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Effect of quorum quenching on the reactor performance, biofouling and biomass characteristics in membrane bioreactors

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ABSTRACT

Enzymatic quorum quenching has recently been shown to be a promising approach to mitigate biofouling in membrane filtration processes. However, its universal effectiveness and mechanisms need further research. In this study, acylase was immobilized into sodium alginate capsules for enzymatic quorum quenching in MBRs operated at typical sludge concentrations (MLSS \approx 10 g/L) for extended period of time. The results showed that quorum quenching influenced sludge characteristics and biofouling, while not impacting pollutant degradation. Better sludge settleability, smaller sludge particle size, less SMP and EPS production, lower apparent viscosity and higher zeta potential of mixed liquor were observed with quorum quenching. Quorum quenching also influenced the characteristics, behavior and function of SMP and EPS, which weakened biofilm formation ability but enhanced membrane filterability.

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1. Introduction

When compared with conventional activated sludge processes, membrane bioreactors (MBRs) have many advantages, such as a smaller footprint, higher volumetric loading, lower sludge production and better effluent quality (Adham et al., 2001; Cote et al., 1998; Meng et al., 2010). As such, MBRs have gained increasing popularity in research and engineering applications in recent years. However, membrane fouling, especially biofouling, decreases membrane permeability and its lifespan. This leads to elevated energy demands, increased physical or chemical cleaning frequency, and increased membrane module replacement rate, which

directly impacts maintenance and operating costs (Drews, 2010; Flemming, 2002).

Biofouling is caused in part by microbial attachment and biofilm growth on the membrane and is now recognized as a bottleneck that limits the development of membrane systems (Xiong and Liu, 2010). To control biofouling, various physical or chemical methods have been investigated and applied, including low-flux operation, periodical backwashing (with air or permeate), intermittent suction, membrane modification, and the addition of coagulant or membrane fouling reducer, etc. (Chang et al., 2002; Lee et al., 2007; Meng et al., 2009; Mishima and Nakajima, 2009). However, these techniques cannot prevent biofilm formation directly since it

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is intrinsically a natural process for bacteria in MBRs to colonize the membrane surface.

Recently, a novel biological paradigm was investigated for biofouling control based on quorum sensing (QS) (Yeon et al., 2009a). QS is bacterial density-dependent cell-to-cell communication using small molecules produced and recognized by microbes. QS has been shown to regulate gene expression mediating some bacterial behaviors such as the production of soluble microbial products (SMP) and extracellular polymeric substances (EPS), exocellular enzyme secretion, and biofilm formation (Hammer and Bassler, 2003; Huang et al., 2008; Tresse, 2009). Accordingly, this discovery opens a new avenue to manage the behaviors of bacteria and control biofouling in membrane systems. Yeon et al. used acylase attached to magnetic carrier to inhibit QS in MBR for advanced wastewater treatment. They showed that this approach, called quorum quenching, reduced biofouling effectively and enhanced the membrane permeability (Yeon et al., 2009b). Since then, this quorum quenching based biofouling control technique has attracted a lot of attention, and it now is viewed as a promising alternative for mitigating membrane biofouling (Choudhary and Schmidt-Dannert, 2010; Kraume and Drews, 2010; Mansouri et al., 2010; Pellegrin et al., 2010; Xiong and Liu, 2010). Xu and Liu (2011) demonstrated that disruption of energy metabolism and subsequent production of QS signaling molecules effectively controlled membrane biofouling. Kim et al. (2011) coupled acylase directly on the nanofiltration (NF) membrane surface for water treatment. They showed that the newly developed membrane with quorum quenching activity could inhibit quorum sensing between microorganisms in the membrane biocake, thereby reducing biofouling. Recently, quorum quenching bacteria were encapsulated into a hollow fiber membrane and biofouling was inhibited successfully (Jahangir et al., 2012; Oh et al., 2012).

However, because quorum sensing regulates a broad spectrum of microbial phenotypes, the characteristics of sludge in the MBR and reactor performance should be examined comprehensively to exclude any potential side effects of quorum quenching on MBR operation (Choudhary and Schmidt-Dannert, 2010). Moreover, there is little information regarding how quorum quenching controls biofouling in MBRs and what will happen if it loses efficacy in the treated MBR.

In this study, acylase immobilized into sodium alginate capsules was used as a bacterial quorum quenching agent in MBR. To verify the applicability of this technology for biofouling control, we ran the reactors at typical activated sludge concentrations (MLSS \approx 10 g/L). The effects of quorum quenching on the membrane bioreactor performance and mixed liquor characteristics were then investigated over a several cycles of MBR operation, significantly extending the results of Yeon et al. (2009b) obtained over short operation time. Considering that bacterial quorum quenching influences SMP and EPS production, which is the dominant fouling agent in membrane filtration processes (Ying et al., 2010), an exhaustive study of the behavior and characteristics of these two groups was further developed to elucidate the origin of biofouling and mechanisms of this innovative biofouling control method.

2. Materials and methods

2.1. Preparation of immobilized acylase and its activity and stability measurements

Porcine kidney acylase I (Sigma, USA), a type of commercial enzyme, was selected for quorum quenching in this study. This enzyme was encapsulated according to the following steps. A volume of 100 mL acylase stock solution (1 mg/mL) was mixed with 300 mL sodium alginate solution (4 wt%), after which the mixture was dropped from a nozzle fed by a peristaltic pump into a calcium chloride solution (2 wt%) with a drop height of 15 cm to form capsules. Next, these spherical capsules with a diameter of 5 mm were maintained in the calcium chloride solution at 4 °C for another 2 h to gel. The capsules were then washed three times with distilled water. To enhance the mechanical strength and stability, capsules were placed into a glutaraldehyde solution (0.25 wt%) for 4 h for cross-linking. Finally, the immobilized acylase capsules were rinsed three times with distilled water, filtered by vacuum suction in a glass funnel, air-dried and kept at 4 °C for further use. Equivalent sodium alginate capsules without acylase were also obtained for the comparison experiments.

The activity and stability of immobilized acylase were measured according to the methods described by Yeon et al. (2009b), with two minor modifications. We used Tris–HCl buffer (pH = 6.8) containing 10 mM CaCl₂ instead of phosphate buffer, after which CoCl₂ at a final concentration of 0.1 mM was added into N-acetyl-L-methionine substrate solution to activate the acylase.

