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Membrane fouling and sludge characteristics in submerged membrane bioreactor under low temperature

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Abstract. This study aimed to investigate the membrane fouling and sludge characteristics in a pilot-scale submerged membrane bioreactor (MBR) operated under low temperature (7°C). To elucidate the mechanisms of membrane fouling at low temperature, we studied the correlation between MBR performances and physicochemical properties of sludge including extracellular polymeric substance (EPS), relative hydrophobicity (RH) and floc size during long-term operation. The MBR was shown able to remove chemical oxygen demand (COD) stably and efficiently (>90 %) in the case of overgrowth of filamentous bacteria (bulking sludge) at low temperature. On the other hand, the occurrence of filamentous bulking greatly accelerated membrane fouling, as indicated by membrane filtration period of 14 days for filamentous bulking at 7°C, in comparison with that of 27 days for non-bulking sludge at 24°C. The overgrowth of filamentous bacteria resulting from low-temperature condition led to an increased release of EPS, higher RH, smaller floc size and lower fractal dimension of sludge. These factors accelerated the formation of compact cake layer on membrane surface in association with performance diminution in terms of increase in transmembrane pressure (TMP) of the membrane and thus the decrease in membrane permeability.

Keywords: membrane bioreactor; membrane fouling; sludge bulking; low temperature; extracellular polymeric substances; hydrophobicity

1. Introduction

Recently, membrane bioreactor (MBR) has been widely adopted for wastewater treatment by virtue of several advantages compared with conventional activated sludge process (Zhang *et al.* 2006). However, the problems associated with membrane fouling remain a major technical obstacle that hampers its engineered applications (Iorhemen *et al.* 2017). Although the factors that affect membrane fouling have been investigated extensively, the physicochemical properties and physiology of activated sludge were demonstrated of great importance in prior studies (Zhang *et al.* 2017).

Great advance has been made on mechanisms of membrane fouling in MBR. For example, Chen *et al.* (2016) reported the interfacial interactions (thermodynamic forces) between foulants and membrane are decisive forces in the process of adhesion/deposition of foulants on the membrane. The gel layer possessed high specific filtration resistance (SFR) and high measured porosity compared with cake layer, based on which a new fouling mechanism (Flory-Huggins theory) was proposed. The filtration resistance of agar gel layer induced by mixed chemical potential was independent of pH and ionic strength, but linearly increased with gel thickness. Zhang *et al.* (2018) used Terahertz time-domain spectroscopy (THz-TDS) technology to characterize the role of calcium ions in alginate fouling, and they found that calcium addition caused structural variation of alginate polymer in solution. In combination with density functional theory (DFT) calculations, they also verified that initial binding of alginate chains induced by calcium ions occurred predominantly in intermolecular but not intramolecular, and two alginate chains cross-linked by a calcium atom tend to stretch in a tetrahedron structure but not parallel to each other. Accordingly, the "chemical potential gap" in Flory-Huggins theory should be responsible for the filtration behaviors of alginate solution in the presence of calcium addition. These theories and results were also supported by a number of literatures published recently (Zhang *et al.* 2017, Chen *et al.* 2017, Shen *et al.* 2015, Teng *et al.* 2018).

Neoh *et al.* (2016) found that sludge bulking cased by overgrowth of filamentous bacteria in sludge suspension had a crucial impact on membrane fouling. Choi *et al.* (2002) found the serious membrane fouling when sludge bulking occurred in a cross-flow MBR. These studies indicated that membrane fouling was, to some extent, related to filamentous sludge bulking. In addition to filamentous bulking, low temperature should be another factor that may affect the performances of MBR in wastewater treatment located in cold regions.

The studies performed in cold north China showed that the temperature had a strong significant impact on performances of a MBR system. For instance, Zhang *et al.* (2014) found that the lower temperature caused an increased energy consumption and membrane fouling

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associated with filamentous bacteria in submerged MBR. It is well known that filamentous microbes have lower half-saturation constant (K_S) and maximum growth rate (μ_{max}). This means that the filamentous bacteria are favored at limited conditions such as low substrate, low dissolved oxygen and low temperature compared with floc-forming bacteria, and vice versa (Wang *et al.*, 2016). This indicates that filamentous bacteria will overgrow easily in a MBR system operated at limited conditions (e. g. low temperature investigated in this study). Regarding the fouling mechanism of bulking sludge, previous studies have shown that the severe membrane fouling taking place under the overgrowth of filamentous bacteria was largely caused by the change of activated sludge properties such as extracellular polymeric substances (EPS), sludge floc size, Zeta potential, and relative hydrophobicity (RH) (Meng *et al.* 2006). However, for a MBR operated under low-temperature condition, less attention has been paid to the relationship between membrane fouling and low-temperature, the factor that may be associated with the overgrowth of filamentous bacteria in a pilot-scale MBR system.

The aim of this study was to investigate the impact of filamentous bulking sludge on the properties of activated sludge and membrane fouling behavior at low temperature in a pilot-scale submerged MBR. The properties of activated sludge including extracellular polymeric substances (EPS), relative hydrophobicity (RH), sludge floc size and fractal dimension were examined as factors to specify the membrane fouling mechanism caused by filamentous bacteria at low temperature.

