

Study on biofouling mechanism in IMBR for wastewater treatment with different temperatures

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Keywords: quorum sensing, extracellular polymeric substances, biofouling, integrated membrane bioreactor, trans-membrane pressure, soluble microbial products.

Abstract: The main goal of the present study was to examine the operating characteristics and mechanisms of membrane fouling in integrated membrane bioreactors (IMBRs) at different temperatures. Two IMBRs, each with identical dimensions and configurations, were used in the study using synthetic domestic sewage at a low temperature (10°C) and high temperature (25°C). The results indicated that the removal efficiency of chemical oxygen demand reached 93–96%, but the membrane contribution rate of IMBR2 (10°C) was higher than that of IMBR1 (25°C). The separation burden of the membrane on organic compounds increased at low temperature, which may have sped up the rate of membrane biofouling. The absolute rate of trans-membrane pressure build-up was faster at low temperature, leading to shorter IMBR operating times. Soluble microbial products (SMPs) and extracellular polymeric substances (EPSs) in the IMBRs significantly increased at low temperature. These substances intensified deflocculation, with an accompanying reduction of floc size and the release of EPSs at low temperature, which facilitated the formation of cake foulants on the surface, covering the entire membrane area. The protein and polysaccharide concentrations of SMPs and EPSs in the IMBRs were correlated with the concentration of C8-HSL. It was demonstrated that temperature affected the concentration of C8-HSL, which controlled the excretion of EPSs and SMPs and thus the membrane biofouling process.

Introduction

Water deficiency and environmental pollution are two major issues confronting some regions of the world due to the limited and uneven distribution of global water resources in conjunction with growing populations (Neslihan et al. 2016, Yun et al. 2016). Wastewater reclamation is greatly effective for overcoming water scarcity problems caused by industrial and agricultural pollution, urban consumer pollution, and soil and water losses. The sustainable use of water resources provides fundamental security for sustainable development and ecosystem balance (Bunani et al. 2013, Haaken et al. 2014). Membrane bioreactors (MBRs) combine biological methods with membrane separation technology. Because of membrane interception, MBR systems offer excellent effluent quality, high volumetric load, and low food/microorganisms, and are therefore becoming increasingly utilised for municipal and industrial wastewater treatment (Sridang et al. 2006, Cyplik et al. 2007, Khan et al. 2011). Today, MBRs are a promising technology to realise wastewater reuse and will play a crucial role in cities with scarce water resources.

However, membrane fouling is a consistent problem in MBRs and can lead to escalating operating and maintenance expenses. An important parameter in the MBR process is the

trans-membrane pressure (TMP), which affects the flux of the entire system. The formation of fouling layers on membrane surfaces results in increased TMP and decreased permeate flux. Low temperatures cause significant decreases in microbial activity and thus a slow degradation rate of pollutants, in turn exacerbating membrane fouling. Wastewater cannot always be maintained at optimal temperature conditions due to the seasonality of wastewater temperature. Temperature variation is the main cause of the deterioration of effluent quality and overall MBR system instability (Brink et al. 2011). Biofouling refers to the irreversible changes of the membrane surface or membrane pore adsorption and deposition characteristics caused by physical and chemical interactions or mechanical interactions of colloidal particles and solute macromolecules during membrane filtration. Biofouling is an intrinsic and natural biological process that is affected by temperature (Lee et al. 2018). Low temperatures affect bacterial activity and sludge flocculation. In MBRs, soluble microbial products (SMPs) and extracellular polymeric substances (EPSs) are the main causes of membrane fouling that are related to microbial activity (Shi et al. 2018, Gao et al. 2013). Studies of advanced molecular biology techniques have demonstrated that the characteristics of biofilms, such as porosity and biological volume, are closely related to membrane fouling in MBRs (Hong et al. 2007, Yu et

al. 2019). In particular, Davies first demonstrated that bacterial quorum sensing mechanisms are involved in the differentiation of *Pseudomonas aeruginosa* biofilm formation (Davies et al. 1998). Quorum sensing (QS) refers to bacterial cell-to-cell communications via signal molecules such as acyl homoserine lactone, which detects their concentration and then triggers a series of group behaviours associated with biofilm formation (Jiang et al. 2013, Li et al. 2017). By monitoring quorum sensing, biofilm growth can be predicted and membrane pollution can be prevented in advance. Despite its importance to membrane biofouling, little information is available concerning the effect of temperature on quorum sensing and the relationship between quorum sensing and EPSs and SMPs at different temperatures.

