New insights into comparison between synthetic and practical municipal wastewater in cake layer characteristic analysis of membrane bioreactor

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GRAPHICAL ABSTRACT

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ABSTRACT

In previous studies, cake layer analysis in membrane bioreactor (MBR) was both carried out with synthetic and practical municipal wastewater (SMW and PMW), leading to different results. This study aimed to identify the comparison between SMW and PMW in cake layer characteristic analysis of MBR. Two laboratory-scale anoxic/oxic MBRs were operated for over 90 days with SMW and PMW, respectively. Results showed that PMW led to rough cake layer surface with particles, and the aggravation of cake layer formation with thinner and denser cake layer. Additionally, inorganic components, especially Si and Al, in PMW accumulated into cake layer and strengthened the cake layer structure, inducing severer biofouling. However, SMW promoted bacterial metabolism during cake layer formation, thus aggravated the accumulation of organic components into cake layer. Therefore, SMW highlighted the organic components in cake layer, but weakened the inorganic functions in practical MBR operation.
1. Introduction

Membrane bioreactor (MBR) is considered as a well-established, mature technology in recent decades, and has been widely applied in many full-scale plants for municipal and industrial wastewater treatment (Deng et al., 2016; Huang and Lee, 2015; Jagatheesan et al., 2016). MBR could achieve high nutrient removal efficiency with complete biomass retention, totally separating hydraulic retention time (HRT) and solids retention time (SRT). Thus, increasing interest in MBR application has been shown in China wastewater treatment market (Meng et al., 2017). Total treatment capacity of large-scale MBRs was over 1.0 million m$^3$/d, and it is expected that the capacity will over 10.0 million m$^3$/d in 2017 (Xiao et al., 2014). Although MBR contains plenty of outstanding advantages, such as complete retention of sludge, high quality of effluent, low footprint, membrane biofouling and its cake layer still remain serious operational obstacles and challenges in the wider spread of MBR (Duan et al., 2013; Tian and Su, 2012; Xia et al., 2008).

During 2010–2015, approximate 600–700 research literatures were published per year related to wastewater treatment with MBRs, and approximate 22% papers were about membrane biofouling and its cake layer (Meng et al., 2017). Cake layer characteristic analysis is regarded as the foundation for understanding the cake layer formation mechanism to carry out novel biofouling mitigation methods (Deng et al., 2016; Gkotsis et al., 2017; Huang and Lee, 2015). Organic components, especially extracellular substances (ES), is considered as the majority of cake layer, and lots of studies are focus on the polysaccharide, protein, humic substances, etc, in ES and their relationship with cake layer (Ng et al., 2010; Ou et al., 2010; Wang et al., 2009). Polysaccharide has been identified as the dominate biopolymers (> 100 kDa) and also the key biofouling-causing substance during low-pressure filtration (Meng et al., 2017). In addition, protein and humic substance are deemed making biofouling and cake layer structure more complex (Liu et al., 2011; Meng et al., 2017). Thus, many biofouling mitigation methods have been carried out to reduce the organic biofouling during MBR operation, such as sludge control, quorum quenching, nanocomposite membrane, etc. (Homayoonfal et al., 2015; Jiang et al., 2013; Meng et al., 2017). However, the studies for cake layer characteristic analysis were operated with different wastewater, especially synthetic and practical municipal wastewater (SMW and PMW) (Campos et al., 2017; Gkotsis et al., 2017; Gurung et al., 2017; Huang and Goel, 2015) has pointed out that different performance would be induced in wastewater treatment system with SMW and PMW, respectively. Additionally, recent literatures (Dang et al., 2014; Du et al., 2017; Zhou et al., 2015) have found that inorganic components, especially Si, Al, Ca, Mg, etc., play the significant role (such as the framework for cake layer structure) in the cake layer. SMW ignored inorganic components in practical MBR operation, leading to understanding variations on cake layer structure and its formation mechanism. Therefore, Villain et al. (2014) has been carried out the study on the comparison between SMW and PMW for MBR performance and membrane biofouling. However, the comparison between SMW and PMW in cake layer characteristic analysis is still unclear and needs further study.