2. Materials and methods

2.1 Experimental MBR set-up

The pilot-scale submerged MBR was shown schematically in Fig. 1. The effective volume of the bioreactor was 30 L. A hollow fiber membrane module (FP-T0008, China) was submerged in the bioreactor and the relevant parameters were provided in Table 1. During experimental period, the synthetic wastewater was continuously supplied by using a peristaltic pump (BT00-600M, Lange Co., Ltd, China) from the storage tank. In practical operation of MBR, it was common practice to set intermittent mode in order to avoid severe drop of pressure and membrane fouling caused by long-term continuous suction. Hence, the membrane-filtered effluent was extracted by a pump of the same mode being operated intermittently with a cycle of 8 min on and 3 min off. The hydraulic retention time (HRT) was kept at 4 h. Unless stated otherwise, the filtration operation of the pilot-scale submerged MBR was conducted at the constant flow rate of 10 L/(m^2h). To maintain a constant liquid level in MBR, a level sensor was applied. The pressure gauges were installed in order to monitor the variation of trans-membrane pressure (TMP) between the membrane and suction pumps. The filtration operation was terminated once the TMP reached 30 kPa.

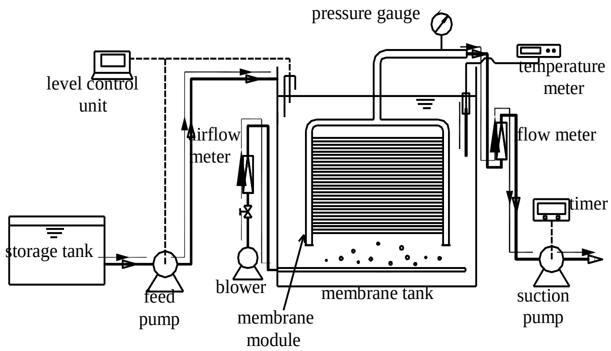


Fig. 1 The schematic illustration of pilot-scale MBR system

Table 1 Characterization of hollow membrane material used in this study

Items	Parameters
Membrane material	Polyvinylidene Fluoride, FP-T0008 fibres
Permeability at 20 ºC (L/hm^{2}bar)	9.5-18
Surface area (m^2)	1.0
Fibber external diameter (mm)	1.1
Fibber internal diameter (mm)	0.6
Pore size (μm)	0.2
Length (mm)	400

2.2 Synthetic wastewater and operations

The composition of synthetic wastewater was glucose, ammonium chloride (NH_4Cl) and dipotassium hydrogen phosphate (K_2HPO_4). The glucose and NH_4Cl served as carbon and nitrogen source, respectively, to support the growth of activated sludge in MBR. This gave rise to an average influent COD of 440 mg/L and COD:N:P of 100:10:2. Sodium bicarbonate ($NaHCO_3$) was also added into synthetic wastewater to maintain the pH of mixed liquor in an appropriate range of 6.8-7.2. The MBR was run with aeration at a flow rate of 0.32 m^3/h, and was inoculated by using aerobic activated sludge collected from the aeration tank of Taiping Wastewater Treatment Plant, Harbin, China. The mixed liquid suspended sludge (MLSS) concentration of activated sludge suspension was kept at 8000±200 mg/L in the suspension of MBR. To investigate the effect of low temperature on the system performance, two identical MBRs were operated in parallel mode with one running at temperature of 7°C by placing the membrane bioreactor in a refrigerator and the other one (control) running at room temperature (24°C).

2.3 Analytical methods

COD, MLSS and mixed liquor volatile suspended solids (MLVSS) were determined according to the standard methods (Walter 1998). Dissolved oxygen (DO) in MBR was monitored by a DO meter (WTW, Germany). The relative density of filamentous bacteria was evaluated by means of microscopic observation (Olympus, CX31). The filament index (FI) was measured and computed using the

Arbitrary Scale method as described by Jin *et al.* (2003). According to this method, the number of filamentous organisms could be rated on a scale of 1-5, where for example, 1 represented very little or no filamentous bacteria, and 5 the excessive growth of filamentous bacteria. The extraction of microbial EPS was carried out according to the formaldehyde/NaOH extraction method reported by Liu and Fang (2002). Thus, the overall amount of EPS (mg/gMLSS) was described and reported by the sum of protein and polysaccharides. The polysaccharides were determined by using phenol/sulphuric acid method and the protein was analyzed using folin method (Lowry *et al.* 1951). The relative hydrophobicity (RH) was assessed using the manner being similar as that reported by Wilén *et al.* (2003) except for several modifications proposed by Meng *et al.* (2006). The RH was expressed as the ratio of MLSS concentration in the aqueous solution after ($MLSS_e$) and before emulsification ($MLSS_i$)

$$RH(\%) = (1 - \frac{MLSS_e}{MLSS_i}) \times 100 \tag{1}$$

The fractal dimension (D_f) of the sludge was estimated to characterize the shape of the sludge (bulking and non-bulking) on the basis of image analysis as described by Motta *et al.* (2001) The calculation of D_f was done according to

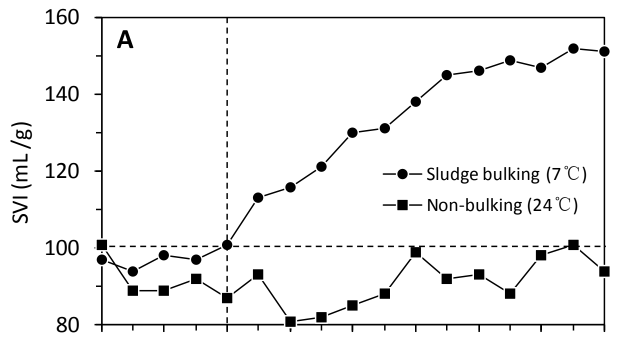
$$A = l^{Df} \tag{2}$$

where A is the projected area of sludge image, l the longest size, and D_f the fractal dimension embedded in two dimension (D=2.0). By taking logarithm on two sides of Equation 2, D_f can be determined from the slope of log$A$-log$l$ line. The floc size of sludge was determined by using Liquid Particle Counting System (Model 9703, Pacific Scientific) with a detection range of 2-300 μm. Each sample was measured three times and reported on an average basis.