In the present study, two identical integrated membrane bioreactors (IMBRs) of exactly the same size were operated at low temperature (10°C) and high temperature (25°C) to identify the mechanisms of membrane fouling in MBRs as a function of temperature. During reactor operations, the following parameters were analysed to investigate the causes of biofouling at both temperatures: chemical oxygen demand (COD), TMP, SMPs, and EPSs. In addition, to analyse the effect of QS in IMBRs, N-octanoyl-DL-homoserine lactone

(C8-HSL), N-hexanoyl-DL-homoserine lactone (C6-HSL), and N-tetradecanoyl-DL-homoserine lactone (C14-HSL) were also measured. One objective of this study was to demonstrate the presence of quorum sensing in the IMBR and to analyse the correlation between QS activity and membrane biofouling.

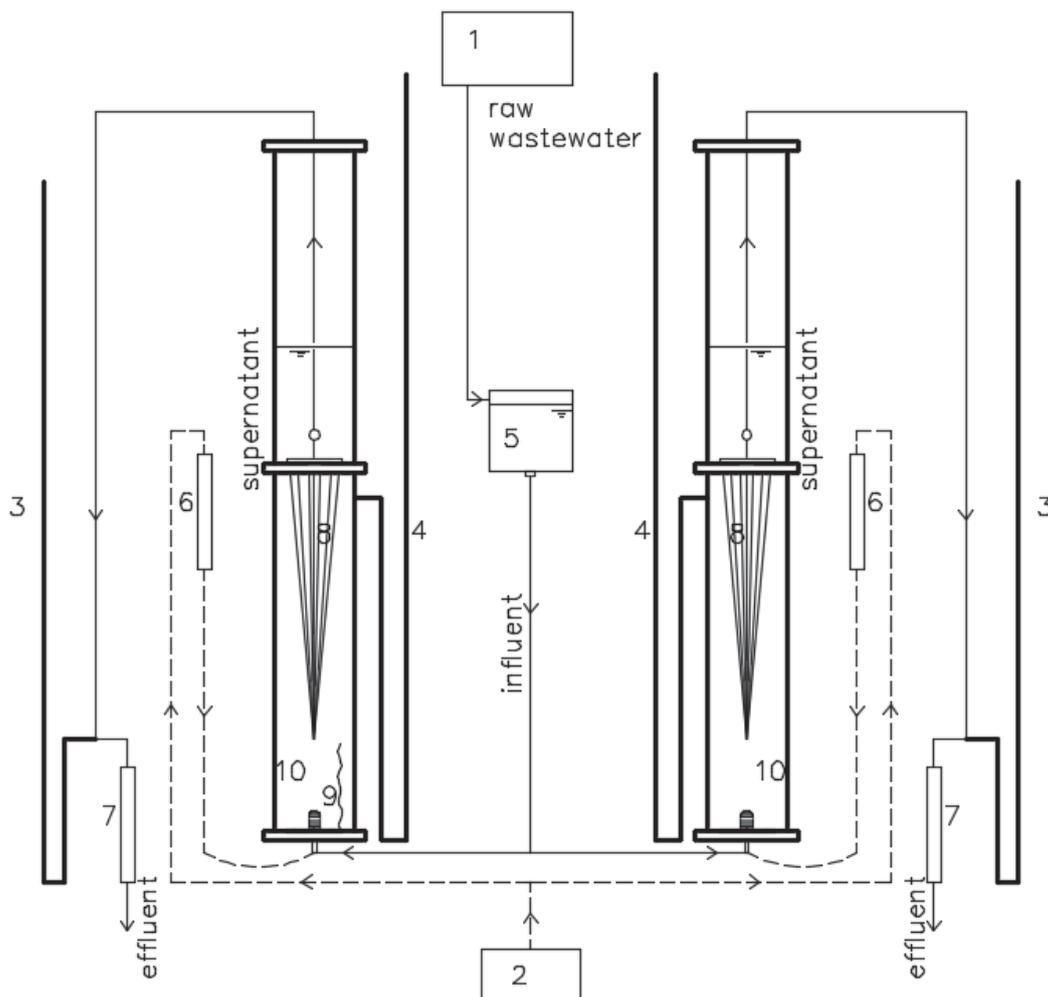
Methods

Experimental installation and operating conditions

To expedite the testing process, two identical reactors were constructed for the present study. Figure 1 presents a schematic diagram of the IMBR. The reactor was composed of plexiglass, with a diameter of 16 cm, column height of 100 cm, and an effective volume of 13 L. A hollow fibre membrane module with a filtration area of 1.0 m² and a nominal pore size of 0.2 µm was made of polyvinylidene fluoride.

Raw water flowed from the inlet tank to the balanced tank by gravity, and the level of the liquid in the reactor was controlled by the balanced water tank. The water outlet was located below the horizontal position of the water inlet to maintain running of the system without power.