2.2. MBR set up and operation

Two identical lab-scale submerged membrane bioreactors with working volumes of 3 L were set up. A PVC hollow fiber membrane module (pore size 0.01 μ m) with a total surface area of 0.07 m² (Litree, China) was placed in every bioreactor and the constant flux was set to 12 L/m² h (LMH). Air was supplied continuously through a perforated pipe under the membrane module at a flow rate of 0.3 m³/h. The influent was fed with a peristaltic pump controlled by a level sensor in the MBR. Filtration was carried out in an intermittent suction mode with 9 min of suction followed by a 3 min release. Hydraulic retention time (HRT) and solids retention time (SRT) were set to 4.8 h and 50 days, respectively. Mixed liquor suspended solids (MLSS) was adjusted to 10 g/L in each MBR at the beginning of the experiment. For quorum quenching, 180 g (approximately 150 mL) of immobilized acylase beads were added to the Quorum Quenching MBR (QQ MBR). For comparison, 180 g of sodium alginate beads without the enzyme were added to the control MBR. To maintain the same acylase content, the wet-weight of beads remaining in the reactors was checked every 48 h, and additional beads were added if needed. When the trans-membrane pressure (TMP) increased to 40 kPa, the membrane modules were removed for physical and chemical cleaning (1% NaClO + 2% NaOH immersion for 50 min) prior to the next run. To compare the effect of quorum quenching on MBR performance and biofouling, after the control MBR was fouled for the second

time (after 113 h of operation), we screened out the sodium alginate capsules from both reactors, cleaned both membrane modules and ran the system for another two cycles lasting 100 h.

The inoculating sludge was drawn from the return activated sludge stream in the Quyang wastewater treatment plant (Shanghai, China). Floating debris was screened out before the inoculation. The synthetic wastewater was composed of 500 mg/L glucose, 25 g/L yeast extract, 25 mg/L bactopectone, 250 mg/L $(\text{NH}_4)_2\text{SO}_4$, 150 mg/L $\text{K}_2\text{H}_2\text{PO}_4$, 150 mg/L KH_2PO_4 , 4.5 mg/L MgSO_4 , 0.2 mg/L FeCl_3 , 35 mg/L NaCl , 1 mg/L CaCl_2 , 2.4 mg/L CoCl_2 and 75 mg/L NaHCO_3 .

2.3. Analytical methods

2.3.1. Extraction of EPS

A mixed liquor sample was centrifuged at 6000g for 5 min, after which the supernatant was filtered through a Millipore filter (0.45 μm). The filtrate content represented soluble microbial products (SMP). Extracellular polymeric substances (EPS) in the mixed liquor were divided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). They were extracted according to a modified thermal extraction method (Zhang et al., 2009). Biocake on the membrane was removed by physical cleaning and subsequent ultrasonic cleaning (SK3300-35 KHz, KUDOS, China) for 10 min. The total EPS in the solution was then directly extracted according to the tightly bound EPS extraction method, after which the content was normalized as mg EPS/m² membrane surface.

2.3.2. Filtration resistance measurements

Filtration resistance can be calculated from the Darcy's law as following:

$$R = \frac{\Delta P}{J\mu} \quad (1)$$

where R is the filtration resistance (m^{-1}), ΔP is the trans-membrane pressure (Pa), J is the permeate flux ($\text{m}^3/\text{m}^2 \text{ s}$), μ is the permeate viscosity (Pa s).

We divided the total filtration resistance into four categories expressed as

$$R = R_m + R_p + R_c + R_i \quad (2)$$

where R_m is the intrinsic resistance of the clean membrane (m^{-1}), R_p is the resistances due to concentration polarization (m^{-1}), R_c is the cake layer resistance (m^{-1}), and R_i is the resistance due to pore-blocking (m^{-1}). To compare the effect of quorum quenching on the distribution of fouling among these categories immediately after second fouling of the control MBR the flux and TMP were measured to yield total filtration resistance (R) from Eq. (1). The activated sludge was then replaced with pure water to estimate the concentration polarization resistance (R_p). After removing the cake layer attached on the membrane surface by physical cleaning and ultrasonic cleaning for 10 min, the flux and TMP were measured again using pure water to obtain R_c . At last, the pressure of a new membrane module was subtracted from the physically cleaned membrane to calculate R_i (Guo et al., 2008).

2.3.3. Relative hydrophobicity (RH) determination

A volume of 50 mL of total EPS was extracted as previously described. An equivalent volume of *n*-hexane was added and agitated uniformly in a separation funnel for 20 min on a shaker. After standing for 30 min, the two phases were separated and the aqueous phase was withdrawn. Content of proteins and polysaccharides in the aqueous phase were determined before and after the extraction. The RH was expressed as

$$\text{RH} = (1 - (C_e/C_i))100\% \quad (3)$$

where C_e is the concentration of proteins or polysaccharides in the water phase after extraction, C_i is the original concentration of proteins or polysaccharides (Arabi and Nakhla, 2008).

2.3.4. EPS characteristics analysis

Proteins and polysaccharides were determined using a modified version of the Lowry method and the phenol–sulfuric acid method, respectively. Molecular weight distribution of SMP and EPS was analyzed using a gel filtration chromatography analyzer (LC-10ADVP, Shimadzu, Japan). A TSK G4000SW type gel column (TOSOH Corporation, Japan) was maintained at 40 °C with Millipore water mobile phase at a flow rate of 0.5 mL/min. The apparent viscosity and particle size distribution were measured at 25 °C using a rotational viscometer (NDJ-1, Shanghai Ande, China) and a particle size analyzer (Accysuzer780, PSS, USA), respectively. SMP samples were used for zeta-potential determination (Zetasizer Nano-Z, Malvern, England) to represent the surface potential of the sludge flocs.

The three-dimensional excitation–emission matrix (EEM) fluorescence spectra were measured using a fluorescence spectrophotometer (Cary Eclipse, Varian Inc., USA). In the test, the fluorescence spectra were recorded using 5 nm slits for both excitation and emission ends with a scanning speed of 1200 nm/min, and the EEM spectra were collected with the scanning emission spectra from 250 nm to 600 nm at 2 nm intervals by varying the excitation wavelengths from 200 nm to 500 nm at 10 nm steps. The contour maps of EEM were plotted using the Origin 8 program.

2.3.5. Other item analysis

Particle size distribution was carried out by a focused beam reflectance measurement (Eyetechnology particle size and shape analyzer, Ankersmid, Holland). Total nitrogen (TN) was monitored using a TOC analyzer (Shimadzu, TOC V-CPN, Japan). For AHL extraction, sludge samples (50 mL) were centrifuged at 1000g for 5 min, after which the supernatant was passed through a 0.45 μm filter membrane. The filtrate was then extracted twice with equivalent volumes of acetic ether (chromatographic grade). The organic phase was evaporated in rotary evaporators at 35 °C (RV 10 basic plus V, IKA, Germany), redissolved in 100 μL methanol–water mixture (1:1), filtered through a 0.45 μm syringe filter and analyzed by ultra-performance liquid chromatography with mass detection (UPLC-MS) (Liu et al., 2009). Bioassay of the QS activity was conducted in the same way as Yeon et al.'s (2009a). Mixed liquor suspended solids (MLSS), sludge volume index (SVI), ammonia nitrogen and chemical oxygen demand (COD) were measured according to the Standard Methods (Chinese-EPA, 2002).

