtration by 0.45 μm filters. Second, the collected fractions were applied to HPLC-Model 1260 (Agilent, Waldbronn, Germany) with a size-exclusion column - Shodex SB-806 M HQ (Showa Denko, Tokyo, Japan) to investigate its molecular weight distribution. The collected fractions were eluted with pure water at 0.5 mL/min and detected via ELSD. Apparent molecular weight was calculated basing on standard curve of Dextran at 1 kDa, 12 kDa, 50 kDa, 500 kDa, 670 kDa, and 1,100 kDa (Sigma-Aldrich, Copenhagen, Denmark). As reference materials, uracil, propanol, aniline, chloroform, and humic acid - Cat. no. 082–04625 (Wako, Osaka, Japan) extracted in NaOH 0.1 mol/L were injected into reversed-phase HPLC.

**Three-dimensional excitation-emission matrix (EEM) fluorescence spectra**

An EPS sample was filtered with a 0.45 μm filter - DISMIC 25CS045AN (Advantec-Toyo, Tokyo, Japan) prior to EEM analysis. The EEM spectrum of the filtered EPS fraction was measured using a fluorescent spectrophotometer- Model FP-8200 (Jasco, Tokyo, Japan), equipped with 150 W of Xe lamp. The EEM spectrum was obtained with subsequent scanning emission spectra from 200 to 550 nm at 5 nm increments by varying the excitation wavelength from 200 to 550 nm at 5 nm increments. The spectrum was scanned at the speed of 5,000 nm/min.

**RESULTS AND DISCUSSION**

**Relatively hydrophobic proteins and polysaccharides**

The average contribution of relatively hydrophobic (RH) substances (e.g., proteins and polysaccharides) in EPS of bulk sludge were comparably higher than that in calcium layers (Fig. 1). It showed that bulk sludge possibly contained a higher level in hydrophobic adhesion than cake layers. The hydrophobic adhesion could enhance adsorption ability of bulk sludge at membrane surface. The result was inconsistent with a previous study of Arabi and Nakhla [11], who found that contributions of hydrophobic substances in bulk sludge and of cake layers are similar and affected by wastewater property. RH proteins in SMP, LB-, and TB-EPS accounted for 50, 24, and 37% in total, respectively (Fig. 1). They were much higher than the contribution of RH polysaccharide (7, 20 and 6%, respectively). On the contrary, in LB-, and TB-EPS of cake layers, RH polysaccharides were present at higher level than corresponding proteins (26 and 17% compared with 13 and 5%, respectively). The result showed that RH polysaccharides tended to adsorb membrane and became a main component of cake layers while RH proteins were highly present in SMP and EPS of bulk sludge. This finding was consistent with finding of Massé [29] in which proteins are reported to possess higher affinity to sludge flocs than hydrophobic polysaccharides. The hydrophobic substances of SMP (mainly proteins, less polysaccharides) could contribute to pore clogging in this case since a high concentration of hydrophobic substances in SMP reportedly contributes to higher fouling rate due to its higher potential for blocking membrane pores [11]. In addition because Liao [30] found that LB-EPS with more hydrophobocity and less negative charge could own low bio-flocculation, causing more release of SMP from EPS matrix. In this study, a high level of hydrophobic proteins in LB-EPS of bulk sludge might cause de-flocculation of sludge flocs, resulting in the high ratio of hydrophobic proteins in the SMP. It implied that hydrophobicity of SMP and LB-EPS of bulk sludge directly relates to fouling development in MBR.