The experiment was conducted in winter, and the ambient temperature of the laboratory ranged from 8 to 10°C. IMBR2



1 – inlet tank, 2 – aeration pump, 3 – piezometric tube, 4 – total piezometric tube, 5 – balanced water tank, 6 – gas flowmeter pipe, 7 – fluid flowmeter, 8 – membrane module 9 – heating bar, 10 – bioreactor

Fig. 1. Schematic diagram of the experimental set-up IMBR1 and IMBR2

did not require a heating device. IMBR1 was operated using a heating bar with the temperature controlled by a thermostat ($25 \pm 0.5^\circ\text{C}$). The reactors were seeded with return-activated sludge obtained from a local municipal sewage treatment plant. The sludge was first sieved (0.3 mm) to remove any debris and large particles and was then introduced into the two reactors. The initial sludge concentration of the two reactors was 6 g/L. To reduce membrane fouling, membrane flux was set at a relatively low value of 4.65 L/(m² h), corresponding to a hydraulic retention time of 2.8 h and an air-water ratio of 20:1.

Wastewater characteristics

To maintain the stability of the raw water quality and to facilitate mechanistic analysis, this study used artificial water to simulate domestic sewage. The feed used in this study was synthesised using tap water and chemicals to simulate domestic wastewater. The source water was prepared in tap water by dissolving 400 mg/L industrial glucose, 50 mg/L urea, 0.8 mg/L CaCl₂, 1 mg/L MgSO₄, and 15 mg/L KH₂PO₄. The synthetic wastewater was supplemented with a number of nutrients and trace elements to provide a balanced feed to the reactors. The synthetic wastewater included very small amounts of Fe₂(SO₄)₃, CuCl₂, CoCl₂, and PtCl₃ supplements. Values of the average COD in synthetic wastewater were 350–380 mg/L. The COD:N:P ratio in synthetic wastewater was approximately 100:7:1.

Analytical methods

Water quality analysis was conducted in the laboratory immediately following collection of the water sample. COD values of raw water, supernatant liquor, and membrane effluent were measured using potassium dichromate (S.E.P.A. 2002). TMP, one of the most important indicators of membrane fouling in the MBR process, was measured manually using piezometric tubes. The date of TMP was recorded to visualise the changes in pressure across the membrane over time.

SMPs samples were obtained by centrifuging the mixed liquor (50 ml) at 4000 rpm for 10 min and then filtering the supernatant through a 0.45 μm filter (Lee et al. 2018). EPSs

samples were collected and extracted from the biomass following methods described elsewhere (Weerasekara et al. 2014). The biomass pellet collected by centrifugation was resuspended in 25 ml of 0.8% NaCl solution and prepared at 80°C in a water bath for 30 min. Subsequently, the solution was centrifuged again at 4000 rpm for 20 min, and the supernatant was filtered through a syringe filter (0.45 μm). The concentrations of polysaccharides and proteins in the SMPs and EPSs samples were measured using the phenol-sulphuric acid and modified Lowry methods, respectively (Yeon et al. 2009).

C8-HSL, C6-HSL, and C14-HSL were measured using ultra-performance liquid chromatography tandem mass spectrometry (Yu et al. 2016). Dissolved fractions were obtained by centrifuging the sludge mixed liquor at 4000 rpm and filtering the supernatant through a 0.45 μm filter. Solid phase extraction was used to concentrate C8-HSL, C6-HSL, and C14-HSL of the particle-free samples.

Results and Discussion

Effects of temperature on COD removal

The average removal efficiencies of COD by the IMBRs at low and high temperature are presented in Figure 2. The characteristics of the influent were similar in the two reactors during the research period, with a mean COD value of 350–380 mg/L. The effluent COD concentrations of IMBR1 and IMBR2 were lower than 20 mg/L, and the COD removal efficiency was approximately 93–96%. The results indicated that the average removal efficiency of COD by IMBR did not significantly differ between the low and high temperature IMBRs (Fig. 2).

The main advantage of IMBR is the excellent effluent quality due to the efficient interception performance of the membrane. The difference between the supernatant COD and effluent COD reflects the organic matter intercepted by the membrane. Membrane interception of organic matter occurs mainly in the supernatant of undecomposed organic substances contained in raw wastewater and microbial metabolites (Xia et al. 2000). The organic matter interception by the membrane