3. Results and discussion

3.1. Comparison of overall reactor performance

The activity and stability of immobilized acylase were measured before dosing it to the reactors. When compared with free enzyme (2 mg/L, 50 mL), which was completely inactivated after four days, the immobilized acylase capsules maintained 66% of its initial activity at the end of the continuous shaking experiment (168 h). Similarly, immobilized acylase still had 62% residual activity after 12 cycles (120 h) in an iterative test. Meanwhile, control sodium alginate capsules (without acylase) showed no significant reduction of N-acetyl-L-methionine. These observations suggest that although part of enzyme was leaking during the examinations, immobilized acylase still exhibited good biocatalytic efficiency and stability.

Considering that quorum quenching affects gene transcription and controls microbial physiology to some extent, we monitored pollutant removal efficiency in both MBRs based on the concentrations in influent and permeate (Table 1). Both reactors exhibited excellent performance, with COD and ammonia removal efficiencies above 95%. Total nitrogen removal (TN) was slightly below 50% most likely due to simultaneous nitrification–denitrification. There was no significant difference between operational performances of both MBRs throughout the entire experimental period. Also, QQ MBR showed similar pollutants removals with quorum quenching (days 1–7) and without (days 8–11). Overall, it can be concluded that quorum quenching had no adverse effects on conventional pollutant removal in the MBRs in this study. However, a longer-term impact of QQ on the MBR performance may need to be studied for the reliability consideration.

The rate of TMP build-up is an important factor in evaluating membrane filterability in MBR systems because it directly reflects the extent of membrane fouling. The TMP variation in Fig. 1 shows that quorum quenching can also mitigate membrane fouling successfully at typical sludge concentrations ($10,000 \pm 1050$ mg/L). In the first two cycles, the TMP in the control MBR increased rapidly, but there was only a small TMP increase in the QQ MBR. Fouling rate ($dTMP/dt$) of QQ MBR was 0.075 kPa/h, which is only 12% of that of control MBR (0.611 kPa/h). It is worth noting that both the control MBR and QQ MBR initially displayed similar trends. The TMP increased slowly and gradually for about 20–30 h, but in the

control MBR it increased abruptly after that time following a common two-step pattern (Le-Clech et al., 2006). The initial increase in TMP was likely due to passive adsorption of colloids and organics, which enables microbes to grow on the membrane surface and in the pores. This growth without quorum quenching, causes formation of a biocake or biofilm and resulted in a further increase in TMP in the second phase of the first and second cycles (Hwang et al., 2008; Sombatsompop et al., 2006). In the third and fourth cycle, when sodium alginate capsules were removed and quorum quenching was not active, the membrane in the QQ MBR was fouled quickly, with no distinct difference from the control MBRs in the TMP evolution. This pattern conclusively demonstrates that quorum quenching could alleviate membrane fouling. The effects of quorum quenching are reversible when it loses its efficacy.

To illustrate the relationship between the QS and TMP change in greater details, we investigated the types and concentrations of QS autoinducers (AHLs) in the MBRs. UPLC-MS analysis revealed that several types of AHLs were present in mixed liquors at the beginning of the MBR operation, with N-octanoyl-homoserine lactone (C8-HSL) having the highest concentration (Fig. S1 in supplementary material). At the end of the second cycle fouled membranes were cut for bioassay of the QS activity. The fouled membrane in the control MBR displayed a darker blue color than the membrane from the QQ MBR. This difference qualitatively showed that the concentrations of QS autoinducers of membrane fouling were much higher in the control MBR (Fig. S2 in supplementary material), which is consistent with Yeon et al.'s (2009a) research. These findings explain that no significant TMP increase occurred in the QQ MBR because quorum quenching prevented microbial attachment and biofilm formation (biofouling).

3.2. Comparison of filtration resistances

The fractions of filtration resistance were measured at the end of the second cycle and the results are displayed in Table 2. It is obvious that two MBRs had different distributions of filtration resistances. In the control MBR, the total resistance (R) and cake layer resistance (R_c) were 3 and 13 times larger than that in the QQ MBR, which means the control reactor suffered from severe biofouling. Also, both R_p and R_i in the control MBR increased around 2 times because of the cake layer formation. According to the theory of Hoek and Elimelech, the cake layer on the membrane hinders back-transport of ions and particles

Table 1 – Comparison of pollutant removal at different times.

Pollutants	Reactor	Time					
		1d (%)	3d (%)	5d (%)	7d (%)	9d (%)	11d (%)
COD	QQ MBR	96.2	98.1	98.2	98.1	98.1	98.1
	Control MBR	97.0	97.0	98.3	98.2	98.1	98.0
Ammonia	QQ MBR	99.3	98.5	99.1	99.4	98.7	99.5
	Control MBR	96.7	98.3	99.8	99.4	98.5	98.8
TN	QQ MBR	46.6	46.5	49.7	47.6	49.0	49.6
	Control MBR	48.0	51.3	53.0	47.9	49.3	51.3

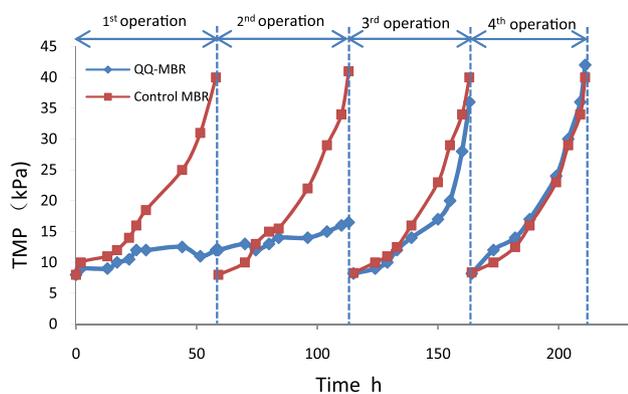


Fig. 1 – Comparison of TMP build-up of QQ MBR and Control MBR.

Table 2 – Comparison of hydraulic resistances between two MBRs.

