Title	Insights into quorum quenching mechanisms to control membrane biofouling under changing organic loading rates
Author(s)	Waheed, Hira; Xiao, Yeyuan; Hashmi, Imran; Stuckey, David; Zhou, Yan
Citation	Waheed, H., Xiao, Y., Hashmi, I., Stuckey, D., & Zhou, Y. (2017). Insights into quorum quenching mechanisms to control membrane biofouling under changing organic loading rates. Chemosphere, 182, 40-47.
Date	2017
URL	http://hdl.handle.net/10220/44110
Rights	© 2017 Elsevier Ltd. This is the author created version of a work that has been peer reviewed and accepted for publication by Chemosphere, Elsevier Ltd. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [http://dx.doi.org/10.1016/j.chemosphere.2017.04.151].

Insights into quorum quenching mechanisms to control membrane biofouling under changing organic loading rates

Hira Waheed[a,b+], *Yeyuan Xiao* [b+], *Imran Hashmi*[a], *David Stuckey*[b,c], *Yan Zhou* [b,d*]

[a] Institute of Environmental Sciences and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology, H-12 Sector, Islamabad, Pakistan.

[b] Advanced Environmental Biotechnology Centre, Nanyang Environment & Water Research Institute, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.

[c] Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK.

[d] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798.

(E-mail: *hira.waheed@iese.nust.edu.pk*; *YYXiao@ntu.edu.sg*; *imranhashmi@iese.nust.edu.pk*; *d.stuckey@imperial.ac.uk*; *ZhouYan@ntu.edu.sg*)

[+] Co-first authors

[b]*Corresponding author

Present/Permanent address: Advanced Environmental Biotechnology Centre, Nanyang Environment & Water Research Institute, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore (E-mail: ZhouYan@ntu.edu.sg).

Abstract

A quorum quenching (QQ) consortium comprised of both acyl homoserine lactones (AHLs)- and autoinducer-2 (AI-2)-degrading bacteria, either immobilized in polymer-coated alginate beads or in liquid suspension, was examined for fouling control in lab-scale MBRs under both steady and changing organic loading rates (OLRs). Under steady conditions the QQ consortium retarded biofouling by a factor of 3. However, a continuous increase in OLR vastly reduced the effectiveness of QQ bacteria; the biofouling was retarded only by factors of 1.4-1.8. A significant increase in extracellular polymeric substance (EPS), especially loosely-bound EPS in mixed liquor together with an increase in polysaccharide content up to 4 times in EPS resulted from the increase in OLR, was attributed to the impaired QQ efficacy. In control MBRs, cake layer resistance was the major factor (>60%) contributing to the increased trans-membrane pressure, as compared with pore blockage resistance and intrinsic membrane resistance. In contrast, the pore blockage resistance became dominant in QQ MBRs (>40%).

Key words

Membrane bioreactor (MBR), Biofouling, Quorum sensing, Quorum quenching, organic loading rate (OLR)

1. Introduction

Membrane bioreactor (MBR) technology is becoming a widely recognized and accepted wastewater treatment technology owing to its distinctive advantages, including high treatment efficiency, low sludge production and a small footprint compared to conventional activated sludge (CAS) (Hong et al., 2002; Lin et al., 2014). However, membrane fouling remains a major impediment that limits the widespread application of MBR due to high energy demand and operational costs associated with fouling control (Drews, 2010; Yu et al., 2010). Complex

interactions between membrane and mixed liquor components like suspended solids, colloids, biopolymers, and solutes introduced from raw wastewater or produced during biomass growth or decay play a significant role in membrane fouling (Hu et al., 2016). It is known that cake layer formation on the membrane surface accounts for over 80% of the total filtration resistance in most MBR studies (Miura et al., 2007; Wang et al., 2007).

In past few years great interest has been focused on mitigating membrane biofouling via quorum quenching (QQ) where bacterial communication is disrupted to reduce cake layer formation on the membrane (Oh et al., 2012). To achieve this, bacteria which produce QQ enzymes were isolated from sludge and applied in MBRs, either in the form of a microbial culture (Cheong et al., 2014; Jahangir et al., 2012), or immobilized in alginate beads (Kim et al., 2013). Successful application of QQ bacteria in terms of biofouling control in MBR systems in lab (Kim et al., 2013, 2015; Maqbool et al., 2015; Oh et al., 2012) as well as pilot scale (Lee et al., 2016) has been reported. These studies have proved the effectiveness of QQ mechanisms for biofouling control under steady conditions; however, the efficacy of QQ under transient conditions has not been studied yet. An increase in organic loading rate (OLR), a typical transient condition, may trigger increased EPS production, causing EPS bulking and increased biofouling in MBRs (Abdollahzadeh Sharghi and Bonakdarpour, 2013; Shariati et al., 2013). This poses a challenge to the application of QQ for biofouling control under transient conditions, as QQ bacteria are believed to disrupt QS molecules, thus reducing EPS production and biofouling (Kim et al., 2013, 2015). Therefore, it is important to comprehensively investigate the factors which may contribute to membrane biofouling, and examine their changes in the presence of QQ bacteria under transient loads.

This study aimed to investigate the impact of OLR on QQ-based biofouling control. To achieve this objective, a consortium of QQ strains comprised of AHLs- and AI-2-degrading bacteria, both in immobilized and suspension forms, was applied to MBRs. The influence of QQ bacteria on EPS production, sludge floc size, and sludge filterability in term of capillary suction time (CST) under both steady and transient organic loads was examined to gain a better understanding of the underlying relationship between QQ and biofouling. The concentrations of AHLs and AI-2 were also monitored to confirm the QQ effects.

2. Material and Methods

2.1. Chemicals

The AHLs including N-butyryl-, N-hexanoyl-, N-heptanoyl-, N-octanoyl-, N-decanoyl-, N-dodecanoyl-, and N-tetradecanoyl-DL-homoserine lactone (C4-, C6-, C7-, C8-, C10-, C12- and C14-HSL); N-(3-oxohexanoyl)-, N-(3-oxooctanoyl)-, N-(3-oxododecanoyl)-, and N-(3-oxotetradecanoyl)-L-homoserine lactone (3OC6-, 3OC8-, 3OC12-, 3OC14-HSL) were obtained from Sigma-Aldrich (Singapore). The precursor of AI-2, 4,5-dihydroxy-2,3-pentanedione (DPD) was purchased from Omm Scientific, Inc (Dallas, TX, USA).