**Hydrophobic/hydrophilic substances of EPS fractions**

Polarities of SMP, LB-, and TB-EPS of bulk sludge differed on reversed phase chromatograms. SMP and MBR permeate possessed a large peak at 5 − 6 min of retention time, relevant to a hydrophobic peak while TB-EPS owned a hydrophilic peak at 2 − 4 min of retention time. LB-EPS of bulk sludge exhibited a bimodal chromatogram of hydro-
philic and hydrophobic substances (Fig. 2). Substances of SMP showed a great hydrophobic property; therefore, the SMP owned a large amount of free hydrophobic groups to form hydrophobic interactions. In bulk sludge, the TB-EPS were highly hydrophilic while LB-EPS showed presence of both hydrophobic and hydrophilic substances. The presence of hydrophobic and hydrophilic substances of LB-EPS can indicate two tendencies of detachment away or involvement in the EPS matrix of bulk sludge. In contrast, both LB- and TB-EPS of cake layers showed a similar chromatogram with a hydrophilic peak, as well as TB-EPS of bulk sludge (Fig. 2). In general, the SMP largely comprised of hydrophobic substances which mainly contain proteins, while the EPS in bulk sludge and cake layers largely contributed by hydrophilic substances. Both results from reverse phase chromatography and relatively hydrophobic analysis indicated that hydrophilic substances of bulk sludge were mainly protein whereas those of cake layers were largely polysaccharides. In this study, the hydrophobic substances in SMP could be adsorbed by the membrane [31], conditioning pores and surface of membrane. The absorbed hydrophobic substances facilitated the accumulation of hydrophilic LB-EPS of bulk sludge (e.g., polysaccharides) on the conditioned membrane to form a cake layer [16]. Hydrophobicity/ hydrophilicity of SMP and EPS is expected to play an important role in membrane fouling [5], which is governed by complicated physical–chemical interactions between the membrane and initial foulants, followed by foulant-foulant interactions [24].

**Molecular weight distribution of EPS fractions**

Molecular weight of SMP was smaller than those of EPS of bulk sludge (Fig. 3). SMP and MBR permeate shared a similar MW distribution, largely containing < 20 kDa substances whereas LB-EPS of bulk sludge mainly comprised of biopolymers of 100 − 670 kDa. TB-EPS contained substances ranging from 670 kDa to over 1,100 kDa. The MW of LB-EPS from bulk sludge was lower than that of cake layers (Fig. 3). The finding of SMP containing lower 100 kDa in our study was consistent with past studies [25, 32], in which by using HPSEC-UV detector and RID, they have reported that these substances can be proteins, polysaccharides, humics. The EPS of cake layers contained large MW than the SMP and their MW distribution ranged from to 450 kDa to over 1,100 kDa which was consistent with previous studies, ranging 488 − 3,397 kDa [25]. Especially Xiong et al. [25] found that the peak of 488 kDa substances in cake layers gradually shift to 838 kDa in a longer-filtration operation. This finding is consistent with the phenomena of the larger MW of cake
Fig. 2 Polarity profile of MBR permeate and EPS fractions of bulk sludge cake layers and reference materials under reverse-phase chromatography. Reference materials included EPS extract (1), Uracil (2), Humic acid extracted in 0.1 mol/L NaOH (3), Propanol (4), Aniline (5), and Chloroform (6).
layers than bulk sludge in our study. Transition of slowly or non-biodegradable substances (proteins, polysaccharides, even glycoprotein) can be a reasonable explanation for the increase in the MW distribution of cake layers. The transition of these substances possibly formed larger molecule compounds, which were not found in LB-EPS of bulk sludge.

**Polarity-molecular weight (MW) profile**

The detected EPS components were plotted in a two-dimensional matrix of polarity and molecular weight (hereinafter called polarity-molecular weight profile). **Figure 4** showed polarity-MW profile of SMP, EPS components of bulk sludge and cake layers. Hydrophobic substances smaller than 20 kDa were mainly found in SMP of bulk sludge (Fig. 4). EEM spectra analysis revealed that the hydrophobic fraction in SMP contained a large ratio of humics (humic acid-like and fulvic acid-like substances) to proteins (e.g., tryptophan protein-like, aromatic protein-like substances). MBR permeate also contained hydrophobic substances smaller than 20 kDa (Figs. 2 and 3). EEM spectra of this fractions in MBR permeate was also similar to those in SMP (data not shown). This indicates that hydrophobic fraction in SMP is transported through micropores and appeared in permeate. Hydrophobic substances smaller than 20 kDa were
also found in LB-EPS of bulk sludge. This implies the close relations of SMP and LB-EPS of bulk sludge. Hydrophobic fractions of 20–450 kDa in LB-EPS of bulk sludge contained humic acid-like and fulvic acid-like substances as well as aromatic protein and tryptophan protein-like substances on EEM spectra (Fig. 5b). These peaks were also found in EEM spectra of SMP. Therefore, hydrophobic fraction of 20–450 kDa in LB-EPS of bulk sludge is possibly one of the potential origin of hydrophobic substances in SMP [5] when the fraction in LB-EPS of bulk sludge is decomposed into smaller molecules.