	R	R _m	R _p	R _c	R _i
QQ MBR (10 ¹² m ⁻³)	4.17	0.73	2.41	0.53	0.51
Percent of R	100%	17.4%	57.7%	12.7%	12.2%
Control MBR (10 ¹² m ⁻³)	17.12	0.73	7.11	7.39	1.89
Percent of R	100%	4.2%	41.5%	43.2%	11.0%

and also alters hydrodynamics. These mechanisms lead to formation of an enhanced concentration polarization layer in reverse osmosis (RO) and NF (Hoek and Elimelech, 2003). Since the origin of concentration polarization in MBR is the same as in RO and NF systems, that is why more membrane foulants (biocake) induced higher concentration polarization in the control MBR. However, our research revealed that mixed liquor viscosity was higher in the control MBR (see below) and contributed also to concentration polarization (Zhao and Zou, 2011). As a result, is difficult to judge the extent to which biocake affected the concentration polarization layer. With a gradual formation of the cake layer and corresponding increase of pressure, more pores were blocked in the membrane which resulted in a larger R_i in the control MBR.

3.3. Comparison of sludge (mixed liquor) characteristics

Sludge characteristics are associated with MBR filterability as well as the removal of pollutants. Quorum sensing, which is

known to regulate bacterial behavior and metabolism, may have an effect on sludge characteristics. To investigate the influence of quorum quenching on sludge characteristics, we measured the sludge volume index (SVI), apparent viscosity, zeta potential and particle size distribution. One batch of samples were collected at the end of the second cycle, when quorum quenching was applied; the other were collected at the end of the fourth cycle, when quorum quenching was idled for 2 cycles. As shown in Table 3, the sludge in the QQ MBR had a somewhat lower SVI, apparent viscosity, and mean particle size, but a higher zeta potential when quorum quenching was working. Meanwhile, after quorum quenching was inactive for 2 cycles, these parameters of QQ MBR became closer to that of control MBR.

In this study, better sludge settleability, lower apparent viscosity and higher zeta potential indicated enhanced filterability, which is consistent with the results of other studies (Li et al., 2008; Meng et al., 2006; Schrader et al., 2005), although the differences between these parameters were not large. Conversely, the effect of quorum quenching on particle size distribution was obvious. Specifically, the mean particle size in the QQ MBR was 163 μm, which was much smaller than that of the control MBR (263 μm). These findings indicate that quorum sensing not only prevented biofilm formation on the membrane, but also induced disaggregation of sludge flocs in the bulk liquid. Despite the view that membrane fouling is mainly the result of deposition of small particles and colloids onto the membrane surface and that membrane fouling resistance increases when sludge particle size decreases (Meng et al., 2006), there was no evidence to corroborate this in the present study.

3.4. Effect of quorum quenching on SMP and EPS characteristics

3.4.1. SMP and EPS content in MBR and on membrane surface
 Extracellular polymeric substances (EPS) and their soluble counterpart, soluble microbial products (SMP), are a complex mixture of polysaccharides, proteins, humic acids, nucleic acids and possibly other compounds. EPS forms a highly hydrated gel matrix, while SMP may form a gel on the membrane surface (Reid et al., 2008). These compounds not only impact the physical chemistry of the mixed liquor, but also provide nutrients and a habitat for microbes on the membrane surface. Considering the important role of these compounds in biofouling, we divided EPS into SMP, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) and then investigated the predominant fouling elements in each. Fig. 2

Table 3 – Comparison of mixed liquor characteristics.

	At the end of 2nd operation		At the end of 4th operation	
	QQ MBR	Control MBR	QQ MBR	Control MBR
SVI (mL/g)	79.35 ± 0.44	87.01 ± 0.75	85.12 ± 1.41	90.00 ± 0.67
Apparent viscosity (mPa s)	13.35 ± 0.21	15.60 ± 0.28	14.70 ± 0.42	15.20 ± 0.28
Zeta potential (mV)	-15.10 ± 0.28	-18.35 ± 0.35	-17.90 ± 0.57	-18.20 ± 0.42
Mean particle size (μm)	163.22	263.60	-	-

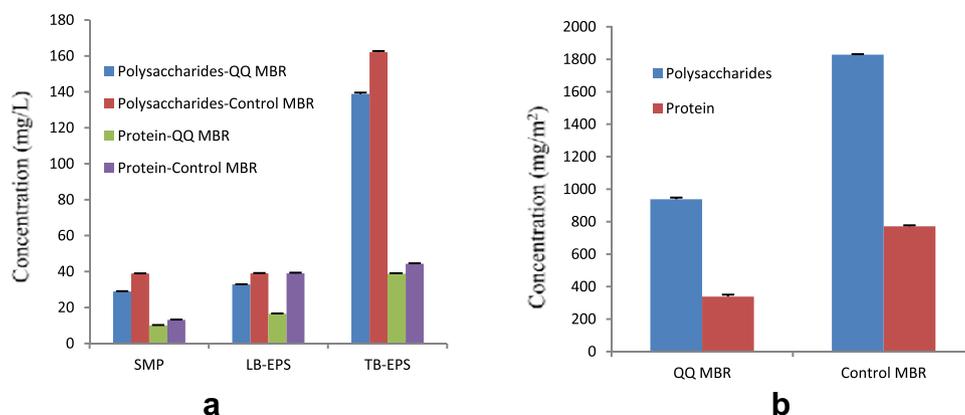


Fig. 2 – EPS content in two MBRs. (a) mixed liquor; (b) foulant on the membrane (biocake).

shows the content of polysaccharide and protein in the mixed liquor and biocake of the two reactors, which sampled at the end of the second cycle.

The results revealed that quorum quenching reduced SMP and EPS production, especially the secretion of proteins (30% less of total EPS than control MBR), and decreased around 50% of EPS accumulation on the membrane surface at the same time. QS was confirmed to be one of the regulatory pathways for EPS production and biofilm formation in microbes (Hooshangi and Bentley, 2008; Marketon et al., 2003; Ruiz et al., 2008; Vu et al., 2009). A recent study showed that quorum sensing played a significant role in the formation of aerobic granules in a sequencing batch reactor, and that these granules produced more galactose than conventional flocs (Liu et al., 2010). Quorum sensing autoinducers (AHLs) secreted from the granules were reported to induce gene expression of bacteria in suspension, causing them to undergo attached growth, which led to the formation of granules and a stable structure (Ren et al., 2010). Based on these findings, it is likely that the reduction of SMP/EPS in both the mixed liquor and membrane biocake is one of the main reasons quorum quenching reduces biofouling.