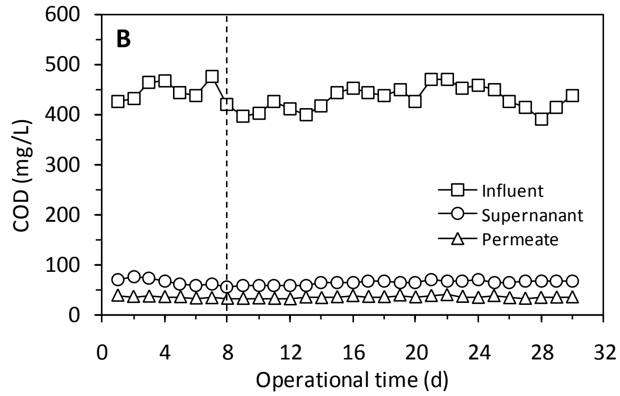
3. Results and discussion

3.1 Sludge bulking and COD removal in MBR

The sludge volume index (SVI) quantitatively describing the bulking sludge caused by filamentous bacteria were monitored and compared between two MBRs operated at temperature of 7 ºC and 24 ºC. As shown in Fig. 2A, the SVI values recorded for both reactors remained below 100 mL/g before day 8, whereas the sludge deterioration took place for the MBR operated at 7 ºC. This could be observed from the occurrence of bulking sludge, i. e. the SVI values were increasing from 97 mL/g on day 8 to 151 mL/g on day 32. In comparison, the sludge in the reactor running at normal temperature (24 ºC) exhibited better settleability and compressibility, which was in line with the SVI values observed always lower than 100 mL/g throughout the tests. This indicated that the low temperature should be the most likely reason responsible for bulking sludge. Despite minimal influence of bulking sludge to treatment performance of MBR, it had a crucial impact to membrane fouling as demonstrated in detail below.



A Variation of SVI in suspended sludge



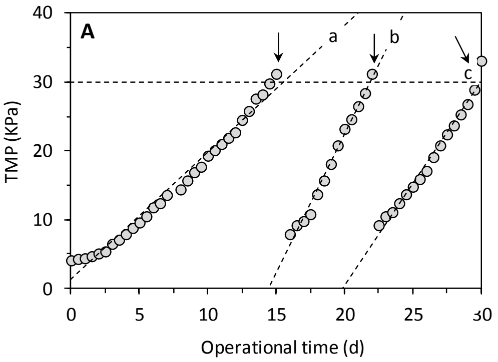
B COD concentration time profile

Fig. 2 Time course of SVI and COD concentration for MBR operated at temperature of 7 ºC.

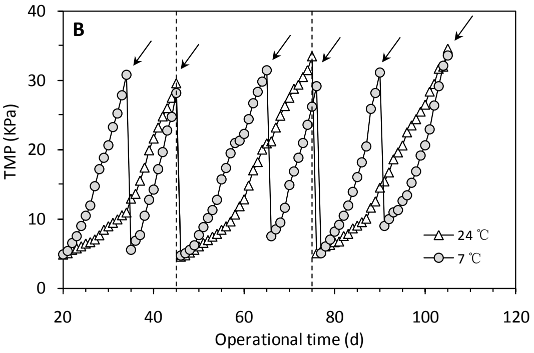
Variation of COD concentration given in Fig. 2B clearly showed a stable organic removal in the MBR where bulking sludge occurred. At low temperature of 7 ºC, COD concentration was measured above 60 mg/L in the supernatant and below 40 mg/L in the permeate, respectively. It is known that low temperature affected negatively the metabolic reactivity of microorganisms, which results in the performance degradation of MBR system. Despite the partial loss of flocculating ability of the sludge, COD could still be removed efficiently during the development of filamentous bulking sludge. It appeared most likely to result from the fact that the abundant filamentous bacteria were more favored to proliferation than other microbial communities under limited environments. The results obtained here were in agreement to the previous studies, which addressed that the organic removal efficiency was increased under sludge bulking condition as a result of excessive filamentous bacteria (Jegatheesan *et al.* 2016). In addition, membrane filtration could also remove a fraction of organic compounds that were non-degradable when the temperature declined (Zhang *et al.* 2014). Therefore, the combination of biological and membrane retention enabled effective COD removal during the filamentous bulking sludge under low temperature.

3.2 Membrane fouling during bulking sludge at low temperature

The transmembrane pressure (TMP) was monitored to compare the dependence of membrane fouling on



A Variation of TMP for filamentous bulking in initial stage (7 ºC)



B TMP of MBRs at different temperatures in ongoing stages (The arrowheads indicated cleaning of fouled membrane)

Fig. 3 Membrane fouling characteristic during sludge bulking

filamentous bulking for two MBRs running at temperature of 7 ºC and 24 ºC. When the TMP reached a critical value being approximately 30 kPa (Fig. 3A), the membrane modules needed to be cleaned physically by flushing with tap water for 30 min, followed by chemical rinsing in 1% NaClO (8 h) and 5% H_2SO_4 solution (2 h). These actions were taken to remove the foulant formed on the membrane surface, thereby water production could be recovered.