This study aims to develop the new insights into the comparison between SMW and PMW in cake layer characteristic analysis of MBR. Two anoxic/oxic MBRs were operated for over 90 days with SMW and PMW, respectively. Cake layer characteristic were identified through cake layer morphology, organic characteristics and inorganic characteristics with ES analysis, SEM-EDX (scanning electron microscopy-energy dispersive X-ray analyzer), X-ray photoelectron spectroscopy (XPS), etc. in this study.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>MBR-SW*</th>
<th>MBR-PW*</th>
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<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Effluent</td>
</tr>
<tr>
<td>COD$_{Cr}$</td>
<td>200 ± 10</td>
<td>7 ± 5</td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td>30 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>TN</td>
<td>35 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>TP</td>
<td>5 ± 1</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>40 ± 4</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>7 ± 3</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Al$^{3+}$</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Si$^{4+}$</td>
<td>1 ± 1</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

* MBR-SW: MLSS = 4.6 g/L, MLVSS = 3.9 g/L, MLVSS/MLSS = 84%.
* MBR-PW: MLSS = 4.0 g/L, MLVSS = 2.7 g/L, MLVSS/MLSS = 68%.

2. Materials and methods

2.1. Reactor set-up and operation

Two laboratory-scale anoxic/oxic MBRs (4.5 L working volume with 1.5 L anoxic tank and 3 L oxic tank) were operated for 90 days with SMW (MBR-SW) and PMW (MBR-PW), respectively. SMW was composed with tap water, containing 200 mg/L glucose, 200 mg/L corn starch, 10 mg/L peptone, 102.75 mg/L NH$_4$Cl and 40 mg/L KH$_2$PO$_4$ as well as trace nutrients such as CaCl$_2$ (8 mg/L), MgSO$_4$·7H$_2$O (9 mg/L), MnSO$_4$·H$_2$O (3.66 mg/L) and FeSO$_4$·7H$_2$O (0.55 mg/L) (SMW characteristics showed in Table 1). Influent was adjusted with NaHCO$_3$ at pH 7.0 (Zhou et al., 2014). PMW was applied with the effluent from an aerated grit chamber of Quyang municipal wastewater treatment plant (WWTP) (Shanghai, China; PMW characteristics are showed in Table 1).

A polyvinlydedene fluoride hollow fiber membrane module (pore size 0.4 μm; 200 dm$^2$ total surface area; manufactured by Li-free Company, Suzhou, China) was immersed in each MBR. A constant fluid flux was maintained at 17 L/(m$^2$ h) with an intermittent suction mode (10 min on/2 min off for each cycle). 0.4 m$^3$/h air was supplied continuously through a cross-flow diffuser. Trans-membrane pressure (TMP) was monitored with a pressure gauge. HRT and SRT were kept at 10 h and 30 days, respectively. The flow rate of recycled mixed liquor from the oxic tank to the anoxic tank was controlled at 200% of the inflow flow rate. Inoculating sludge was collected from the return activated sludge stream in the Quyang WWTP (Shanghai, China). The newly inoculated A/O-MBR was initially operated to achieve steady state for sludge acclimatization. Membrane module was then replaced with a new and similar unit for the experiment. When TMP reached 40 kPa, the membrane module was cleaned with physical (washing with tap water) and chemical methods (2% NaClO followed by 1% citric acid immersion, each for 4 h prior to the next run). Basic operational information of MBRs are showed in Table 1.

2.2. Extracellular substances (ES) in cake layer

ES in cake layer was extracted based on the modified thermal extraction method (Zhou et al., 2014). The cake layer was scraped from membrane surface and dissolved in 40 mL 0.9% NaCl. Solution was treated with ultrasound (DSS101DT, 40 kHz, 300W, Shanghai, China) for 8 min, then shaken at 150 rpm for 10 min. Solution was next heated at 80 °C for 30 min and centrifuged at 12,000g (MILTIFUGE X1R, Thermo Electron Corporation, USA) for 20 min. Supernatant was considered as ES of cake layer. Polysaccharide and protein are regarded as the significant roles in ES of cake layer (Deng et al., 2016).
Poly saccharide and protein were measured with the phenol-sulfuric acid method and Bradford method, respectively. Three-dimensional excitation-emission (EEM) fluorescence spectra of ES in cake layer were measured via a fluorescence spectrophotometer (FluoroMax-4, HORIBA, Japan). EEM spectra were measured with the scanning emission spectra from 200 nm to 550 nm at 5 nm increments by varying the excitation wavelengths from 200 nm to 500 nm at 5 nm sampling intervals.