2.2. Experimental setup

Experiment was conducted under varying (Phase I) and fixed OLR (Phase II) in duplicate. (Table 1) In Phase I four MBRs, each with a working volume of 450 mL; (i) control MBR (C-MBR), (ii) control MBR with empty beads (Cb-MBR), (iii) MBR with addition of QQ bacteria entrapped in polymer-coated alginate beads (QQb-MBR), and (iv) MBR with addition of QQ bacteria in broth (QQs-MBR) were set up and operated simultaneously. Under fixed OLR (Phase II), two more QQb-MBR were added in addition to the four MBRs; when C-MBR, Cb-MBR and QQs-MBR fouled, one of the QQb-MBR was stopped, and the level of EPS and other fouling

parameters in both MBRs were analyzed to gain better understanding of the QQ mechanism along with the operation cycle. In Phase I, the OLR was gradually increased from 4 to ~ 7 mg COD/ g VSS • hr via wasting sludge from the MBRs; while in Phase II, the OLR was maintained constantly via supplementing the seed sludge.

Hollow fiber membranes with a pore size of 0.1 μm and surface area of 0.0045 m^2 were used in the present study. Sludge (collected from Ulu Pandan Water Reclamation Plant, Singapore) was acclimatized to a synthetic wastewater, comprising glucose (500 mg/L), NH_4Cl (190 mg/L), KH_2PO_4 (55.6 mg/L), $CaCl_2$ (5.5 mg/L), $MgSO_4.7H_2O$ (5.7 mg/L), $FeCl_3$ (1.5 mg/L), $MnCl_2$ (1mg/L) and $NaHCO_3$ to maintain pH in the range of 7.0-7.5, for one month before being used as the seed sludge. During the study, the solids retention time (SRT) and hydraulic retention time (HRT) were maintained at 30 d and 12 h, respectively, at an operational flux of 8.5 L/m^2/h (LMH) unless otherwise mentioned. A constant flux of 8.5 LMH was maintained to keep fouling at a reasonable rate.

Table 1: Experimental setup and operating conditions

	Phase I	**Phase II**
Experimental conditions	Varying Organic loading rate	Fixed organic loading rate
MBRs setup	i. Control MBR (C-MBR) ii. Control MBR with vacant beads (Cb-MBR) iii. MBR with QQ beads (QQb-MBR) iv. MBR with QQ sludge (QQs-MBR)	
Reactor volume (mL)	450	450
Membrane type	Hollow fiber, PVDF	Hollow fiber, PVDF
Flux (LMH)	8.5	8.5
HRT (h)	12	12
SRT (d)	30	90

2.3. Bacterial immobilization

A QQ consortium comprising of AHLs degrading strains *Enterobacter cloaca* (QQ13), *Microbacterium sp*. (QQ1), *Pseudomonas sp*. (QQ3), which were isolated from activated sludge in the authors' lab (the 16S rRNA gene accession number: KR058854, KR058848 and KR058846, respectively), *Rhodococcus* sp. RBH4 (Kim et al., 2015) and an AI-2 degrading *E. coli* strain *ΔlsrRΔluxS* (Thompson et al., 2015) was selected for the study. Polymeric beads, entrapped with a single QQ strain, were prepared according to the method described by Kim et al. (2015) with some modifications. Briefly, fresh QQ bacterial culture, grown in Luria Bertanni (LB) broth with an optical density of 1.00 measured at 400 nm, was centrifuged at 4000 rpm for 30 min and re-suspended in a phosphate buffer solution (PBS). For QQs-MBR, strain suspensions were directly added into the sludge, keeping sludge to volume ratio at 2%. Whereas for QQ beads, 5 mL of bacterial suspension was mixed with sterile sodium alginate (2% w/v) and the final suspension dropped into $CaCl_2$ solution (4% w/v) through a nozzle at a rate of 1 mL/min using a peristaltic pump. For polymeric coating, pellets of polysulfone were dissolved in N-methyl-2-pyrrolidone (8% w/v) at 60 ^{o}C; finally, alginate beads were dipped in polymeric solution for 15 s and stored in deionized water at 4 ^{o}C. The QQ strains were entrapped separately in polymeric beads to avoid non-homogenous mixing, and added in equal proportions to MBRs keeping the overall ratio of beads to original MBR working volume at 2%.

2.4. Membrane resistance analysis

Membrane resistances were evaluated in terms of their fouling potential using a simple resistance in series model (Choo and Lee, 1996) based upon Darcy's Law:

$$R = \frac{\Delta P}{J\mu}$$

where R is hydraulic resistance (1/m), ΔP is the TMP rise (Pa), J is the operational flux ($m^3/m^2/s$), and μ is the permeate dynamic viscosity (Pa.s).

$$R$$

where, R_t is the total hydraulic resistance, comprised of cake layer resistance (R_c), pore blockage resistance (R_p) and intrinsic membrane resistance (R_m). R_m was measured by filtering clean water through new membrane, while R_t was determined at the end of an experiment by measuring flux and TMP when deionized (DI) water was filtered through the membrane. The cake layer was then removed via sonication and the membrane submerged in deionized (DI) water to measure R_m+R_p. Finally, R_c was calculated by subtracting $R_m + R_p$ from R_t.

2.5. Quantification of signal molecules

AHLs were extracted from the biocake and activated sludge (50 mL) of the MBR as per the method described by Waheed et al. (2016). Samples were concentrated 100 times and analyzed using an Agilent 6460 (USA) QQQ triple quadrupole mass spectrometer (MS) equipped with an AJS electro-spray interface. The instrument settings were optimized as follows: interface voltage, 3.5 kV; drying gas temperature, 325 °C; drying gas (N_2) flow rate, 8 L/min; sheath gas temperature, 300°C; sheath gas (N_2) flow rate, 11 L/min. An Agilent 1290 Infinity II HPLC was used for chromatographic separation, which was achieved on a 100x2.1 mm Kinetex 2.6 µm Bi-phenyl column (Phenomenex, USA) using 0.1% formic acid (A) and methanol with 0.1% formic acid (B) as the mobile phase at a gradient of 5 to 85% B within 12 min. The limit of quantification (LOQ) for this method was 0.01 µg/L.