Fig. 3A illustrated two distinct stages of TMP variation with time at the beginning of operation at 7 ºC. The first stage was characterized by a relatively slow TMP increase from 4.1 kPa to 6.5 kPa within 3 days. In the second stage on day 4-15, the TMP was observed to ascend steeply over a short period of time. Such difference in TMP variation appeared unlikely to be consistent with the previous study by Sombatsompop *et al.* (2006). They found the increase in TMP during the first stage was as slow as that during 3-15 days at mesophilic conditions (24 ºC), and the second stage lasted from day 15 to 26 along with an exponential change of the TMP with respect to time. In this stage, the TMP increased with time linearly. This clearly indicated that the membrane fouling taking place at low temperature was more serious than that at mesophilic temperature. Additionally, as revealed in Fig. 2A, the filamentous bacteria started to grow excessively on day 8, yet this did not accompany well with the quickly increased TMP observed on day 4. We attributed such inconsistence to the effect of EPS content as discussed below.

The change of TMP behaved different in periodic variations with respect to running time and operating conditions for two MBRs (Fig. 3B). At the beginning stage of each cycle, the TMP increased with time, reaching the maximum of about 30 kPa, followed by being recovered to its initial level of 5 kPa after cleaning. As recalled in Fig. 2A and Fig. 3A, the MBR running at 7 ºC started to suffer from bulking sludge on day 8, and this led to a drastic increase in TMP compared with that running at 24 ºC during each repeatable cycle. Before the TMP reached 30 kPa, the cleaning period required for MBR (7 ºC) was approximately 14 days, a value half time of that for MBR (27 days, 24 ºC). Within the tested period, the membrane fouling rate expressed by dTMP/dt of 1.89 kPa/d for 7 ºC and 0.97 kPa/d for 24 ºC was derived from Fig. 3B upon averaging the values obtained from each individual cycle. Cleaning period paired with fouling rate suggested a half shorter working period when the reactor was operated at low temperature. This did represent more energy consumption to eliminate the negative impact of bulking sludge on membrane fouling. Owing to serious membrane fouling, the effluent flux was decreased from 10 L/m^{2}h to 9 L/m^{2}h. However, the qualified effluent could still be achieved for HRT of 4 h by adjusting the liquid level in the MBR. Chu and Li (2005) addressed that the fouling rate could be increased as the membrane filtration flux increased. These results confirmed that the membrane fouling proceeded more dramatically under the condition of filamentous bulking and low temperature.

The close correlation between temperature and filamentous bulking suggested that the membrane fouling was more severe when filamentous bacteria grew abundantly in the sludge. This could be demonstrated by the increase of FI from 2 to 5 (Table 2), leading to a shortening of the membrane filtration period from 27 d to 14 d (Fig. 3B). The results were in good agreement with previous study reported by Meng *et al.* (2006) who showed that the thick cake layer formation caused by filamentous bacteria was non-porous. The filaments played a role of backbone and framework in the cake layer. The filamentous bacteria preferred to attach onto the membrane foulants, causing a tight adherence and penetration within membrane foulants. Thus, the cleaned membrane fouled fast at filamentous-bulking condition led to almost disappearance of the first stage and the shortening of the second stage in TMP curves (Fig. 3A).

Based on the analysis above, the order of membrane fouling tendency and degree followed the order as normal sludge at mesophilic temperature < normal sludge at low temperature < filamentous bulking sludge at low temperature.

3.3 Effect of EPS on filamentous bulking and membrane fouling

EPS is known as a kind of complex substances containing a variety of polymeric materials such as carbohydrates, proteins, lipids and nucleic acids. In this work, the sum of total carbohydrates and proteins was used to evaluate the total amount of EPS due to their typically dominant components in the extracted EPS.

Table 2 Summary of chemical composition of EPS extracted from the sludge in MBRs

Sludge type	FI	Carbohydrates (mg/gMLSS) [a]	Proteins (mg/gMLSS)	Total EPS (mg/gMLSS)	P/C [b]	RH (%)
Non-bulking sludge at 24 ºC	2	8.91	19.15	28.06	2.15	41
Non-bulking sludge at 7 ºC	2	18.52	42.16	60.68	2.27	43
Bulking sludge at 7 ºC	5	46.07	131.31	177.38	2.85	92

[a] The MLVSS/MLSS ratio was about 0.7-0.73.
[b] P/C represents the ratio of proteins to carbohydrates.

Table 2 summarizes the concentration of carbohydrate and protein measured in microbial flocs of normal and filamentous bulking sludge. Clearly is visible that the bulking sludge (FI=5) containing EPS being 192 % higher than normal sludge (FI=2). The change of filamentous bacteria density is correlated to the change in the microbial products. Choi *et al.* (2002) addressed that the filamentous bacteria produced more foulants (i. e. EPS) than floc-forming bacteria, and thus a higher amount of lipid-like substances existed in the mixed liquor of filamentous bulking sludge than in normal sludge. It is known that the metabolic activity of microorganisms was inhibited under lower temperature. Hence, more EPS excreted from the bacterium accumulated not only onto the outside site of cell membrane but also in the sludge suspension. Both polysaccharide and protein content increased when temperature was lowered to 7 ºC, which was in line with Brink *et al.* (2011) who found the negative correlation between polysaccharide and temperature during membrane fouling. On the other hand, it was most likely that the EPS accumulated because the microorganisms were not metabolizing the EPS as fast as they normally would at a higher temperature.