Hydrophilic substances as large as 100–670 kDa were found only in LB-EPS of bulk sludge (Fig. 4). EEM spectra showed this fraction contained aromatic and tryptophan protein-like substances (Fig. 5). Interestingly, this fraction was not found in cake layer nor in permeate. Therefore, this fraction is possibly trapped inside the membrane or degraded on the membrane surface, as discussed in next section.

Hydrophilic substances larger than 450 kDa were found in TB-EPS of bulk sludge as well as LB- and TB-EPS of cake layers. These fractions had similar EEM spectra, having a high level of aromatic protein and tryptophan protein-like but no humics (Fig. 4 and 5c). Moreover, hydrophobic substances larger than 450 kDa were also found in the three samples and showed similar EEM spectra. These results indicated that LB- and TB-EPS of cake layers were highly similar with TB-EPS of bulk sludge (Fig. 4). Therefore, TB-EPS of bulk sludge is probably deposited to be a main component of EPS in cake layer.

Several studies have investigated relations of polarity and molecular weight of membrane foulants by using adsorbent resins (e.g., XAD resin) [12, 33–36]. In these studies, a sample was first fractioned with resin into two fractions: hydrophilic and hydrophobic ones, then MW of each fraction was analyzed with size-exclusion HPLC. In this study, preparative reverse-phase HPLC was employed to fraction a sample by polarity. Its chromatogram could offer more detailed profile of a sample, and compounds separated by their polarity. Subsequently, we can decide the number of fractions to be collected from the obtained chromatogram. Therefore, we can get precisely targeted fractions by using this method. Moreover, polarity-MW profile from the method can show MW distribution of hydrophobic and hydrophilic fractions as in previous studies [12, 34].

![Fig. 4 Polarity–molecular weight profiling of EPS fractions in bulk sludge and cake layers (n=4). Dot size represents relative presence of relevant substances in EPS fractions.](image-url)
Fig. 5 Excitation emission matrix (EEM) spectra of polarity-molecular weight fractions of EPS fraction in bulk sludge and cake layers. (*) EEM of polarity-molecular weight fractions in SMP was similar with that in MBR permeate; (**) EEM of polarity-molecular weight fractions in TB-EPS of bulk sludge shared similarity with those in both LB- and TB-EPS of cake layers.
method also allow collect polarity-MW fractions for further analysis such as EEM in the study (Fig. 5).

**Role and fate of EPS components in MBR fouling**

Soluble microbial products in supernatant of bulk sludge could contain hydrophobic substances smaller than 100 kDa (Fig. 4), which are expected to freely permeate UF and MF membrane in a MBR. However, hydrophobic humics smaller than 20 kDa are found to adsorb on membrane surface and onto membrane pore [37]. Inside of membrane pores, these hydrophobic humic substances are adsorbed on the membrane (Fig. 6a). There are two evidences for membrane conditioning by SMP. The first evidence was that MW distribution of SMP was much larger than that of permeate for the initial 5 days after chemical cleaning (Fig. S1). This suggests that a part of SMP was adsorbed or rejected by the membrane. The second evidence was that EEM spectra and MW distribution of MBR permeate changed every day by day at the initial stage of filtration, while those of SMP did not change (Fig. S1). This is probably because membrane surface condition had changed every day, suggesting that some substances attached on the membrane pores.

The adsorbed humic substances can incorporate with other hydrophilic compounds to narrow the pore of membrane and cause internal membrane clogging (Fig. 6b). LB-EPS in bulk sludge contained a 100 − 670 kDa hydrophilic fraction. This fraction probably plays an important role in membrane fouling, because it was not found in MBR permeate nor on cake layer. This size of fraction can enter membrane pores or be deposited on the blocked pore. When it enter the membrane pore, it possibly attached on the internal membrane structure which has been conditioned by hydrophobic humic substances (Fig. 6b). Because backwash effluent did not contain this fraction, it can be a major irreversible foulant. When this fraction is deposited on the blocked pore or membrane surface, it is probably degraded into smaller molecules. This degraded fraction was possibly trapped on the internal structure of membrane or pass through the membrane. Analyses of the backwash effluent showed presence of hydrophobic and hydrophilic substances of 35 kDa (Fig. S2). This 35 kDa fraction in backwash effluent is probably a degraded fraction of LB-EPS, because its size is larger than SMP. Conse-
sequently, the deposited fraction of LB-EPS in bulk sludge was decomposed into smaller molecules, pulled into conditioned micropores, then remain inside the pores.