One milliliter of sample was used to analyze the concentration of AI-2 in the sludge supernatant via the bioluminescence method using the reporter strain *Vibrio harveyi* BB170 (Taga and Xavier, 2011). Briefly, the fresh culture of BB170 (OD_{600} = ~0.3 to 0.5) was diluted

2000 times in a freshly-prepared AB medium. Duplicate mixture containing 10 % (v/v) sample along with 90 % (v/v) diluted BB170 culture was added to a microtitre plate and incubated at 30 ^{o}C with rotary mixing at 150 rpm. The same amount of Milli-Q water was used as for negative controls. The luminescence produced in each sample was measured using a microtitre plate reader (Infinite M200 Pro, Tecan, Switzerland) at a wavelength of 490 nm. Measurements were recorded every 30 min from 5 to 8 h, until the negative control samples showed the lowest amount of luminescence. A precursor of AI-2, 4,5-dihydroxy-2,3-pentanedione (DPD) (Omm Scientific, Dallas, TX, USA), was used as the calibration standard.

2.6. Sludge characterization & water quality analysis

The heat extraction method (Li and Yang, 2007) was used for extraction of extracellular polymeric substances (EPS). Briefly, 15 mL of sludge was collected from the MBRs, centrifuged at 4000 rpm and 4 ^{o}C for 30 min (this condition was also used for the following centrifugation steps unless otherwise stated), and supernatant stored as soluble EPS. The sludge pellet was re-suspended in 0.05 % (w/v) NaCl and homogenized; then the sample was centrifuged (4000 rpm for 15 min at 4 ^{o}C) and supernatant stored as loosely bound (LB) EPS. The sludge pellet was re-suspended in same NaCl solution and incubated at 60^{o}C for an hour; after centrifugation the supernatant was stored as tightly bound (TB) EPS. For protein (PN) analysis, the Lowry method (Lowry et al., 1951) with the folin-ciocalteu phenolic reagent was used, and absorbance at 750 nm was measured via a spectrophotometer (UV-2600, Shimadzu). For the quantification of polysaccharides (PS), the absorbance at 490 nm was measured using the phenol sulfuric acid method (DuBois et al., 1956).

The dissolved oxygen (DO) and specific oxygen uptake rate (SOUR) were monitored using a multi meter (Fisher Scientific, XL600). The chemical oxygen demand (COD), sludge volume

index (SVI), mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) were analyzed as per the Standard Methods (APHA, 2012). Sludge dewaterability was evaluated in terms of CST using a CST apparatus (Ofite 294-50, OFI Testing Equipment Inc., US). The sludge particle size distribution was monitored using a particle size analyzer (SALD-3101, Shimadzu).

3. Results and discussion

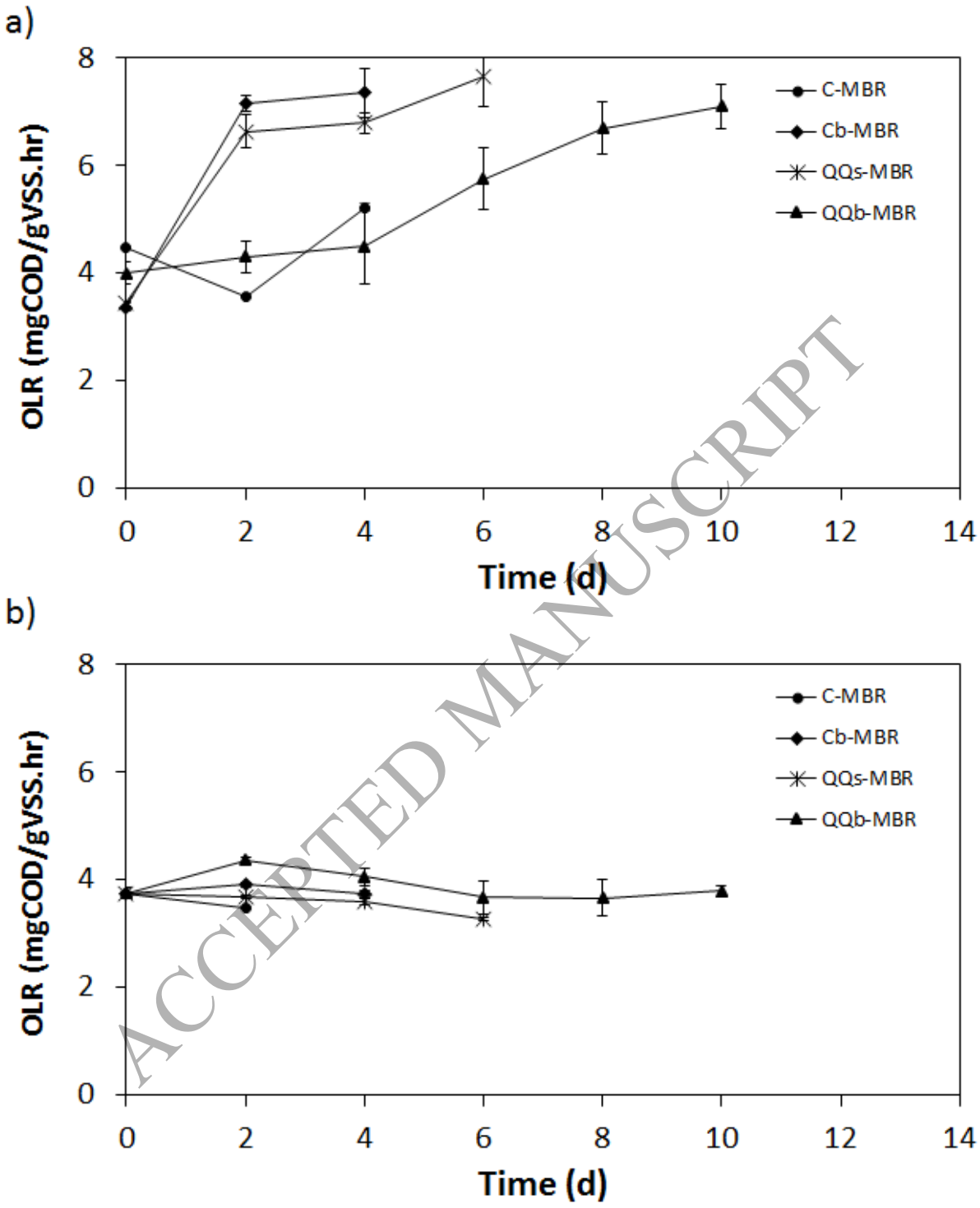
3.1. MBR operation and trans-membrane pressure (TMP) trends