In this study, the accumulation of EPS should be responsible for membrane fouling observed for low temperature, as indicated by correlation between the increase in TMP (Fig. 3A) and total amount of EPS (Table 2). The first stage and total period for TMP curve obtained at 7 ºC were found much shorter than that at 24 ºC (Fig. 3B). In consistence, Gasmi *et al.* (2015) found that EPS was the key factor to membrane fouling, and the total EPS content of 60.68 mg/gMLSS (86.69 mg/gMLVSS) for normal sludge at the initial stage was much higher than that of 19.2 mg/gMLSS (27.43 mg/gMLVSS) at mesophilic temperature (Sombatsompop *et al.* 2006). Hence, the membrane fouling evolved more severely as EPS content increased at low temperature. On the contrary, the overgrowth of filamentous bacteria resulted in a dramatic increase in EPS content of 177.38 mg/gMLSS (253.40 mg/gMLVSS; Table 2). The most likely reason should be that the filamentous bacteria attaching onto the membrane foulants promoted the formation of a non-porous cake layer (Meng *et al.* 2006). Besides, EPS is also of critical importance in the formation of cake layer (Allison and Sutherland 1987) as the EPS adsorbed onto membrane surface makes the attachment of bacteria easier and thus the readily formation of a sludge cake. As a result, the excessive filamentous bacteria combined with the abundant EPS caused the severe membrane fouling when sludge bulking occurred at low temperature. The results obtained herein were seen in consistence with several previous studies reported by Lin *et al.* (2014) and Ramesh *et al.* (2007).

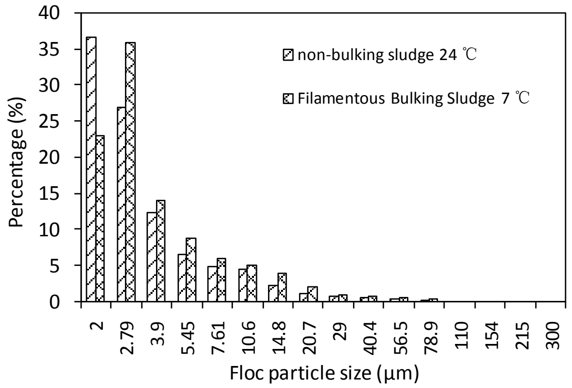
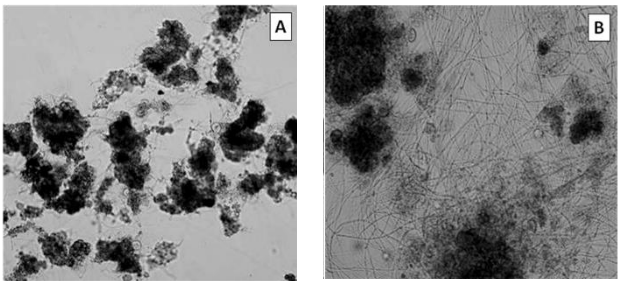


Fig. 4 Sludge floc size distribution in the suspension of normal and filamentous bulking sludge

3.4 Effect of hydrophobicity on filamentous bulking and membrane fouling

To examine the effect of filamentous bacteria on the surface properties of sludge flocs, the relative hydrophobicity (RH) of sludge flocs was investigated. The RH of normal sludge with FI=2 and bulking sludge with FI=5 were 43% and 92% (Table 2), respectively. The sludge flocs attached by more filamentous bacteria appeared more hydrophobic. Higher RH of sludge flocs rendered a stronger adherence of floc particles with one another as well as onto the membrane surface driven by trans-membrane pressure, thereby augmenting the membrane fouling. Emanuelsson *et al.* (2003) demonstrated that the surface of attached biofilm was much more hydrophobic than that of the suspended sludge. Being consistent with the results reported by Wilén *et al.* (2003) and Choi *et al.* (2002), the sludge flocs with higher-degree hydrophobicity (RH) tended to deposit onto the membrane surface due to strong interaction between hydrophobic groups involved in EPS (e. g. acyl group) and membrane surface. Thus the cake layer became thicker when there was additional amount of filamentous bacteria developed in the activated sludge. This should be one of the most likely reasons for more serious membrane fouling observed for filamentous bulking sludge at low temperature.

Cai *et al.* (2017) reported that hydrophobicity of sludge flocs was correlated to polymeric substances like EPS and proteins involved in sludge flocs. The proteins contained in EPS have a positive influence on the hydrophobicity of



A Non-bulking sludge (24 ºC) B Bulking sludge (7 ºC)

Fig. 5 Optical images of the sludge morphology

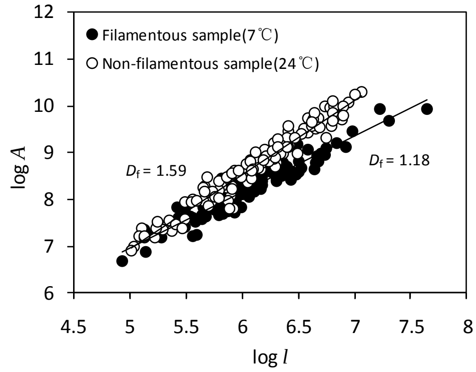


Fig. 6 Estimation of fractal dimension from log-log plot of area-size power law for filamentous and non-filamentous sludge samples