2.3. Inorganic elements concentrations

Concentrations of inorganic elements in the influent, effluent and cake layer were analyzed with an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 2100 DV, Perkin Elmer, USA) according to the Standard Methods (China-NEPA, 2002).

2.4. XPS measurement

The sample was scraped from the fouled membrane modules and freeze-dried for 48 h prior to XPS (PHI-5000 ESCA, Perkin Elmer, USA) analysis described in Zhou et al. (2015). XPS data was analyzed using the software of Auger Scan 320 Demo.

2.5. SEM-EDX (scanning electron microscopy-energy-dispersive X-ray analyzer) analysis

A piece of the membrane fiber was cut from the middle part of the membrane module after frozen in liquid-nitrogen. Membrane fiber was analyzed with SEM (XL30, Philips, Netherlands) coupled with an EDX (Oxford Isis, UK).

2.6. Other analytical methods

Determination of chemical oxygen demand (CODcr), ammonia nitrogen (NH4+-N), total phosphorus (TP), total nitrogen (TN) and mixed liquid suspended solid (MLSS) were conducted in accordance with Standard Methods (China-NEPA, 2002). Dissolved oxygen (DO) and pH were identified with DO-pH analyzer (HQ44d, HACH, USA). Molecular weight (MW) distribution of ES in cake layer was measured with a gel filtration chromatography (GPC, with a TSK G4000SW type gel column (TOSOH Corporation, Japan) and a liquid chromatography spectrometer (LC-10ATVP, SHIMADZU, Japan)) analyzer. Viscosity of cake layer was identified with DV-C viscometer (Brookfield, USA).

2.7. Statistical analysis

Person’s product momentum correlation coefficient \( r_p \) (Eq. (1)) was carried out for the linear correlation between two parameters.

\[
r_p = \frac{\sum (x-x_{avg})(y-y_{avg})}{\sqrt{\sum (x-x_{avg})^2\sum (y-y_{avg})^2}}
\]

where \((x, y)\) is a sample of paired data, and \(x_{avg}\) and \(y_{avg}\) are mean value. Value of \(r_p\) oscillate between 1 and +1, as \(r_p = -1\) or \(r_p = +1\) indicates a perfect correlation, and 0 showed no correction. If \(-0.4 < r_p < +0.4\), the correction is assumed weak and ignored. The positive \(r_p\) presents a direct proportionality, while the negative \(r_p\) shows an inverse proportionality.

3. Results and discussion

3.1. Component comparison between synthetic and practical municipal wastewater

Table 2 shows the component comparison between synthetic and practical municipal wastewater. All insoluble components in wastewater were remained effectively with reactor and membrane. Plenty of precipitate was accumulated at the bottom of reactor, thus regular bottom cleaning was needed for MBR-PW. Compared with insoluble components of wastewater, soluble components were easier for bacterial utilization. Higher soluble CODcr and NH4+-N in SMW indicated that SMW contained richer nutrient, and lower concentration of CODcr and NH4+-N in MBR-SW predicted the stronger material utilization efficiency. Additionally, inorganic element analysis with ICP indicated that Ca, Mg, Al and Si were the major inorganic elements in both SMW and PMW. Table 1 shows Si and Al, especially the insoluble parts, were the majority of the inorganic components in SMW. But SMW mainly contained soluble Ca as the major inorganic component. Tables 1 and 2 also present that membrane separation could not intercept soluble component with Ca and Mg, the majority of which flew out with effluent. Insoluble inorganic components with Ca, Mg, Al and Si were intercepted in MBR system with membrane separation. Therefore, the difference between PMW and SMW mainly led to the variations of inorganic components in the influent. In addition, compared with PMW, SMW offered richer nutrient for activated sludge in MBR system.