The experiment was conducted in two separate phases, as described in Table 1. The DO levels in all reactors throughout the phases were maintained within 5.5 to 6.5 mg/L. The COD removal efficiency was more than 90 % in all MBRs (Table 2), and similar COD removal in the QQ MBRs indicate that QQ beads or sludge did not affect the performance of MBRs. A gradual decrease in mixed liquor volatile suspended solids (MLVSS) concentration was carried out purposely in Phase I via wasting sludge and controlling a SRT of 30 d, thus creating a continuous increase in the OLR. While in Phase II, all MBRs were supplemented with the seed sludge at regular intervals to maintain a constant MLVSS and OLR (Figure 1). To evaluate MBR performance, TMP was monitored for both experiments as the extent of membrane fouling is directly linked to TMP increase. Figure 2 shows the TMP profiles of MBRs of both Phases which were terminated when the TMP reached ~30 kPa. In Phase I (Figure 2a), no significant difference was observed between both control MBRs, as the overall fouling rates ($\Delta TMP/\Delta t$) for C-MBR and Cb-MBR were found to be 5.81 and 5.12 kPa/d, respectively. However, addition of QQ sludge or QQ beads in MBRs reduced the fouling rate by 29% and 49%, respectively, as compared to C-MBR under transient conditions. While in steady state (Phase II in Figure 2b), addition of QQ consortium, either in culture broth or in immobilized beads, significantly reduced

the overall fouling rate by more than 66 % as compared to C-MBR, with the QQ beads slightly better than the QQ sludge. The vacant beads in Cb-MBR also reduced the fouling rate by 35%. It was found that OLR caused mainly external fouling due to accumulation of colloidal and dissolved organic molecules (Trussell et al., 2006), thereby, MBRs at low OLR during Phase II operated longer than MBR at high or changing OLR.

Table 2 Sludge characterization and performance efficiency of MBRs (n=3)

	Phase I				Phase II			
	C-MBR	**Cb-MBR**	**QQs-MBR**	**QQb-MBR**	**C-MBR**	**Cb-MBR**	**QQs-MBR**	**QQb-MBR**
SOUR (mg/hr/g)	32.4	40.7	35.8	79.9	70.2	77.5	122.3	160.3
CST (s)	15.0	8.9	18.5	12.0	19.8	16.0	12.5	13.5
PSA mean (μm)	50.6	87.5	125.3	48.6	69.0	38.5	60.6	35.0
COD removal (%)	97.5	96.9	98.0	85.2	90.5	90.6	91.5	92.0



197

198 **Figure 1 The organic loading rate (OLR) of (a) Phase I and (b) Phase II**

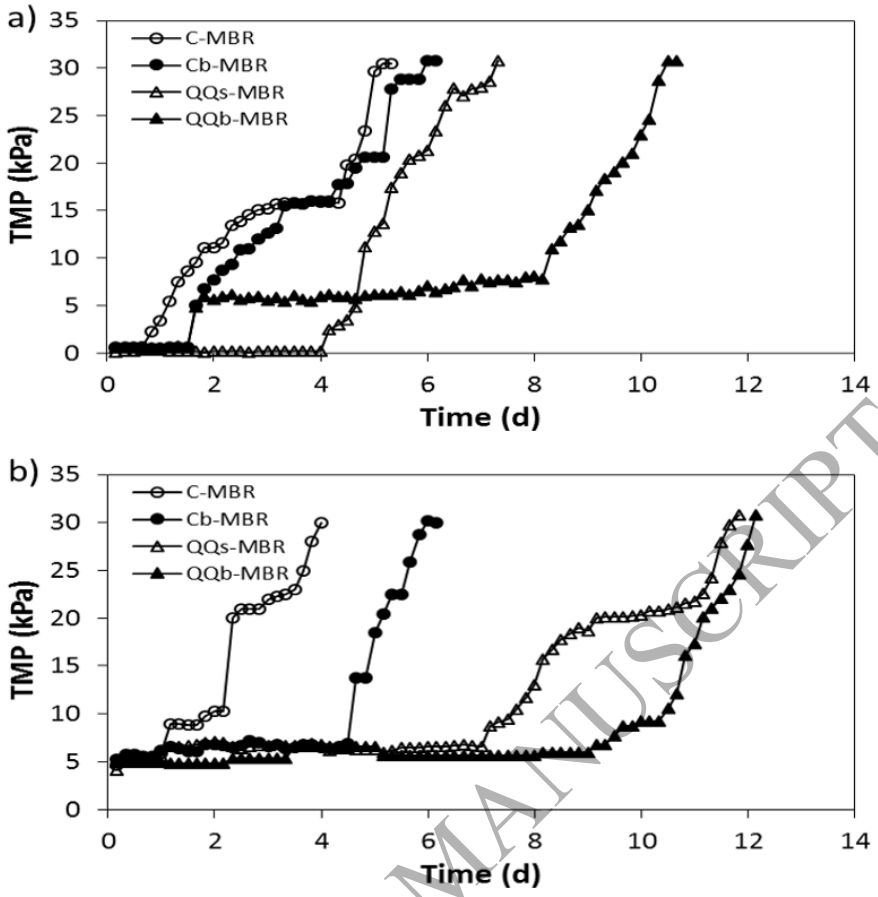


Figure 2 TMP profiles of (a) Phase I and (b) Phase II

The similar TMP profiles of QQs-MBR and QQb-MBR in Phase II suggest that the QQ consortium was effective in fouling control when the MBR was in steady state, irrespective of whether it is in broth or in immobilized beads. However, under transient conditions, robust fouling control was achieved only when the QQ consortium was in immobilized beads. QQ strains may grow well in immobilized form since beads provide protective environment under harsh conditions Whereas, activity/growth of QQ strains was suppressed when they were added directly to sludge, which could be due to the presence of predators or QQ enzyme suppressors released by other competitors. Previous studies (Gao et al., 2013; Zhang et al., 2006) demonstrated three dominant stages in overall TMP profiles, including an initial rise due to pore

blockage (stage 1), a slow rise due to accumulation of EPS on the membrane surface (stage 2), and a sharp jump resulted from biofilm formation (stage 3). In this study, a slower rise in TMP during stage 2 in the QQ MBRs compared to control MBRs was observed for both Phases, indicating that QQ may have played a role in retarding biofouling by postponing cake layer formation.

3.2. Membrane resistance and foulant characterization

Membrane resistances from all six reactors of Phase II were compared to further elucidate the mechanism of QQ (Figure 3). A clear decrease in the fraction of pore blockage resistance (Rp/Rt) with time was observed in QQb-MBR, from >70% on Day 4, to ~ 45% on Day 6, and further to ~ 40% on Day 12 when it was fouled. The fraction of cake layer resistance (Rc/Rt) remained very low (<7%) on Day 4 and 6, but increased to ~30% on Day 10. This clearly indicates the strong inhibition of cake layer formation by QQ in the early stage. Compared to other MBRs, the fraction of pore blockage resistance (Rp/Rt) of both QQ MBRs (>40%) were the greatest, when they were fouled. This suggests that more molecules or small particles had blocked the membrane pores in QQ MBRs, as the QQ had postponed the cake layer formation. Hence in QQ MBRs, the pore blockage may be the leading factor that had led to the final fouling.