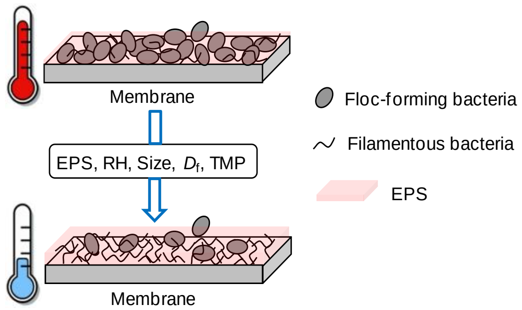


Fig. 7 Schematic illustration of membrane fouling at low temperature

sludge flocs, but such impact is minimal to carbohydrate because the proteins constitute the major components of hydrophobic amino acids in EPS. Table 2 listed that the proteins/carbohydrates ratio (P/C) of bulking sludge was slightly higher than that of normal sludge, indicating that the change of P/C value also affected the RH of sludge suspension. Therefore, the excessive growth of filamentous bacteria produced much more EPS, and thus caused higher hydrophobicity of the sludge flocs.

3.5 Floc size distribution and fractal dimension of activated sludge

Sludge flocs with particle size in the range of 2-300 µm and fractal dimension were further characterized. As shown in Fig. 4, sludge floc with particle size smaller than 40 µm predominated (>98 %) in normal sludge (24 ºC) and filamentous bulking sludge (7 ºC). The main size of sludge flocs was larger than 2.79 µm in bulking sludge, and smaller than 2 µm in normal sludge, respectively. The increase in floc size should be attributed to the overgrowth of filamentous bacteria in sludge suspension (Meng *et al.* 2006).

The highest fraction of floc size was over the range of 2-3 µm in both normal and bulking sludge, a value much smaller than that observed at 24 ºC. The largest size of the sludge flocs was larger than 30 µm in normal sludge and increased to 78.9 µm when bulking sludge occurred at 24 ºC (Fig. 4). Zhang *et al.* (2015) found that the difference in floc size should result from different physiological properties of sludge and hydraulic shearing force onto sludge flocs at different HRTs and SRTs. With regard to such effect, Choi *et al.* (2002) showed that the floc size having maximum quantity was 2 µm at mesophilic temperature, representing a value much smaller than other studies. This should be attributed to the implementation of short SRT (6 d), great air flow rate and high DO concentration (>3 mg/L). Palmarin and Young (2016) studied the effect of operating parameters on membrane fouling in a cross-flow microfiltration system, and observed that the floc particles having a size smaller than 50 µm yielded greater specific resistance and higher cake resistance. Seminaro *et al.* (2002) investigated the pore blocking and permeability reduction in cross-flow microfiltration of bentonite suspension using polysulfone membranes with nominal pore size of 0.2 µm. The gradual blocking of the pores by small particles could be identified and the pores became more easily blocked by particles with smaller size, resulting in a rapid drop of permeability. In general, the sludge suspension with smaller-size flocs was not propitious for membrane filtration process (Jeong *et al.* 2016). This also illuminated the reasons for much severe membrane fouling observed at low temperature.

The observation of sludge morphology shows the normal sludge with compact and flocculating aggregates (Fig. 5A) while the bulking sludge with a large amount of filamentous microorganisms (Fig. 5B), corresponding to the D_f value of 1.59 and 1.18 (Fig. 6), respectively. The correlation between D_f, FI and SVI suggested that the bulking sludge containing a large quantity of filaments had great FI (5) and SVI (>150 mL/g), as well as low D_f (1.18) value. Specifically, the low D_f implies a more open structure of activated sludge (Jin *et al.* 2003). In comparison, the non-bulking sludge was characterized by higher D_f (1.59), which accorded to more compact and dense structure of the flocs with better settleability (SVI<100 mL/g). Since the D_f embedded in 2D space was in the range of 1-2, the sludge with D_f being close to D=1 had more porous and filamentous properties. In other words, the lower D_f value implied the overgrowth of filamentous bacteria with more developed porous and open structure, and higher surface area to adhere onto the membrane surface causing membrane fouling in MBR.

Taken together, the relationship between low temperature and membrane fouling was established in this study. At low temperature, the filamentous bacteria grew more rapidly than floc-forming bacteria due to lower half-saturation constant (K_S) and maximum growth rate (μ_{max}) value (Wang *et al.* 2016) in kinetic selection theory. The decline in temperature from 24 ºC to 7 ºC resulted in the decrease in D_f from 1.59 to 1.18, indicating the overgrowth of filamentous bacteria at low temperature. This could be also in line with observation of a larger amount of EPS produced by filamentous bacteria (Nagaoka *et al.* 1998). This would enhanced membrane fouling because of the formation of compact cake layer via the interaction between hydrophobic groups involved in EPS (e. g. acyl group) and membrane surface (Wilén *et al.* 2003). The membrane fouling in the MBR operated at low temperature was schematically illustrated in Fig. 7.

4. Conclusions

The present study investigated the membrane fouling and sludge characteristics in a pilot-scale submerged membrane bioreactor (MBR) operated under low temperature (7°C). The main conclusions could be drawn as follows. The MBR could remove COD stably and efficiently (>90 %) in the case of overgrowth of filamentous bacteria at low temperature. The overgrowth of filamentous bacteria resulting from low-temperature condition led to an increased release of EPS, higher RH, smaller floc size and lower fractal dimension of sludge. These factors accelerated the formation of compact cake layer on membrane surface in association with performance diminution in terms of increase in transmembrane pressure (TMP) of the membrane and thus the decrease in membrane permeability. This study provides a demonstration of membrane fouling associated with bulking sludge occurring at low temperature in MBR, which prompts development of strategy to control filamentous bulking and thus mitigate membrane fouling.