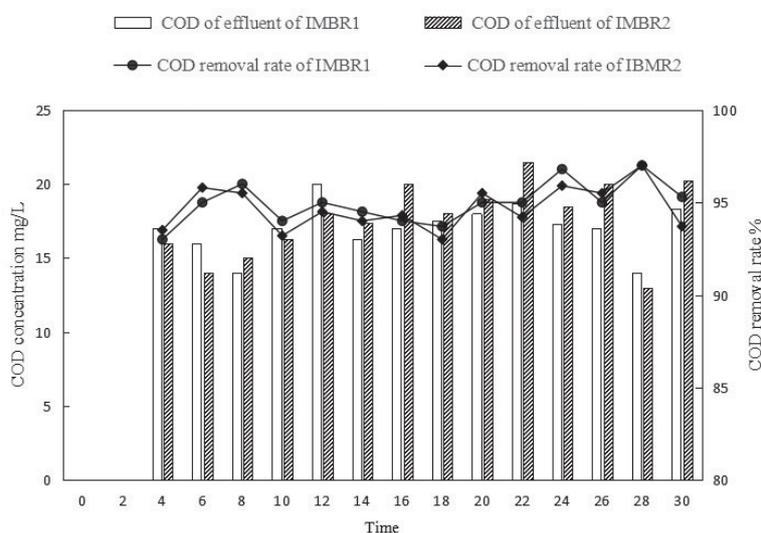


Fig. 2. Removal efficiency of COD in IMBR1 and IMBR2

was not zero under the two temperature conditions, and the difference between the supernatant and effluent COD reached 34.6 mg/L in IMBR2 (Fig. 3). The removal of pollutants by IMBR can be divided into two phases, biological removal and membrane removal. Membrane removal strongly affects the removal of pollutants, including screening via the membrane and the sorption of the deposited layer formed on the membrane surface. Biological removal is strongly affected by the operating temperature. Microbial activity declines as operating temperature decreases, and the ability of microorganisms to degrade the material is reduced. However, the total removal rates of COD in IMBR1 and IMBR2 did not significantly differ between the two temperatures (Fig. 2). By contrast, the difference between the supernatant COD and effluent COD of IMBR2 (10°C) was higher than that of IMBR1 (25°C) (Fig. 3). The contribution of membrane interception to pollutant removal under low-temperature regulation was greater than that at high temperature. Therefore, the separation burden of the membrane on organic compounds increased, which may have sped up the rate of membrane biofouling at low temperature.

The synthetic raw wastewater used in this study contained glucose, which is typically easily decomposed. Therefore, the difference between supernatant COD and effluent COD may be attributed to the metabolites produced by microbial biological reactions. The difference between supernatant COD and effluent COD increased and later tended to decrease in both IMBR1 and IMBR2 (Fig. 3). When the rate of biodegradation of metabolites was greater than its accumulation, the amount of metabolites in the supernatant declined. During the initial stage of the reaction, metabolites produced by microbial biological reactions were not effectively degraded and instead accumulated in the IMBR supernatant, leading to an increase in membrane fouling. As the reaction progressed, the difference of COD in the supernatant and effluent decreased, indicating that the role of biodegradation increased. The experimental analysis demonstrated that as the reaction progressed, the activated sludge was cultured and acclimated to improve the biodegradation efficiency of metabolites, and the dissolved accumulated metabolites in the IMBR could be biodegraded. However, this process required a long adaptive period.

To reflect the distribution of COD removal between biological and membrane interception in the IMBRs, two concepts of the biological contribution rate (P_S) and membrane contribution rate (P_M) were used:

$$P_S = (C_0 - C_{sup}) / (C_0 - C_e) \quad (1)$$

$$P_M = (C_{sup} - C_e) / (C_0 - C_e) \quad (2)$$

where:

C_{sup} represents the supernatant COD (mg/L),

C_0 represents raw water COD (mg/L),

C_e represents effluent COD (mg/L).

The microorganisms in the IMBRs played a major role in the removal of organic matter under the two temperature conditions, with an average contribution rate of over 92% (Fig. 4). Although the contribution of membrane interception to COD removal was relatively small, significant differences were observed between the two temperature conditions. The membrane contribution rate of IMBR2 (10°C) was higher than that of IMBR1 (25°C), because the formation and accumulation of SMPs was facilitated at low temperature (Fig. 6). The level of microorganism degradation decreased at low temperature, resulting in an increase in the separation rate of solutes at the membrane, enhancing the effect of screening via the membrane. The removal efficiency of COD in the effluent of the IMBRs at low and high temperature did not significantly differ (Fig. 2). However, the degradation of SMPs by microbes increased the burden of membrane separation of organisms, which caused severe membrane fouling under low temperature conditions (Ma et al. 2012, Brink et al. 2011).

Effects of temperature on TMP

The TMP of IMBR1 (25°C) only began to increase after 12 days, gradually reaching 25 kPa after 20 days (Fig. 5). The TMP of IMBR2 (10°C) began to rise to alarming levels after 10 days and was higher than 30 kPa at 14 days (Fig. 5). Initially, the TMP of IMBR2 (10°C) was slightly higher than that of IMBR1 (25°C), but then rapidly increased even further.