3.4.2. Relative hydrophobicity (RH)

Relative hydrophobicity (RH), which is another important characteristic of sludge, affects microbial adhesion and may impact membrane fouling. We evaluated RH of polysaccharides and proteins in the mixed liquor and of the biocake on the membrane surface (Fig. 3). Relative hydrophobicities of the polysaccharides and proteins in the QQ MBR were much lower than those in the control MBR, not only in the mixed liquor, but also on the membrane surface. Additionally, the RH values of all components in the biocakes were notably higher than in the mixed liquor for both MBRs. Consistent with these results, quorum quenching has been shown to reduce the microbial hydrophobicity in other studies (Limsuwan and Voravuthikunchai, 2008; Marketon et al., 2003; Nithya et al., 2010; Tielen et al., 2010). It should be mentioned that although quorum quenching decreased RH of polysaccharides more compared to protein in the mixed liquor, the same trend was not observed for the biocake (foulants), which originated from the mixed liquor. Lee et al. (2001) found that

protein in EPS had a strong positive influence on the hydrophobicity of microbial flocs, while carbohydrates had no appreciable influence. Also, proteins were much more hydrophobic than polysaccharides in this study. Therefore, increased hydrophobicity of protein is more likely to enhance attachment of flocs on the membrane surface than polysaccharides. It is well known that hydrophobic microbial flocs increase the membrane fouling rate (Le-Clech et al., 2006); thus, the reduction of hydrophobicity due to quorum quenching could also have a positive impact on membrane fouling.

3.4.3. Excitation–emission matrix (EEM) fluorescence spectra

In this study, three-dimensional EEM fluorescence spectroscopy was applied to compare the composition of SMP and EPS and membrane foulants between the control and QQ MBRs. As shown in Fig. 4, the spectra from two MBRs are quite different. Five main peaks appeared in the SMP samples. The first main peak was located at the excitation/emission wavelengths (Ex/Em) of 280/320 nm (Peak A), while the second main peak was observed at the Ex/Em of 240/352 nm (Peak B). The two peaks have been reported as aromatic protein-like substances (Peak A) and tryptophan protein-like substances (Peak B) (Chen et al., 2003; Wang et al., 2009). Peak C, located at

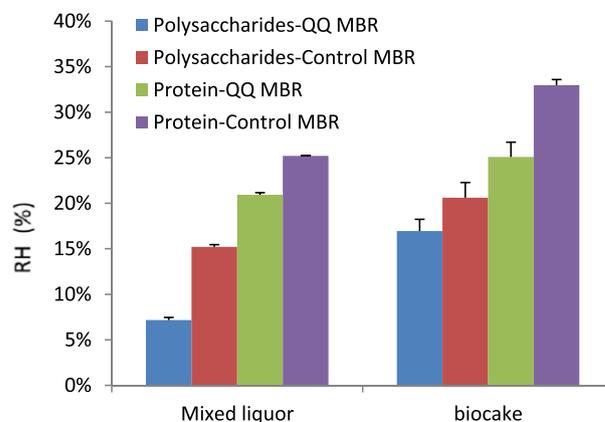


Fig. 3 – Comparison of relative hydrophobicity (RH) of EPS in mixed liquor and biocake (foulants).

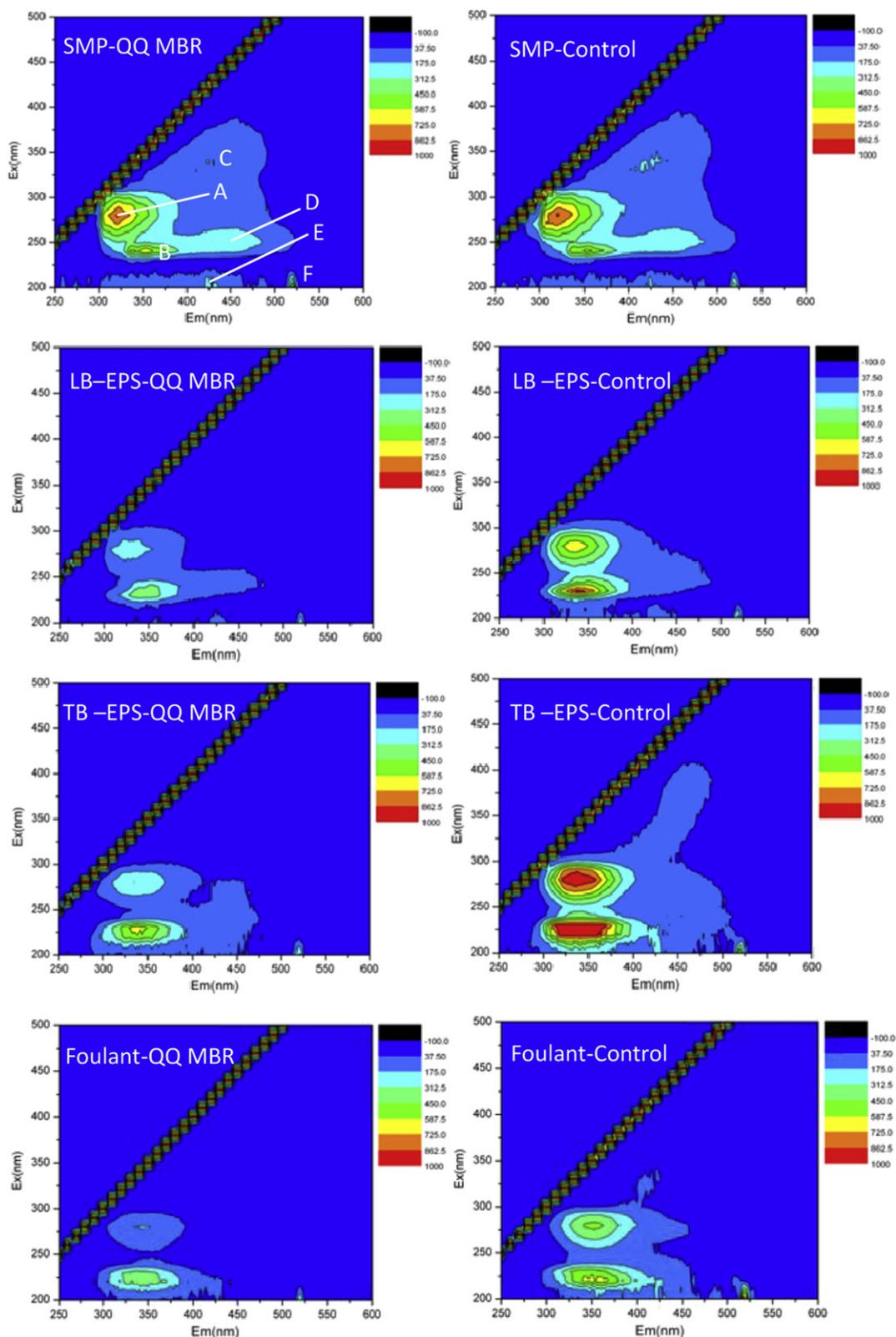


Fig. 4 – EEM fluorescence spectra of SMP, LB-EPS, TB-EPS and membrane foulants.