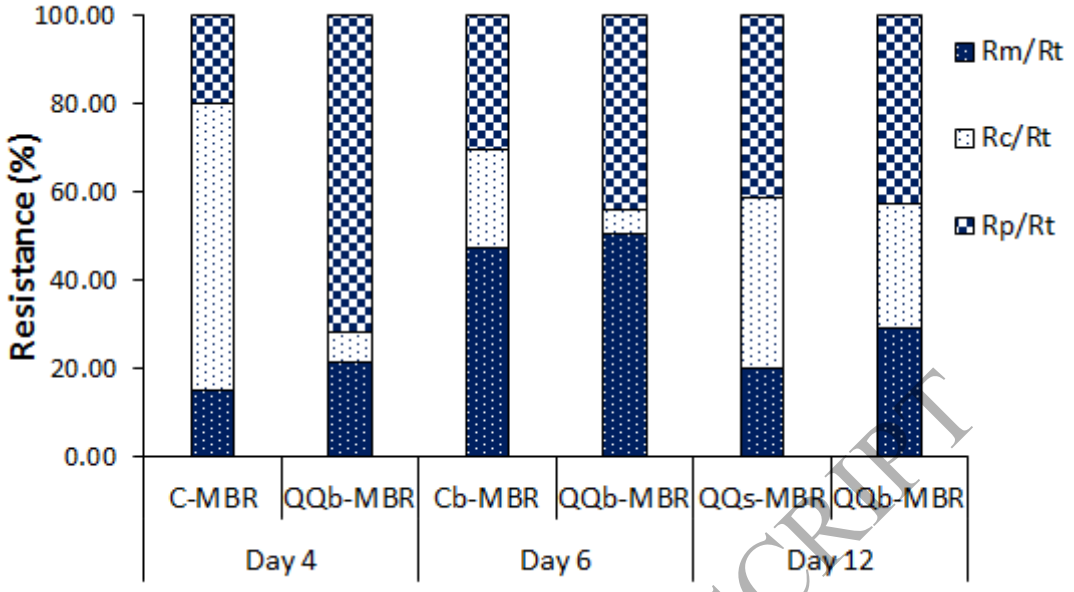


Figure 3 Membrane resistance analysis under steady conditions

The significantly higher portion of cake layer resistance (Rc/Rt) in C-MBR as compared to Cb-MBR indicates that beads scouring had greatly reduced the cake layer formation. This was also observed when QQs-MBR was compared to QQb-MBR.

Fouled membranes from all reactors were analyzed via Fourier transform infrared (FTIR) spectroscopy (AIM-8800, Shimadzu) to detect any foulant composition changes posed by quenching mechanism. QQ strains either immobilized or added as a suspension did not modify the membrane foulants, as the organic or functional groups in all membranes were found to be same, as shown in Figure S2 in the Supplementary Information. It can be inferred that quenching activity delayed biofilm formation, but kept the foulant composition intact.

3.3. Effect of QQ on QS signal molecules

Biofilm formation (biofouling) could be mitigated substantially by disrupting signal molecules such as AHLs, oligopeptides or AI-2 using enzymes released by QQ bacteria (Shrout

and Nerenberg, 2012). In the present study, the role of QQ bacteria was confirmed by monitoring the concentrations of two signal molecules, AHLs and AI-2 using LC-MS and *V. harveyi* bioluminescence assays, respectively.

3.3.1. Quenching of AHLs

AHLs in the seed sludge were analyzed to screen the major types of QS signal molecules; C4-HSL, 3OC6-HSL, 3OC8-HSL and 3OC12-HSL were detected as the dominant AHLs present. Table S1 presents the detailed quantitative results of AHLs in the biocake, mixed liquor and effluents from each reactor. Various AHLs including C4-, 3OC6-, C6- and 3OC14-HSL were detected in the mixed liquor of C-MBR and Cb-MBR, whereas they were not detected or lower than the quantification limit in QQs-MBR or QQb-MBR. This indicates that of the QQ consortium had quenched these QS molecules, thus reducing the biofilm formation. High concentrations of C4-HSL and 3OC6-HSL in the biocake sample of C-MBR and Cb-MBR as compared to that in QQ MBRs may signify the role of small chain signal molecules in the formation of matured biofims.

3.3.2. AI-2

The AI-2 concentration was determined by bioluminescence assay with 4,5-dihydroxy-2,3-pentanedione (DPD), a precursor of AI-2, as the calibration standard. The effectiveness of QQ bacteria in reducing the AI-2 signal molecules is shown in Figure 4. The AI-2 concentrations in QQb-MBR were the lowest when all MBRs were fouled in both Phases. However, the AI-2 in QQs-MBR of Phase II was higher than that of C-MBR, though it was still lower than both C-MBR and Cb-MBR in Phase I. Higher concentrations of AI-2 in QQs-MBR could be due to the less survival rate of the *E. coli* strain *ΔlsrRΔluxS,* since the cells were not protected in the beads. A slow but gradual increase in AI-2 concentrations with time in QQb-MBR was also noticed.

These may suggest that the QQ consortium were effective in quenching AI-2, but only at the early stage; with time the quenching effect vanished. Their quenching effect was more robust when they were immobilized in beads.

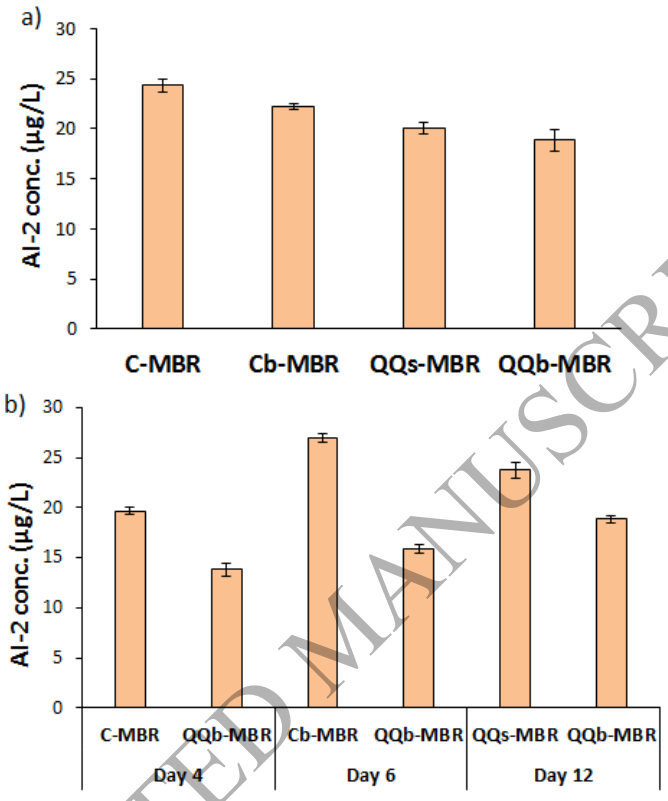


Figure 4 Level of autoinducer (AI-2) in the mixed liquor of MBRs, (a) Phase I and (b) Phase II