One of the main causes of the high resistance of membrane filtration at low temperature is increased liquid viscosity at low temperatures (Brink et al. 2011). IMBR2 (10°C) exhibited

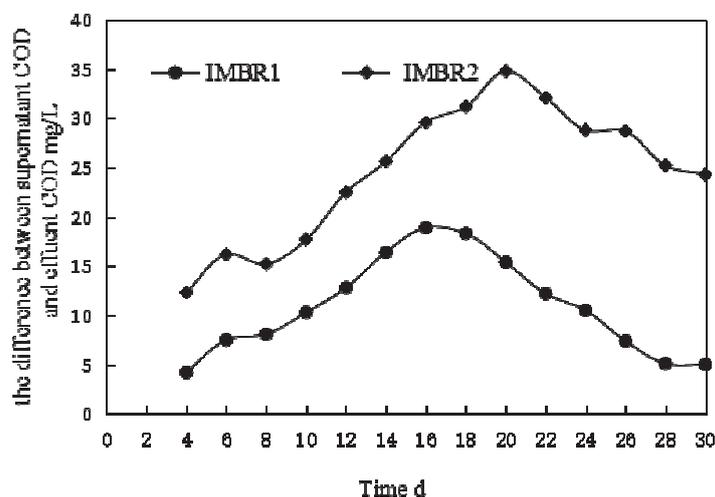


Fig. 3. The difference between supernatant COD and effluent COD

much higher values and higher rates of TMP increase compared to IMBR1 (25°C) over the entire experimental period (Fig. 5). It suggested that the operating temperature clearly and strongly affected TMP. When the IMBR was operated at low temperature, sludge viscosity increased, which directly blocked the membrane pores in the IMBR. This blockage intensified deflocculation, with an accompanying reduction of floc size and the release of EPSs at low temperature (Figs. 6 and 7), which facilitated the formation of filter cakes on the surface covering the entire membrane area.

On the other hand, the mass transfer rate decreased at low temperature, as the Brownian diffusion coefficient is linear to absolute temperature. Consequently, the particle back-transport velocity was reduced, causing severe membrane fouling at low temperature in the IMBR (Jiang et al. 2005). Concentration polarisation was one main cause of membrane fouling. At the higher temperature, the material migration coefficient was larger and the concentration polarisation was lower (Chang et al. 2001), thus relieving the formation of gel polarity on the membrane surface.

Effects of temperature on SMPs and EPSs

The concentrations of SMPs and EPSs were monitored throughout the experiment, as these substances are important factors affecting membrane fouling in IMBRs (Silva et al. 2017,

Massé et al. 2006). The concentration of SMPs in IMBR1 was significantly lower than that in IMBR2, with the same reaction time (Fig. 6). These results indicate that more macromolecular substances were produced by microbial metabolism and autolysis in IMBR2 at a low operating temperature. Similar to the total concentration of SMPs, protein and polysaccharide concentrations in SMP samples at low temperature were significantly higher than at high temperature. The protein concentration of SMP samples in the reactor was significantly higher than the polysaccharide concentration. SMPs were produced during endogenous respiration and matrix catabolism (i.e., biomass-associated products and substrate utilisation-associated products, respectively) (Shin and Kang 2003). The existence of SMPs in the supernatant of the biological treatment has been confirmed in many studies, and the main component of SMPs is recalcitrant macromolecular organic matter (Liang et al. 2007). The membrane plays an important role in retaining SMPs, which cause the membrane fouling serious at low temperature.

EPSs extracted from the two IMBRs at different temperatures exhibited the same pattern as SMPs (Fig. 7). EPS (proteins and polysaccharide) levels in IMBR2 (10°C) were significantly higher than values in IMBR1 (25°C). EPSs, also known as the gel matrix, are the main component of the biofilm (Yeon et al. 2009). Consequently, the biofouling may have worsened due to greater EPS production at the lower