the Ex/Em of 340/426 nm, were reported to be the hydrophobic acid fractions (Chen et al., 2003). It is interesting to note that the intensity of Peak C in QQ MBR was 10% lower compared to the control MBR, suggesting that it contained less hydrophobic

acid materials and this provides further support for quorum quenching's ability to enhance hydrophilicity of SMP and EPS. The other three peaks (Peaks D–F) were recognized as fulvic acid-like materials (Chen et al., 2003). Peak C disappeared in

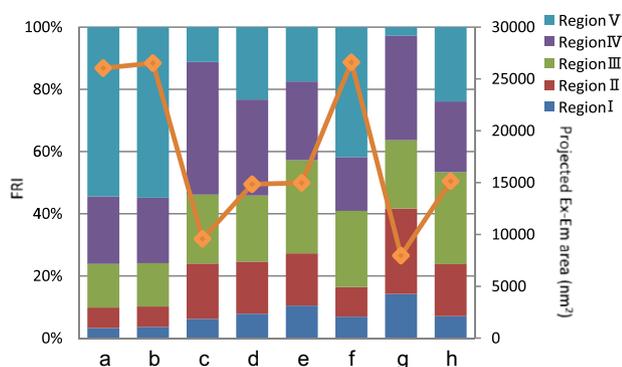


Fig. 5 – FRI distribution of: (a) SMP-QQ MBR; (b) SMP-Control MBR; (c) LB-EPS-QQ MBR; (d) LB-EPS-Control MBR; (e) TB-EPS-QQ MBR; (f) TB-EPS-Control MBR; (g) Total EPS-QQ MBR; (h) Total EPS-Control MBR.

LB-EPS samples from two MBRs. With respect to the spectra of TB-EPS and foulants on the membrane, control MBR exhibited a larger fluorescence field and a peak located in Region V, which is related to humic acid-like substances (Chen et al., 2003).

Fluorescence regional integration (FRI) and fractional projected excitation–emission area were applied to analyze the EEM spectra quantitatively (Chen et al., 2003) (Fig. 5). Except for SMP, the other samples displayed significantly different FRI distributions between control and QQ MBRs. The biggest difference is the FRI ratio of Region V (humic acid-like substances), which was much smaller in samples from the QQ MBR. As quorum quenching promoted deflocculation of sludge flocs into small pieces, more humic acid-like substances were desorbed and appeared in the bulk liquid. Because humic-acid compounds have a high membrane fouling potential (Chuang et al., 2009; Wu and Lee, 2011), the control MBR with a higher concentration of humic acid-like substances suffered from more severe fouling. It should be noted that the total projected excitation–emission area of the control MBR was much bigger than that of QQ MBR (except for

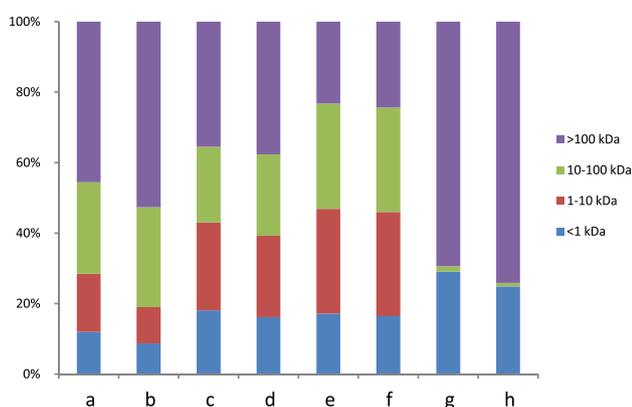


Fig. 6 – SMP/EPS molecular weight (MW) distribution of: (a) SMP-QQ MBR; (b) SMP-Control MBR; (c) LB-EPS-QQ MBR; (d) LB-EPS-Control MBR; (e) TB-EPS-QQ MBR; (f) TB-EPS-Control MBR; (g) Foulant-QQ MBR; (h) Foulant-Control MBR.

Table 4 – Comparison of molecular weight distributions of foulants.

	Mn	Mw	Mw/Mn
QQ MBR (kDa)	8.6	33197.7	3860.2
Control MBR (kDa)	11.8	1954.8	165.7

the SMP sample). Based on this, although FRI of Regions I & II (protein-like substances) and region IV (soluble microbial by-product like material) in the control MBR is smaller than that of the QQ MBR, the actual integration area of these foulants in control MBR was still 10–50% larger, which is consistent with conclusion that more EPS and foulants were produced in the control MBR.

3.4.4. Molecular weight (MW) distribution

Since considerable SMP and EPS concentrations were present in the QQ MBR, but biofouling and TMP increase was minimal, we analyzed another important EPS parameter, molecular weight (MW) distribution, in the mixed liquor and on the membrane surface. As shown in Fig. 6, SMP and LB-EPS in the mixed liquor primarily consisted of high MW (>100 kDa) compounds in both reactors, and these macromolecules were retained on the membrane surface. In addition, the percentage of high MW (>100 kDa) compounds in SMP, LB-EPS, TB-EPS and membrane foulants in the control MBR was higher than in the QQ MBR. Since there is no such tendency for low MW (<10 kDa) compounds, it is likely that the high MW compounds induced membrane biofouling more. It is interesting to notice that SMP samples contained more high MW compounds than LB/TB-EPS in both MBRs, and that these high MW molecules accumulated on the membrane. These findings are consistent with the observations of Arabi and Nakhla (2008), who found that increased retention of high MW EPS on membrane resulted in higher pore-blocking resistance and increased fouling.

To understand the mechanism of MW distributions on membrane fouling better, number-average molecular weight (Mn), weight-average molecular weight (Mw) and the coefficient of MW distribution (Mw/Mn) of the samples were used and are summarized in Table 4. Foulants in QQ MBR had lower Mn, but 10 times larger Mw and Mw/Mn, which indicates a much wider distribution of MWs. It seemed that part of macromolecules on membrane of control MBR disappeared, which induced a much smaller Mw than QQ MBR. It is possible that degradation by bacteria deposited on the membrane (biofouling) resulted in this effect (Okamura et al., 2010), since more biomass was membrane attached in the control reactor. Foulants from QQ MBR contained more low MW molecules, which increased the possibility for bulk liquid permeating through the biocake and membrane pores (Zhu et al., 2011). Therefore, QQ MBR showed better filterability.