Acknowledgments

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References

Allison, D.G. and Sutherland, I.W. (1987), "The role of exopolysaccharides in adhesion of freshwater bacteria", *Microbiol*, **133**, 1319-1327. https://doi.org/10.1099/00221287-133-5-1319.

Brink, P.D., Satpradit, O.A., Bentem, A., Zwijnenburg, A., Temmink, H. and Loosdrecht, M. (2011), "Effect of temperature shocks on membrane fouling in membrane bioreactors", *Water Res.*, **45**, 4491-4500. https://doi.org/10.1016/j.watres.2011.05.046.

Cai, X., Shen, L.G., Zhang, M.J., Chen, J.R., Hong, H.C. and Lin, H.J. (2017), "Membrane fouling in a submerged membrane bioreactor: An unified approach to construct topography and to evaluate interaction energy between two randomly rough surfaces", *Bioresour. Technol.*, **243**, 1121-1132. https://doi.org/10.1016/j.biortech.2017.07.054.

Chen, J. R., Zhang, M.J., Li, F.Q., Qian, L., Lin, H.J., Yang, L.N., Wu, X.L., Zhou, X.L., He, Y.M. and Liao, B.Q. (2016), "Membrane fouling in a membrane bioreactor: High filtration resistance of gel layer and its underlying mechanism", *Water Res*, **102**, 82-89. https://doi.org/10.1016/j.watres.2016.06.028.

Chen, J.R., Lin, H.J., Shen, L.G., He, Y.M., Zhang, M.J. and Liao, B.Q. (2017), "Realization of quantifying interfacial interactions between a randomly rough membrane surface and a foulant particle", *Bioresour. Technol.*, **226**, 220-228. https://doi.org/10.1016/j.biortech.2016.12.025.

Choi, J.G., Bae, T.H., Kim, J.H., Tak, T.M. and Randall, A.A. (2002), "The behavior of membrane fouling initiation on the crossflow membrane bioreactor system", *J. Membr. Sci.*, **203**, 103-113. https://doi.org/10.1016/S0376-7388(01)00790-6.

Chu, P.H. and Li, X.Y. (2005), "Membrane fouling in a membrane bioreactor (MBR): Sludge cake formation and fouling characteristics", *Biotechnol. Bioeng.*, **90**(3), 323. https://doi.org/10.1002/bit.20409.

Emanuelsson, E.A.C., Arcangeli, J.P. and Livingston, A.G. (2003), "The anoxic extractive membrane bioreactor", *Water Res.*, **37**(6), 1231. https://doi.org/10.1016/S0043-1354(02)00487-6.

Gasmi, A., Heran, M., Hannachi, A. and Grasmick, A. (2015), "Fouling analysis and biomass distribution on a membrane bioreactor under low ratio COD/N", *Membr. Water Treat.*, **6**, 263-276. https://doi.org/10.12989/mwt.2015.6.4.263.

Iorhemen, O.T., Hamza, R.A. and Tay, J.W. (2017), "Utilization of aerobic granulation to mitigate membrane fouling in MBRs", *Membr. Water Treat.*, **8**, 395-409. https://doi.org/10.12989/mwt.2017.8.5.395.

Jegatheesan, V., Pramanik, B.K., Chen, J.Y., Navaratna, D., Chang, C.Y. and Shu, L. (2016), "Treatment of textile wastewater with membrane bioreactor: A critical review", *Bioresour. Technol.*, **204**, 202-212. https://doi.org/10.1016/j.biortech.2016.01.006.

Jenkins, D., Richard, M.G. and Daigger, G.T. (1986), "Manual on the causes and control of activated sludge bulking and foaming", Ridegeline Press, Lafayette, CA, USA.

Jeong, S.H., Cho, K.J., Bae, H.W., Keshvardoust, P., Rice, S.A., Vigneswaran, S., Lee, S.H. and Leiknes, T. (2016), "Effect of microbial community structure on organic removal and biofouling in membrane adsorption bioreactor used in seawater pretreatment", *Chem. Eng. J.*, **294**, 30-39. https://doi.org/10.1016/j.cej.2016.02.108.

Jin, B., Wilén, B.M. and Lant, P. (2003), "A comprehensive insight into floc characteristics and their impact on compressibility and settleability of activated sludge", *Chem. Eng. J.*, **95**, 221-234. https://doi.org/10.1016/S1385-8947(03)00108-6.

Lin, H.J., Zhang, M.J., Wang, F.Y., Meng, F.G., Liao, B.Q., Hong, H.C., Chen, J.R., Gao, W.J. (2014), "A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: Characteristics, role in membrane fouling and control strategies", *J. Membr. Sci.* **460**, 110-125. https://doi.org/10.1016/j.memsci.2014.02.034.

Liu, H. and Fang, H.H. (2002), "Extraction of extracellular polymeric substances (EPS) of sludges", *J. Biotechnol.*, **95**, 249. https://doi.org/10.1016/S0168-1656(02)00025-1.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951), "Protein measurement with the Folin phenol reagent", *J. Biol. Chem.*, **193**, 265.

Meng, F.G., Zhang, H.M., Yang, F.L., Li, Y.S., Xiao, J.N. and Zhang, X.W. (2006), "Effect of filamentous bacteria on membrane fouling in submerged membrane bioreactor", *J. Membr. Sci.*, **272**, 161-168. https://doi.org/10.1016/j.memsci.2005.07.041.