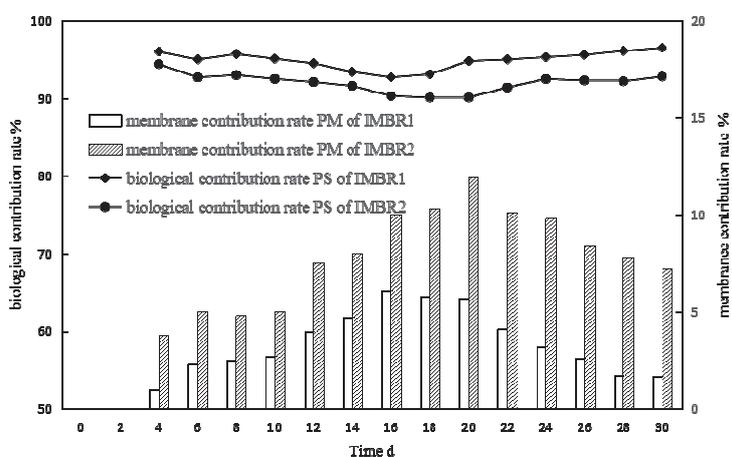


Fig. 4. Membrane and biological contribution rate of IMBR1 and IMBR2

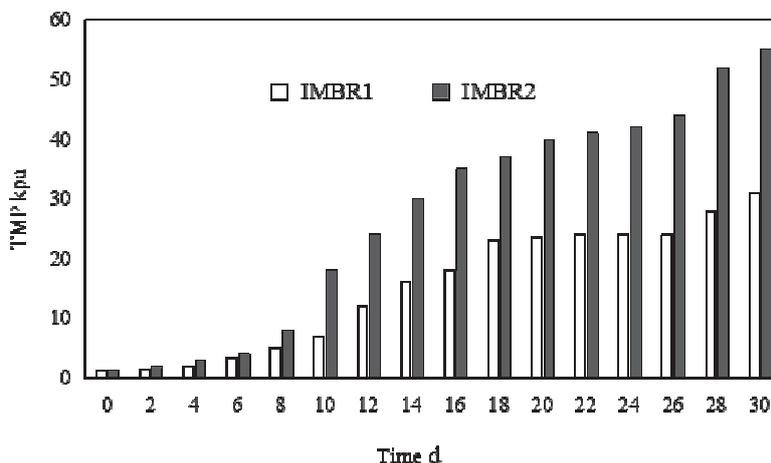


Fig. 5. Changes of TMP of IMBR1 and IMBR2

temperature. These results were consistent with previous investigations. For example, Ma et al. reported that membrane fouling was triggered by higher EPS and SMP concentrations at lower temperatures (Ma et al. 2013). A recent study found that biofouling at 12°C was more severe than that at 25°C due to the higher EPS concentration, when membrane bioreactors were cultivated with synthetic wastewater containing glucose, yeast extract and bacto peptone (Nahm et al. 2019).

The synthetic wastewater composition not only determines the total yield of EPS, but also influences the composition of EPS, namely the relative proportion of proteins and polysaccharide. In this experiment, glucose was the only organic carbon source of synthetic wastewater. It was found that EPS was dominantly composed of proteins even if proteins were absent in the feed (Fig. 7). These results were in agreement with literature studies (Ayse et al. 2016). On the other hand, Ayse found that the protein to polysaccharide ratio in EPS was clearly higher in the feed containing proteins (Ayse et al. 2016).

Effects of temperature on quorum sensing

Quorum sensing is the most important information system for microorganisms. Moreover, quorum sensing is the primary

control of the release of EPSs, which in turn affects the formation of bacterial micelles and the generation of biofilms in sewage treatment (Nahm et al. 2019). To further confirm the relationship between quorum sensing and biofouling at different temperatures in the IMBRs, C8-HSL, C6-HSL, and C14-HSL were extracted and tested.

During operation, only C6-HSL and C8-HSL were detected in the IMBRs. C14-HSL was not detected in the reactor, indicating that this substance was degraded during the reaction process and its concentration was below the lower limit of detection. Quorum sensing bacteria (secretory signal molecules) and quorum sensing quenching bacteria (degradation signal molecules) are present in a large number of wastewater treatment bioreactors. The interaction between the two types of bacteria ultimately determines the concentration of signalling molecules in the reactor (Song et al. 2014). The results indicated that C14-HSL was sterilised and degraded by quorum sensing quenching bacteria in the IMBR.

The concentration of C6-HSL at 10°C and 25°C ranged from 16.3–22.3 ng/L and 22.1–25.7 ng/L, respectively (Fig. 8). C6-HSL concentrations did not significantly differ at low and high temperatures. By contrast, C8-HSL concentrations in