3.4. Effect of QQ on sludge characteristics

The mixed liquor was sampled regularly to monitor the effect of QQ on the different sludge characteristics as listed in Table 2. The SOUR was measured as a representative of sludge respirometric activity, and Phase II showed higher activity compared to the Phase I. The SOUR of QQb-MBR was almost 2 times as many as that of control MBRs in both Phases. This shows that QQ phenomenon or disruption in bacterial signaling had a direct impact on metabolic

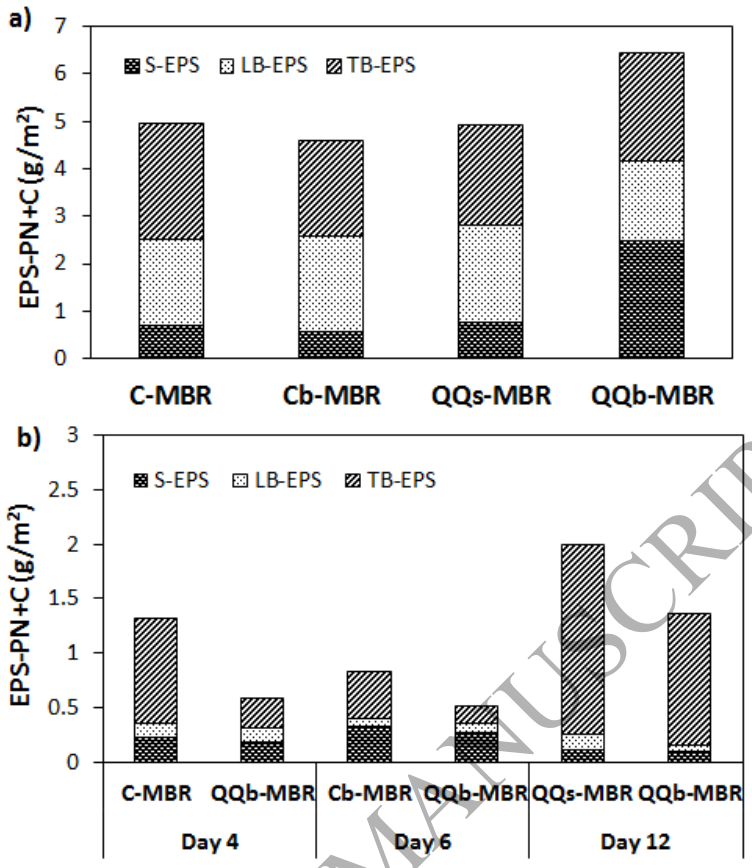
activity of microbial cells; which could be related to starving conditions developed during interruption in bacterial communications, or dominance of strains those require very high OUR (Garcia-Ochoa et al., 2010; Gomez et al., 2006).

Mean floc size for the QQb-MBR was found to be 49 and 35 μm for Phases I and II respectively. Overall, the sludge floc sizes in Cb-MBR and QQb-MBR were smaller than C-MBR and QQs-MBR in both Phases, respectively; the presence of beads in the former two MBRs contributed to this reduction in floc size. The dewaterability of sludge determined by CST is also an indication of its permeability in the MBR (Table 2), and a high EPS content will increase sludge viscosity thus reducing its permeability. However, no clear impacts of the QQ consortium on CST could be observed. Reduction in floc size still resulted in the better sludge dewaterability, however, its effects on sludge settling property cannot be ignored.

3.5. QQ effects on EPS production

3.5.1. EPS concentrations in the biocake

EPS, either in bound or soluble form, is considered to be the major component in cell flocs and biofilms which ultimately leads to membrane fouling in MBRs. In this context, the soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) extracted from the mixed liquor and sludge cake were analyzed for all phases. The protein and carbohydrate distributions in different EPS fractions extracted from the sludge cake is shown in Figure 5. EPS fractions retained on the membranes in the form of a fouling layer led to a high resistance on the membrane surface, and a reduction in permeate flux (Lin et al., 2014). In the biocake sample, bound EPS was higher than soluble EPS in all phases. Bound EPS facilitates more in floc formation, and an increase in floc size tends to enhance floc deposition on the membrane surface, which ultimately leads to biocake formation (Chu and Li, 2005).



299

300 **Figure 5 EPS concentration in the biocake samples (a) Phase I and (b) Phase II**

301 The total biocake EPS in Phase I was more than 3 times higher than Phase II, which
302 indicates that the change in OLR had a dominant effect on the total biocake EPS content.
303 However, the TB-EPS were in the same range (~0.95-2.2 g/m^2) amongst all MBRs in both
304 phases except a little lower amount was observed in Cb-MBR of Phase II (Figure 5). The high S-
305 EPS and LB-EPS contents in Phases I could have resulted from new or loose sludge flocs being
306 deposited on the surface of the compacted biocake. It also suggests that the scouring effects of
307 the beads were minimal for fouling control, which corresponded well to the TMP profiles in
308 Figure 2.

The total and especially the TB-EPS in QQb-MBR were much lower on Day 4 and 6 as compared to the control reactors of the same day (Figure 5b), but they increased to levels higher than the control reactors when it was fouled. This corresponded well with the membrane resistance analysis.

3.5.2. EPS concentrations in the mixed liquor

Figure 6 shows the protein-carbohydrate ratio (EPS_P/EPS_C), as well as soluble and bound EPS concentrations at different time intervals in the mixed liquor. When the MBRs were started, the bound-EPS (more dominantly for LB-EPS) contents of all MBRs in Phase II were lower than those of Phase I, which could be the reason of the higher SOUR. The prominent increases (from Day 0 to Day 2) in LB- and TB-EPS in Cb-MBR and QQs-MBR of Phase I corresponded well to the significant decreases in MLSS in the MBRs (Figure S1). But after that, both LB-EPS and TB-EPS in the two MBRs gradually decreased. The LB- and TB-EPS in QQb-MBR also increased since the start of the MBR, but started to decrease from Day 8 and 4, respectively. The initial increase could be partially due to the decrease in MLSS concentrations, which led to an increase in OLR and severe biofouling. Similar results were also reported in sludge granulation (Kim et al., 2013) and MBR operation (Trussell et al., 2006).