3.2. Cake layer morphology

Cake layer surface in MBR-SW and MBR-PW are shown in SEM images (E-supplementary data for this work can be found in e-version of this paper online). MBR-SW had a smooth cake layer surface with SMW, but the cake layer surface in MBR-PW was irregular. Previous studies (Zhou et al., 2015, 2014) reported that particles, especially the inorganic ones, in PMW accumulated easily onto cake layer surface during suction process. In other hand, Mg2+ addition in SMW even aggravated the sludge granulation to mitigate membrane biofouling, but the cake layer surface was still smooth (Sajjad and Kim, 2015). This predicts that the comparison between SMW and PMW would cause an obvious morphology variation of cake layer surface. Table 1 also shows that MBR-PW had lower MLVSS/MLSS rate than MBR-SW, indicating more inorganic components were in the MBR-PW, which was due to the inorganic component accumulation in the reactor. Therefore, components, especially the inorganic, in the influent play the significant role on the morphology of cake layer surface.

Fig. 1 further presents TMP and cake layer thickness variation between MBR-SW and MBR-PW. TMP increase rate is the index of cake layer formation process. The mathematical equation between day \(x; \) and TMP \(y; \) kPa was, respectively, \(y = 2.2x - 68.9\) \((R^2 = 0.9715)\) and \(y = 2.66x - 97.5\) \((R^2 = 0.9632)\) in MBR-SW and MBR-PW. The higher slope in MBR-PW indicated that PMW induced severer membrane biofouling behavior. Fig. 1 also shows that MBR-PW had the thicker cake layer than MBR-SW. The correlated formula between day \(x; \) and cake layer thickness \(y; \) \(\mu m\) was \(y = 3.6x - 115.9\) \((R^2 = 0.9304)\) and \(y = 4.0x - 154.9\) \((R^2 = 0.9642)\), respectively, in MBR-SW and MBR-PW, predicting that...
PMW aggravated cake layer formation. Consequently, PMW induced the severer membrane biofouling than SMW. In addition, Fig. 1 presents the relationship between TMP (μm) and cake layer thickness (μm) in MBR-SW and MBR-PW was as y = 1.6x − 0.2 (R² = 0.9288) and y = 1.5x − 10.1 (R² = 0.9464), respectively. The gradient result indicated that the component variation in the influent had no obvious effects on the relationship between TMP and cake layer thickness.

### 3.3. Organic characteristics of cake layer

Table 3 shows that MBR-PW had the higher organic rate in the cake layer than MBR-SW, indicating that SMW promoted the organic components accumulated into cake layer. Additionally, ES, as the key organic mixture in cake layer, plays a significant role in cake layer formation (Guo et al., 2012; Zhang et al., 2008). Table 3 presents that ES variations between MBR-SW and MBR-PW. Polysaccharide, protein and TOC of ES in MBR-SW were higher than those in MBR-PW, which was because SMW contained lots of biodegradable components for bacterial metabolism and secretion (Villain et al., 2014). TMP growth had been considered as the index of membrane biofouling process (Zhou et al., 2015). Mathematical analysis of Table 3 results shows that the correlation coefficients between organic rate and TMP were > 0.4, predicting that organic components, especially polysaccharide, showed the direct positive proportionality with membrane biofouling. However, mathematical analysis also presented that PMW decreased the correlation coefficients between TMP and organic components of ES, meaning that organic behavior might be mitigated in MBR-PW. Cake layer organic rate decrease but severer membrane biofouling with PMW further indirectly proved the inorganic biofouling behavior enhancement in MBR-PW. Additionally, MBR-SW also had the higher cake layer viscosity (Table 3), which was due to higher organic component in cake layer. High viscosity of cake layer, having the positive correlation with membrane biofouling (0.53 and 0.46 in MBR-SW and MBR-PW, respectively), was one of the reasons for dense cake layer structure with SMW, especially humic substances (Kimura et al., 2005; Zhang et al., 2017).