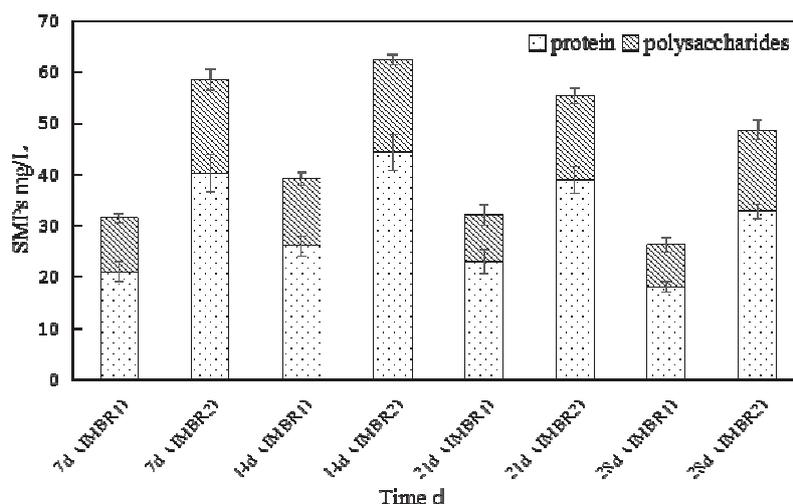


Fig. 6. SMPs in IMBR1 and IMBR2 under different temperatures

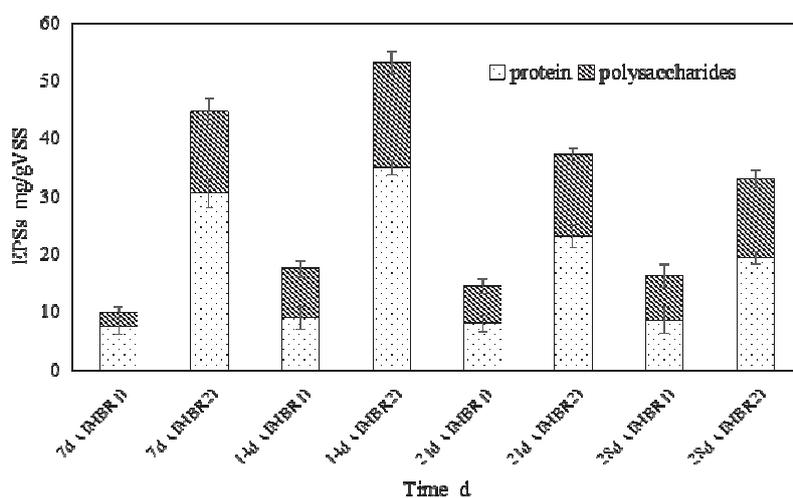


Fig. 7. EPSs in IMBR1 and IMBR2 under different temperatures

IMBR1 (range: 21.3–28.4 ng/L) were significantly lower than those in IMBR2 (38.2–42.1 ng/L), indicating that variation in operating temperature affected C8-HSL concentrations. This finding may be due to corresponding levels of secretion and degradation of C8-HSL by activated sludge. Among the different signal molecules, C8-HSL was selected as a model quorum sensing signal molecule, as it was predominant in the IMBRs (Yeon et al. 2009).

Figure 9 presented the correlation between SMPs and C8-HSL in the reactors using regression analyses. Protein and polysaccharide levels in SMPs were clearly correlated with the concentration of C8-HSL, with R^2 values of 0.81 and 0.73, respectively. The intensity of microbe quorum sensing in the reactor affected the secretion of SMPs and in turn membrane contamination.

Previous studies have demonstrated that EPS secretion in membrane bioreactors is influenced and regulated by microbe quorum sensing in the reactor (Koutsoudis et al. 2006). Protein and polysaccharide levels in EPSs were also correlated with the concentration of C8-HSL, with R^2 values of 0.81 and 0.91, respectively (Fig. 10). According to the analysis in the previous section, the concentration of EPSs in the reactor directly affected membrane biofouling. By analysing the correlations among C8-HSL, EPSs, and SMPs at different temperatures, we demonstrated that temperature affected the concentration of C8-HSL in MBRs, which

controlled the secretion of EPSs and SMPs and was closely related to membrane biofouling.

Conclusions

The main objectives of this study were to examine the performance of IMBRs for treating synthetic wastewater at low (10°C) and high temperature (25°C). The characteristics of membrane fouling at different temperatures were investigated. The following conclusions are based on our experimental data. The effluent COD concentrations in both IMBR1 and IMBR2 were lower than 20 mg/L, and COD removal efficiency was approximately 93–96%. The contribution of membrane interception to COD removal at low temperature was significantly higher than at high temperature. The separation burden of the membrane on organic compounds that have not been spread increased at low temperature, what may have sped up the rate of membrane biofouling.

SMP and EPS (protein and polysaccharide) levels increased at low temperature, leading to a decline in permeate flux and increased TMP. The membrane played an important role in retaining SMPs and EPSs, and biofouling was more likely to occur at low temperature. During the operation, only C6-HSL and C8-HSL were detected in the IMBRs. The concentration of C8-HSL varied significantly in the IMBRs under different temperatures. Protein and polysaccharide levels in SMP and

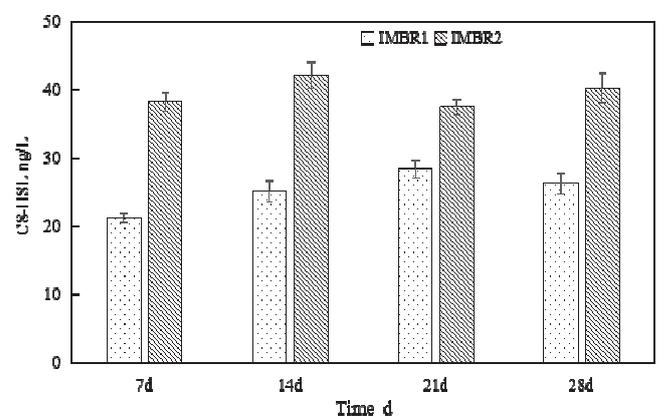
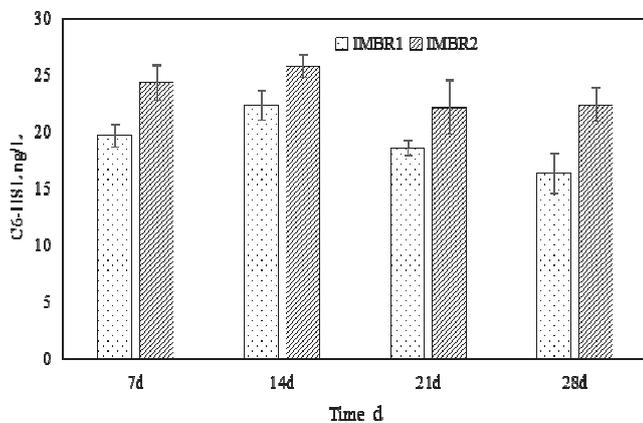