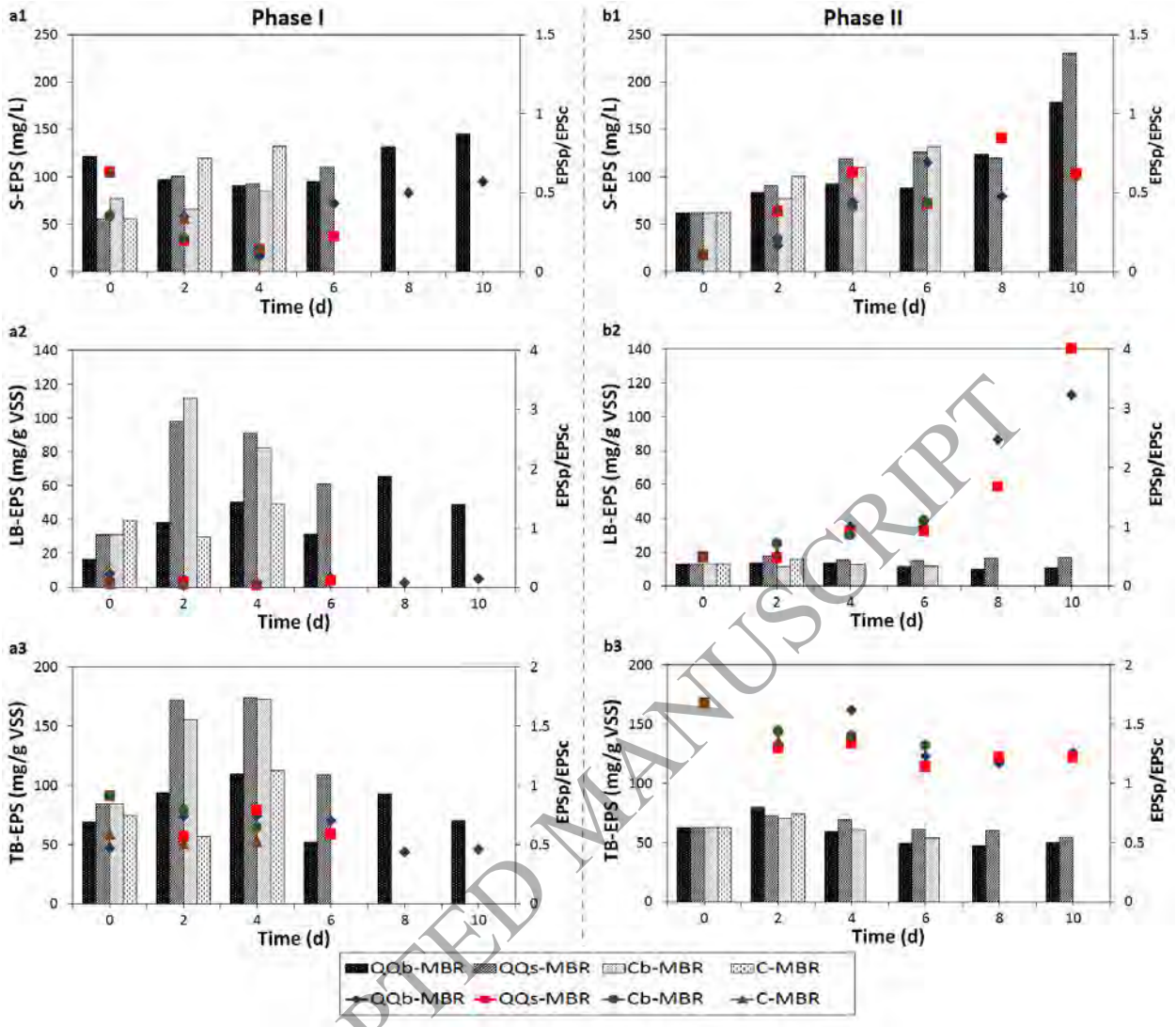


Figure 6 Profiles of extracellular polymeric substances (EPS) in terms of the total concentrations of polysaccharide and protein (histograms) and the ratio of protein to polysaccharide (scattered-dots) in the mixed liquor of (a) Phase I and (b) Phase II. From top to bottom are the soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), respectively.

In Phase II, decreases in LB- and TB-EPS in all MBRs were clearly seen from the beginning of the operation. However, no significant differences between the QQ MBRs and the

control MBRs were observed. Instead, the S-EPS gradually increased in all MBRs in both Phases except of a slight decrease in QQb-MBR in Phase I before Day 4. Many studies have directly correlated the S-EPS with biomass concentrations (Drews et al., 2008; Geng and Hall, 2007; Jeong et al., 2007). In our study, the MLSS levels dropped substantially in Phase I; however, continuous rise in S-EPS were still observed. These results suggest that intensive aeration and fluidization of polymeric beads significantly reduced the sludge floc size (Table 2), and the dissociation of sludge flocs had resulted in the decrease in bound EPS and increase in S-EPS and eventually contributed to membrane pore blockage (Meng et al., 2009; Metzger et al., 2007).

The ratio of EPS_P (EPS-protein) to EPS_C (EPS-carbohydrate) was also investigated to gain better understanding of QQ mechanism. In Phase I, all MBRs showed a decrease in EPSp/EPSc ratio of the S-EPS with an increase in OLR, except for that of QQb-MBR, which slightly increased (Figure 6:a1). In contrast, a clear increase in the EPS_P/EPS_C in the sludge supernatant was observed in Phase II (Figure 6:b1). The gradual increase in EPS_P/EPS_C of the LB-EPS was also observed in all MBRs in Phase II, while it remained unchanged in Phase I. However, for TB-EPS, a slight decrease in EPS_P/EPS_C was observed in both Phases. These results suggest that the increase in OLR had more impacts on LB-EPS and S-EPS; it had maintained the high fraction of polysaccharides in the LB-EPS, which contributed to fouling more than protein-like substances (Rosenberger et al., 2006; Yigit et al., 2008). The EPSp/EPSc ratio in the LB-EPS corresponded well to the higher concentrations of polysaccharides than protein in the biocake in Phase I, and the higher protein contents in Phase II. This suggests that LB-EPS were more significantly correlated with membrane fouling compared with TB- and S-EPS, as they contributed to flocculation and sedimentation processes, which was also reported in literatures (Lin et al., 2014; Ramesh et al., 2007; Wang et al., 2009).