Fluorescence spectra (Fig. 2) shows that Peak A (excitation/emission wavelengths of 270–285/320–330 nm), reported as tryptophan-like substances (Xia et al., 2015), was the major fluorescent organic component in cake layer. Tryptophan-like substance works as an important role in the bacterial metabolism (Schrocksnadel et al., 2006; Xia et al., 2015). As an α-amino acid applied in the biosynthesis of proteins, tryptophan-like substance is a measurement of biological activity through either bacterial production or algal growth and its grazing (Baker et al., 2007). Higher tryptophan-like substance in MBR-SW (Fig. 2) indicated activated bacterial behavior because of rich nutrient in SMW (Section 3.1). Additionally, there is no obvious variation of molecular weight (MW) distribution of ES in cake layer between MBR-SW and MBR-PW (Fig. 3), predicting that the comparison between SMW and PMW had no obvious effects on the MW distribution of ES in cake layer, even with rich nutrient in SMW. Moreover, only fraction ranging < 1 kDa of ES in MBR-SW were little higher than that in MBR-PW. Tryptophan-like substance is considered to be correlated with the autochthonous generation of small colloidal and dissolved organic matter in microbial activity (Baker et al., 2007). Thus, higher tryptophan-like substance in SMW would lead to the < 1 kDa ES increase. In all, SMW highlighted the organic components in cake layer with bacterial metabolism promotion.
3.4. Inorganic characteristics of cake layer

XPS results shows that MBR-SW and MBR-PW had similar inorganic components in the cake layer, such as SiO\(_2\), Al\(_2\)O\(_3\), CaCO\(_3\), CaSO\(_4\) and Mg(II) (E-supplementary data for this work can be found in e-version of this paper online). Guo et al. (2012) has reviewed that inorganic components with Ca and Mg played the dominate role in cake layer structure, especially MBR for SMW treatment. SiO\(_2\), Al\(_2\)O\(_3\), CaCO\(_3\), CaSO\(_4\) and Mg(II) had been also reported as common inorganic components in activated sludge in MBR operation (Zhou et al., 2015), and thus the similar inorganic components in cake layer of both MBRs was because of the same inorganic form between MBR-SW and MBR-PW. Consequently, comparison between SMW and PMW would not induced obvious variation of inorganic form in cake layer. As a mathematical analysis, line scan EDX was carried out to identify element cross-sectional distribution in cake layer. As XPS results show, Si, Al, Ca and Mg were the major inorganic components of cake layer in both MBRs. SEM images shows that cross-sectional cake layer (inner structure) was smooth in MBR-SW, but that in MBR-PW was rough (E-supplementary data for this work can be found in e-version of this paper online). This result was consistent with cake layer surface SEM images.

Fig. 4 presents that MBR-SW and MBR-PW had the different distributions of Si and Al. Tables 1 and 2 show that PMW contained plenty of insoluble Si and Al, and most of them was remained in MBR reactor. Zhou et al. (2015) also reported that Si and Al were in the form of SiO\(_2\)-Al\(_2\)O\(_3\) crystals in PMW and cake layer. Therefore, MBR-PW had similar Si and Al distribution along the cross-sectional cake layer, but MBR-SW contained lower Si and Al in its cake layer. However, because of ion form in SMW, Al was evenly distributed along cake layer in MBR-SW. Initial Si accumulation in both MBRs (Fig. 4(b)) was due to original Si components of sludge, and later Si accumulation in MBR-PW was mainly because of the Si in PMW. Additionally, inorganic components (especially Si), working as the framework of cake layer, intensify cake layer structure and causing denser cake layer.

4. Conclusion

This study identified comparison between SMW and PMW in cake layer characteristic analysis of MBR. PMW led to rough cake layer.
surface with particles, and the aggravation of cake layer formation with thinner but denser cake layer. Inorganic components, especially Si and Al, in PMW accumulated into cake layer and strengthened the cake layer structure. SMW promoted bacterial metabolism with rich nutrient, causing obvious organic accumulation onto cake layer. Therefore, SMW highlighted the organic components in cake layer, but weakened the inorganic functions in practical MBR operation.
Table 4
Correlation coefficient ($r$) between TMP and inorganic elements (Ca, Mg, Al and Si) in the influent. ($n$ = 10).

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<th>MBR-SW</th>
<th>MBR-PW</th>
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<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Mg</td>
</tr>
<tr>
<td>Soluble in Influent</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Insoluble in Influent</td>
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<td>-</td>
</tr>
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</table>

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.08.069.

References