Fig. 8. Concentrations of C6-HSL and C8-HSL in IMBR1 and IMBR2

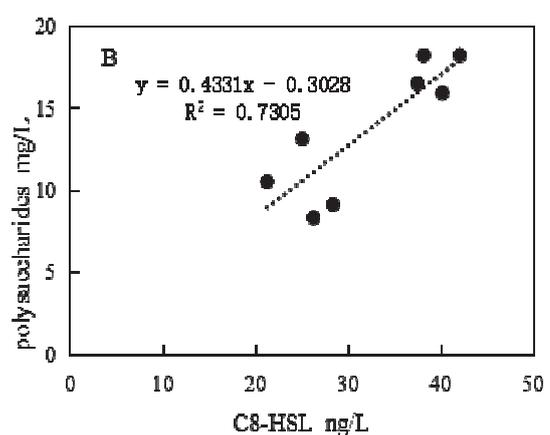
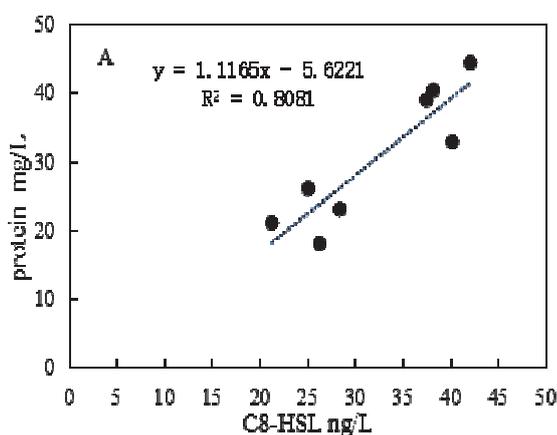


Fig. 9. Correlation analysis between SMPs (protein and polysaccharides) and C8-HSL

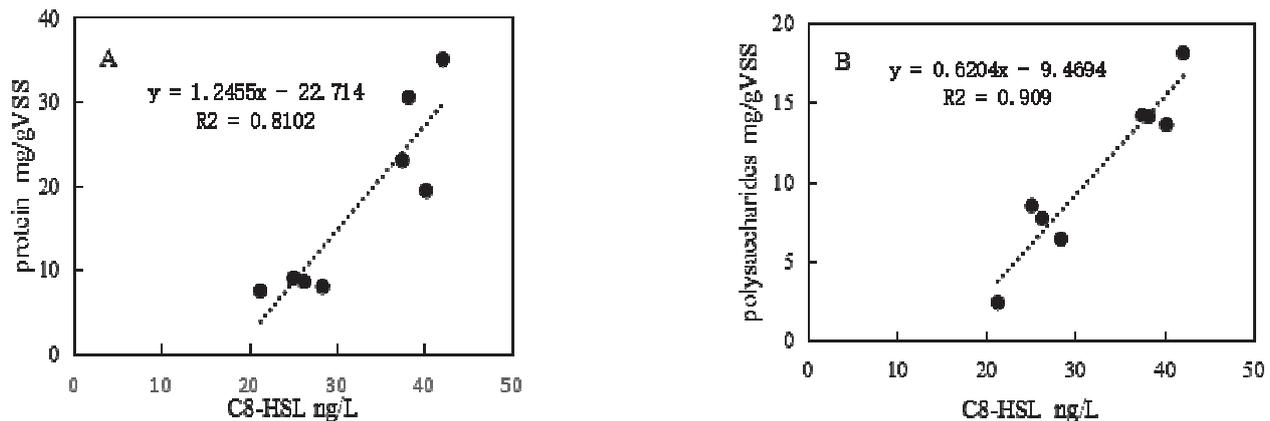


Fig. 10. Correlation analysis between EPSs (protein and polysaccharides) and C8-HSL

EPS samples were strongly correlated with the concentration of C8-HSL. Temperature affected the concentration of C8-HSL in MBRs, which in turn controlled the secretion of EPSs and SMPs and was closely related to membrane biofouling.

Acknowledgments

The work was supported by Joint Open Fund of Jiangsu Collaborative Innovation Center for Ecological Building Material and Environmental Protection Equipments and Key Laboratory for Advanced Technology in Environmental Protection of Jiangsu Province.

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