4. Conclusions

In summary, we demonstrated the effectiveness of quorum quenching for biofouling control in MBRs under typical sludge concentrations (MLSS \approx 10 g/L). Quorum quenching had no

apparent side effects on effluent quality but led to slightly increased zeta potential of mixed liquor, increased settleability, decreased production of polysaccharides and proteins, as well as reduced apparent viscosity and relative sludge hydrophobicity. Excitation–Emission Matrix (EEM) fluorescence and molecular weight (MW) distribution analysis indicated that quorum quenching could change the composition of SMP and EPS of sludge and biocake on the membrane. All of these factors likely led to the inhibition of biofilm formation and the enhanced membrane permeability that was observed compared with the control reactor. Moreover, the reduction in biofouling by quorum quenching was found to be reversible, and the subsequent membrane performance was not (positively or negatively) affected when quorum quenching ceased.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2012.09.050>.

REFERENCES

- Adham, S., Gagliardo, P., Boulos, L., Oppenheimer, J., Trussell, R., 2001. Feasibility of the membrane bioreactor process for water reclamation. *Water Science and Technology* 43 (10), 203–209.
- Arabi, S., Nakhla, G., 2008. Impact of protein/carbohydrate ratio in the feed wastewater on the membrane fouling in membrane bioreactors. *Journal of Membrane Science* 324 (1–2), 142–150.
- Chang, I.S., Le Clech, P., Jefferson, B., Judd, S., 2002. Membrane fouling in membrane bioreactors for wastewater treatment. *Journal of Environmental Engineering-ASCE* 128 (11), 1018–1029.
- Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science & Technology* 37 (24), 5701–5710.
- Chinese-EPA, 2002. *Water and Wastewater Monitoring Methods*, fourth ed. Chinese Environmental Science Publishing House, Beijing, China.
- Choudhary, S., Schmidt-Dannert, C., 2010. Applications of quorum sensing in biotechnology. *Applied Microbiology and Biotechnology* 86 (5), 1267–1279.
- Chuang, S.H., Chang, W.C., Chang, M.C., Sung, M.A., 2009. The effects of soluble organic matters on membrane fouling index. *Bioresource Technology* 100 (5), 1875–1877.
- Cote, P., Buisson, H., Praderie, M., 1998. Immersed membranes activated sludge process applied to the treatment of municipal wastewater. *Water Science and Technology* 38 (4–5), 437–442.
- Drews, A., 2010. Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures. *Journal of Membrane Science* 363 (1–2), 1–28.
- Flemming, H.C., 2002. Biofouling in water systems – cases, causes and countermeasures. *Applied Microbiology and Biotechnology* 59 (6), 629–640.
- Guo, J.F., Xia, S.Q., Wang, R.C., Zhao, J.F., 2008. Study on membrane fouling of submerged membrane bioreactor in treating bathing wastewater. *Journal of Environmental Sciences (China)* 20 (10), 1158–1167.
- Hammer, B.K., Bassler, B.L., 2003. Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Molecular Microbiology* 50 (1), 101–114.
- Hoek, E.M.V., Elimelech, M., 2003. Cake-enhanced concentration polarization: a new fouling mechanism for salt-rejecting membranes. *Environmental Science & Technology* 37 (24), 5581–5588.
- Hooshangi, S., Bentley, W.E., 2008. From unicellular properties to multicellular behavior: bacteria quorum sensing circuitry and applications. *Current Opinion in Biotechnology* 19 (6), 550–555.
- Huang, Y.L., Ki, J.S., Case, R.J., Qian, P.Y., 2008. Diversity and acyl-homoserine lactone production among subtidal biofilm-forming bacteria. *Aquatic Microbial Ecology* 52 (2), 185–193.
- Hwang, B.K., Lee, W.N., Yeon, K.M., Park, P.K., Lee, C.H., Chang, I.S., Drews, A., Kraume, M., 2008. Correlating TMP increases with microbial characteristics in the bio-cake on the membrane surface in a membrane bioreactor. *Environmental Science & Technology* 42 (11), 3963–3968.
- Jahangir, D., Oh, H.S., Kim, S.R., Park, P.K., Lee, C.H., Lee, J.K., 2012. Specific location of encapsulated quorum quenching bacteria for biofouling control in an external submerged membrane bioreactor. *Journal of Membrane Science* 411, 130–136.
- Kim, J.H., Choi, D.C., Yeon, K.M., Kim, S.R., Lee, C.H., 2011. Enzyme-immobilized nanofiltration membrane to mitigate biofouling based on quorum quenching. *Environmental Science & Technology* 45 (4), 1601–1607.
- Kraume, M., Drews, A., 2010. Membrane bioreactors in waste water treatment – status and trends. *Chemical Engineering & Technology* 33 (8), 1251–1259.
- Le-Clech, P., Chen, V., Fane, T.A.G., 2006. Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science* 284 (1–2), 17–53.
- Lee, J., Ahn, W.Y., Lee, C.H., 2001. Comparison of the filtration characteristics between attached and suspended growth microorganisms in submerged membrane bioreactor. *Water Research* 35 (10), 2435–2445.
- Lee, W.N., Chang, I.S., Hwang, B.K., Park, P.K., Lee, C.H., Huang, X., 2007. Changes in biofilm-architecture with addition of membrane fouling reducer in a membrane bioreactor. *Process Biochemistry* 42 (4), 655–661.
- Li, H.F., Yang, F.L., Li, Y.Z., Wong, F.S., Chua, H.C., 2008. Impact of biological constituents and properties of activated sludge on membrane fouling in a novel submerged membrane bioreactor. *Desalination* 225 (1–3), 356–365.
- Limsuwan, S., Voravuthikunchai, S.P., 2008. *Boesenbergia pandurata* (Roxb.) Schltr., *Eleutheria americana* Merr. and *Rhodomyrtus tomentosa* (Aiton) Hassk. as antibiofilm producing and anti-quorum sensing in *Streptococcus pyogenes*. *Fems Immunology and Medical Microbiology* 53 (3), 429–436.
- Liu, H.X., Hu, Y., He, Y.Q., Liu, Y., Li, W., Yang, L., 2009. Ultra-performance liquid chromatographic-electrospray mass spectrometric determination (UPLC-ESI-MS) of O-demethylated metabolite of paeonol in vitro: assay development, human liver microsome activities and species differences. *Talanta* 79 (5), 1433–1440.