Motta, M.D., Pons, M.N., Roche, N. and Vivier, H. (2001), "Characterisation of activated sludge by automated image analysis", *Biochem. Eng. J.*, **9**, 165-173. https://doi.org/10.1016/S1369-703X(01)00138-3.

Nagaoka, H., Yamanishi, S. and Miya, A. (1998), "Modeling of biofouling extracellular polymers in a membrane parathion activated sludge system", *Water Sci. Technol.,* **38**, 497-504.

Neoh, C.H., Noor, Z.Z., Mutamim, N.S.A. and Lim, C.K. (2016), "Green technology in wastewater treatment technologies: Integration of membrane bioreactor with various wastewater treatment systems", *Chem. Eng. J.*, **283**, 582-594. https://doi.org/10.1016/j.cej.2015.07.060

Palmarin, M.J. and Young, S. (2016), "Influence of mixed liquor suspended solids on the removal efficiency of a hybrid membrane bioreactor", *Membr. Water Treat.*, **7**(1), 11-22. https://doi.org/10.12989/mwt.2016.7.1.011.

Ramesh, A., Lee, D.J. and Lai, J.Y. (2007), "Membrane biofouling by extracellular polymeric substances or soluble microbial products from membrane bioreactor sludge", *Appl. Microbial Biotechnol.*, **74**(3), 699-707. https://doi.org/10.1007/s00253-006-0706-x.

Seminario, L., Rozas, R., Bórquez, R. and Toledo, P.G. (2002), "Pore blocking and permeability reduction in cross-flow microfiltration", *J. Membr. Sci.*, **209**, 121-142. https://doi.org/10.1016/S0376-7388(02)00285-5.

Shen, L.G., Lei, Q., Chen, J.R., Hong, H.C., He, Y.M. and Lin, H.J. (2015), "Membrane fouling in a submerged membrane bioreactor: Impacts of floc size", *Chem. Eng. J.*, **269**, 328-334. https://doi.org/10.1016/j.cej.2015.02.002.

Sombatsompop, K., Visvanathan, C. and Ben Aim, R. (2006), "Evaluation of biofouling phenomenon in suspended and attached growth membrane bioreactor systems", *Desalination*, **201**(1-3), 138-149. https://doi.org/10.1016/j.desal.2006.02.011.

Teng, J.H., Shen, L.G., Yu, G.Y., Wang, F.Y., Li, F.Q., Zhou, X.L., He, Y.M. and Linn, H.J. (2018) "Mechanism analyses of high specific filtration resistance of gel and roles of gel elasticity related with membrane fouling in a membrane bioreactor", *Bioresour. Technol.*, **257**, 39-46. https://doi.org/10.1016/j.biortech.2018.02.067.

Walter, W.G. (1998), *APHA Standard Methods for the Examination of Water and Wastewater*, Health Laboratory Science, Washington DC, USA.

Wang, P., Yu, Z.S., Qi, R. and Zhang, H.X. (2016), "Detailed comparison of bacterial communities during seasonal sludge bulking in a municipal wastewater treatment plant", *Water Res.*, **105**, 157-166. https://doi.org/10.1016/j.watres.2016.08.050.

Wilén, B.M., Jin, B. and Lant, P. (2003), "The influence of key chemical constituents in activated sludge on surface and flocculating properties", *Water Res.*, **37**(9), 2127-2139. https://doi.org/10.1016/S0043-1354(02)00629-2.

Zhang, C.Q., Wang, G.Z. and Hu, Z.Q. (2014), "Changes in wastewater treatment performance and activated sludge properties of a membrane bioreactor at low temperature operation", *Environ. Sci- Prog. Impacts*, **16**, 2199-2207. https://doi.org/10.1039/C4EM00174E.

Zhang, H.F., Wang, B., Yu, H.H., Zhang, L.H. and Song, L.F. (2015), "Relation between sludge properties and filterability in MBR: Under infinite SRT", *Membr. Water Treat.*, **6**(6), 501-512. https://doi.org/10.12989/mwt.2015.6.6.501.

Zhang, J., Chua, H.C., Zhou, J. and Fane, A.G. (2006), "Factors affecting the membrane performance in submerged membrane bioreactors", *J. Membr. Sci.*, **284**(1-2), 54-66. https://doi.org/10.1016/j.memsci.2006.06.022.

Zhang, M.J., Hong, H.C., Lin, H.J., Shen, L.G., Yu, H.Y., Ma, G.C., Chen, J.R. and Liao, B.Q. (2018), "Mechanistic insights into alginate fouling caused by calcium ions based on terahertz time-domain spectra analyses and DFT calculations", *Water Res.*, **129**, 337-346. https://doi.org/10.1016/j.watres.2017.11.034.

Zhang, M.J., Lin, H.J., Shen, L.G., Liao, B.Q., Wu, X.L. and Li, R.J. (2017), "Effect of calcium ions on fouling properties of alginate solution and its mechanisms", *J. Membr. Sci.*, **525**, 320-329. https://doi.org/10.1016/j.memsci.2016.12.006.

Zhang, W.X., Tang, B. and Bin, L.Y. (2017), "Research Progress in Biofilm-Membrane Bioreactor: A Critical Review", *Ind. Eng. Chem. Res.*, **56**, 6900-6909. https://doi.org/10.1021/acs.iecr.7b00794.

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