Hydrophilic substances larger than 670 kDa, which were main components of the TB-EPS in bulk sludge, were also found in LB- and TB-EPS of cake layers (Fig. 4). This result indicated that TB-EPS in bulk sludge was deposited on the membrane surface and formed cake layers (Fig. 6c). After the initial deposition of cake layer, EPS production from live microbe cells as well as intercellular compounds from dead cells can make biocake layers thicker, causing serious fouling (Fig. 6c). EPS matrix of cake layers were mainly comprised of large hydrophilic substances, which contained a high proportion of polysaccharides and were larger than 450 kDa or even over 1,100 kDa. These large hydrophilic substances can originate from (i) substances in SMP rejected by membrane [18], (ii) aggregation of non-degradable molecules of bulk sludge [37], (iii) metabolites of microbe aggregates [5], (iv) inner-cellular compounds or cell body of dead cells in the layers [38].

Physical cleaning can only remove biocake layers on fouled membrane but not foulants in membrane pores, which are considered as irreversible foulants, when physically cleaned membrane is applied for a new filtration cycle (Fig. 6d). The irreversible fouling quickly becomes severe, caused by abundant biopolymers and humic substances in SMP readily plugging the narrowed pores. Hydrophobic and electrostatic interactions of humic and hydrophilic polysaccharides [24] are considered to keep the foulants in the membrane pores [16; this study]. Consequently, the non-covalent network formed by hydrophilic proteins and polysaccharides can make irreversible foulants more complicated [24, 39].

The foulants inside the membrane were collected by backwashing with pure water to determine their characteristics of protein and polysaccharide components, EEM, polarity and MW distribution. The foulants contained 20–100 kDa hydrophobic and hydrophilic substances of protein and polysaccharide (Fig. S2). Under EEM spectra, tryptophan protein-like substances and aromatic proteins were detected but humic acid-like substances were not. This implied that humic acid foulants cannot be removed by only physical cleaning nor backwash. Kimura et al. [15] reported that NaClO obtains a higher removal efficiency for irreversible foulants of humic substances than NaOH and HCl. These facts in our study indicate low MW hydrophobic and hydrophilic substances contribute to pore clogging and irreversible fouling. Firstly, small hydrophobic humics are adsorbed by membrane, which conditions membrane surface and narrows membrane pores. Subsequently, larger hydrophilic compounds (protein and polysaccharides) not only plug the narrowed pores but also accumulate on the conditioned membrane surface. However, complicated interactions between membrane and foulants, as well as those among foulants, still need to be understood. Especially biotransformation of the biopolymers in EPS of cake layers requires more researches in the future. The finding in polarity-MW profile of this study also implies that any method can encourage the absorption SMP into EPS matrix of bulk sludge to reduce SMP quantity in supernatant can be a good strategy to control biofouling. In addition, inhibiting formation of gel layer by reducing or breaking hydrophobic and electrostatic interaction of humics and 100 – 450 kDa hydrophilic substances on MBR membrane surface would be also effective to reduce irreversible fouling. Minimizing hydrophobic content of SMP and EPS of bulk sludge, for instance by quorum quenching [14] may reduce attachment of hydrophilic biopolymers into/onto membrane, lessening membrane fouling rate in MBR.

CONCLUSIONS

In this study, polarity-molecular weight profile of EPS fractions in bulk sludge and cake layers were plotted in a two-dimensional matrix. Based on the polarity-molecular weight profiling of EPS fractions, fate of EPS components in fouled MBR and potential source of irreversible foulants could be tracked. The results showed that hydrophobic substances as large as 100 – 670 kDa was found only in LB-EPS of bulk sludge but not in that of cake layers nor in MBR permeate. Hydrophobic substances smaller than 20 kDa were mainly found in SMP in bulk sludge. Hydrophilic substances larger than 670 kDa was mainly found in TB-EPS of bulk sludge and in LB- and TB-EPS of cake layers. These findings suggest the possible mechanism of fouling process, in which: after conditioning of micropores of virgin membrane by hydrophobic substances smaller than 20 kDa in SMP, hydrophilic biopolymers as large as 100 – 670 kDa in bulk sludge plug the narrowed micropores of the conditioned membrane, causing irreversible fouling.

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SUPPLEMENTARY MATERIALS

Supplementary figures for this article can be accessed at the journal website as a separate file. https://www.jstage.jst.go.jp/article/jwet/16/1/16_17-020/_article/-char/en

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