- Liu, J.R., Nguyen, D., Paice, M., 2010. Aerobic granule formation in a sequencing batch reactor treating newsprint effluent under low phosphate conditions. *Water Science and Technology* 62 (11), 2571–2578.
- Mansouri, J., Harrisson, S., Chen, V., 2010. Strategies for controlling biofouling in membrane filtration systems: challenges and opportunities. *Journal of Materials Chemistry* 20 (22), 4567–4586.
- Marketon, M.M., Glenn, S.A., Eberhard, A., Gonzalez, J.E., 2003. Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti*. *Journal of Bacteriology* 185 (1), 325–331.
- Meng, F.G., Chae, S.R., Drews, A., Kraume, M., Shin, H.S., Yang, F.L., 2009. Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. *Water Research* 43 (6), 1489–1512.
- Meng, F.G., Liao, B.Q., Liang, S.A., Yang, F.L., Zhang, H.M., Song, L.F., 2010. Morphological visualization, componential characterization and microbiological identification of membrane fouling in membrane bioreactors (MBRs). *Journal of Membrane Science* 361 (1–2), 1–14.
- Meng, F.G., Zhang, H.M., Yang, F.L., Zhang, S.T., Li, Y.S., Zhang, X.W., 2006. Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors. *Separation and Purification Technology* 51 (1), 95–103.
- Mishima, I., Nakajima, J., 2009. Control of membrane fouling in membrane bioreactor process by coagulant addition. *Water Science and Technology* 59 (7), 1255–1262.
- Nithya, C., Begum, M.F., Pandian, S.K., 2010. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of *Pseudomonas aeruginosa* PAO1. *Applied Microbiology and Biotechnology* 88 (1), 341–358.
- Oh, H.S., Yeon, K.M., Yang, C.S., Kim, S.R., Lee, C.H., Park, S.Y., Han, J.Y., Lee, J.K., 2012. Control of membrane biofouling in MBR for wastewater treatment by quorum quenching bacteria encapsulated in microporous membrane. *Environmental Science & Technology* 46 (9), 4877–4884.
- Okamura, D., Mori, Y., Hashimoto, T., Hori, K., 2010. Effects of microbial degradation of biofoulants on microfiltration membrane performance in a membrane bioreactor. *Environmental Science & Technology* 44 (22), 8644–8648.
- Pellegrin, M.L., Wilf, M., Aguinado, J., Greiner, A.D., Zhang, K., Gluck, S., Min, K., McCandless, R., Burbano, M.S., Diamond, J., 2010. Membrane processes. *Water Environment Research* 82 (10), 1159–1234.
- Reid, E., Liu, X.R., Judd, S.J., 2008. Sludge characteristics and membrane fouling in full-scale submerged membrane bioreactors. *Desalination* 219 (1–3), 240–249.
- Ren, T.T., Yu, H.Q., Li, X.Y., 2010. The quorum-sensing effect of aerobic granules on bacterial adhesion, biofilm formation, and sludge granulation. *Applied Microbiology and Biotechnology* 88 (3), 789–797.
- Ruiz, L.M., Valenzuela, S., Castro, M., Gonzalez, A., Frezza, M., Souleire, L., Rohwerder, T., Queneau, Y., Doutheau, A., Sand, W., Jerez, C.A., Guiliani, N., 2008. AHL communication is a widespread phenomenon in biomining bacteria and seems to be involved in mineral-adhesion efficiency. *Hydrometallurgy* 94 (1–4), 133–137.
- Schrader, G.A., Zwijnenburg, A., Wessling, M., 2005. The effect of WWTP effluent zeta-potential on direct nanofiltration performance. *Journal of Membrane Science* 266 (1–2), 80–93.
- Sombatsompop, K., Visvanathan, C., Ben Aim, R., 2006. Evaluation of biofouling phenomenon in suspended and attached growth membrane bioreactor systems. *Desalination* 201 (1–3), 138–149.
- Tielen, P., Rosenau, F., Wilhelm, S., Jaeger, K.E., Flemming, H.C., Wingender, J., 2010. Extracellular enzymes affect biofilm formation of mucoid *Pseudomonas aeruginosa*. *Microbiology* 156, 2239–2252.
- Tresse, O., 2009. Biofilms and quorum sensing regulation. *FEBS Journal* 276, 73.
- Vu, B., Chen, M., Crawford, R.J., Ivanova, E.P., 2009. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules* 14 (7), 2535–2554.
- Wang, Z.W., Wu, Z.C., Tang, S.J., 2009. Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy. *Water Research* 43 (6), 1533–1540.
- Wu, S.C., Lee, C.M., 2011. Correlation between fouling propensity of soluble extracellular polymeric substances and sludge metabolic activity altered by different starvation conditions. *Bioresource Technology* 102 (9), 5375–5380.
- Xiong, Y.H., Liu, Y., 2010. Biological control of microbial attachment: a promising alternative for mitigating membrane biofouling. *Applied Microbiology and Biotechnology* 86 (3), 825–837.
- Xu, H.J., Liu, Y., 2011. Control and cleaning of membrane biofouling by energy uncoupling and cellular communication. *Environmental Science & Technology* 45 (2), 595–601.
- Yeon, K.M., Cheong, W.S., Oh, H.S., Lee, W.N., Hwang, B.K., Lee, C.H., Beyenal, H., Lewandowski, Z., 2009a. Quorum sensing: a new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment. *Environmental Science & Technology* 43 (2), 380–385.
- Yeon, K.M., Lee, C.H., Kim, J., 2009b. Magnetic enzyme carrier for effective biofouling control in the membrane bioreactor based on enzymatic quorum quenching. *Environmental Science & Technology* 43 (19), 7403–7409.
- Ying, W., Yang, F., Bick, A., Oron, G., Herzberg, M., 2010. Extracellular polymeric substances (EPS) in a hybrid growth membrane bioreactor (HG-MBR): viscoelastic and adherence characteristics. *Environmental Science & Technology* 44 (22), 8636–8643.
- Zhang, H.M., Xia, J., Yang, Y., Wang, Z.X., Yang, F.L., 2009. Mechanism of calcium mitigating membrane fouling in submerged membrane bioreactors. *Journal of Environmental Sciences (China)* 21 (8), 1066–1073.
- Zhao, S.A.F., Zou, L.D., 2011. Relating solution physicochemical properties to internal concentration polarization in forward osmosis. *Journal of Membrane Science* 379 (1–2), 459–467.
- Zhu, X.F., Wang, Z.W., Wu, Z.C., 2011. Characterization of membrane foulants in a full-scale membrane bioreactor for supermarket wastewater treatment. *Process Biochemistry* 46 (4), 1001–1009.