The biofouling was deferred by factors of 3.04 ± 0.5 and 2.96 ± 0.7 (mean ± standard deviation, n=2) in QQb-MBR and QQs-MBR, respectively, compared to the C-MBR for Phase II. However, these factors decreased to 1.8 ± 0.3 and 1.4 ± 0.1 in Phase I when the OLR kept increasing. A continuous increase in OLR, which resulted in significant EPS production and higher content of polysaccharide in LB-EPS, was the major factor resulting in the impaired efficiency of immobilized QQ strains. The reduction in S-EPS concentration in the mixed liquor in MBRs containing QQ bacteria were reported previously (Lee et al., 2016; Maqbool et al., 2015). However, it was not observed in our study; instead, the QQ consortium retarded the EPS accumulation on the membranes.

4. Conclusions

- The QQ consortium in immobilized beads showed robust fouling control capacity under both steady state and transient condition. When added in broth, they remained effective only under steady state.
- The QQ consortium significantly reduced the AHL levels in mixed liquor inside MBR and in sludge cake on membrane surface.
- LB-EPS of the mixed liquor was the major contributor to membrane fouling, and within the EPS, polysaccharides contributed more to membrane fouling than proteins.
- A continuous increase in OLR resulted in higher EPS levels and higher polysaccharide ratios in the LB-EPS, which in turn lead to faster fouling in the MBRs.

Acknowledgments

The authors would like to acknowledge the support and funding from International Research Support Initiative Program (IRSIP), Higher Education Commission (HEC), Pakistan; and Environment & Water Industry Programme Office (EWI), SEO Nanyang Technological

University Singapore.

References

Abdollahzadeh Sharghi, E., Bonakdarpour, B., 2013. The study of organic removal efficiency and halophilic bacterial mixed liquor characteristics in a membrane bioreactor treating hypersaline produced water at varying organic loading rates. Bioresour. Technol. 149, 486–495. doi:10.1016/j.biortech.2013.09.110

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), 2012. Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington, DC.

Cheong, W.-S., Kim, S.-R., Oh, H.-S., Lee, S.H., Yeon, K.-M., Lee, C.-H., Lee, J.-K., 2014. Design of quorum quenching microbial vessel to enhance cell viability for biofouling control in membrane bioreactor. J. Microbiol. Biotechnol. 24, 97–105.

Choo, K.-H., Lee, C.-H., 1996. Membrane fouling mechanisms in the membrane-coupled anaerobic bioreactor. Water Res. 30, 1771–1780. doi:10.1016/0043-1354(96)00053-X

Chu, P.H., Li, X.-Y., 2005. Membrane fouling in a membrane bioreactor (MBR): sludge cake formation and fouling characteristics. Biotechnol. Bioeng. 90, 323–331. doi:10.1002/bit.20409

Drews, A., 2010. Membrane fouling in membrane bioreactors—Characterisation, contradictions, cause and cures. J. Membr. Sci. 363, 1–28.

Drews, A., Vocks, M., Bracklow, U., Iversen, V., Kraume, M., 2008. Does fouling in MBRs depend on SMP? Desalination, Selected Papers Presented at the 4th International IWA Conference on Membranes for Water and Wastewater Treatment, 15-17 May 2007, Harrogate, UK. Guest Edited by Simon Judd; and Papers Presented at the International Workshop on Membranes and Solid-Liquid Separation Processes, 11 July 2007, INSA, Toulouse, France. Guest edited by Saravanamuthu Vigneswaran and Jaya Kandasamy 231, 141–149. doi:10.1016/j.desal.2007.11.042

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356. doi:10.1021/ac60111a017

Gao, D.-W., Wen, Z.-D., Li, B., Liang, H., 2013. Membrane fouling related to microbial community and extracellular polymeric substances at different temperatures. Bioresour. Technol. 143, 172–177. doi:10.1016/j.biortech.2013.05.127

Garcia-Ochoa, F., Gomez, E., Santos, V.E., Merchuk, J.C., 2010. Oxygen uptake rate in microbial processes: An overview. Biochem. Eng. J. 49, 289–307. doi:10.1016/j.bej.2010.01.011

Geng, Z., Hall, E.R., 2007. A comparative study of fouling-related properties of sludge from conventional and membrane enhanced biological phosphorus removal processes. Water Res. 41, 4329–4338. doi:10.1016/j.watres.2007.07.007

Gomez, E., Santos, V.E., Alcon, A., Martin, A.B., Garcia-Ochoa, F., 2006. Oxygen-Uptake and Mass-Transfer Rates on the Growth of Pseudomonas putida CECT5279: Influence on

Biodesulfurization (BDS) Capability. Energy Fuels 20, 1565–1571. doi:10.1021/ef050362y

Hong, S.P., Bae, T.H., Tak, T.M., Hong, S., Randall, A., 2002. Fouling control in activated sludge submerged hollow fiber membrane bioreactors. Desalination 143, 219–228. doi:10.1016/S0011-9164(02)00260-6

Hu, Y., Wang, X.C., Yu, Z., Ngo, H.H., Sun, Q., Zhang, Q., 2016. New insight into fouling behavior and foulants accumulation property of cake sludge in a full-scale membrane bioreactor. J. Membr. Sci. 510, 10–17. doi:10.1016/j.memsci.2016.02.058

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. J. Membr. Sci. 411–412, 130–136. doi:10.1016/j.memsci.2012.04.022

Jeong, T.-Y., Cha, G.-C., Yoo, I.-K., Kim, D.-J., 2007. Characteristics of bio-fouling in a submerged MBR. Desalination 207, 107–113. doi:10.1016/j.desal.2006.07.006

Kim, K.S., Sajjad, M., Lee, J., Park, J., Jun, T., 2013. Variation of extracellular polymeric substances (EPS) and specific resistance to filtration in sludge granulation process to the change of influent organic loading rate. Desalination Water Treat. 52, 4376–4387. doi:10.1080/19443994.2013.801795

Kim, S.-R., Lee, K.-B., Kim, J.-E., Won, Y.-J., Yeon, K.-M., Lee, C.-H., Lim, D.-J., 2015. Macroencapsulation of quorum quenching bacteria by polymeric membrane layer and its

application to MBR for biofouling control. J. Membr. Sci. 473, 109–117. doi:10.1016/j.memsci.2014.09.009

Lee, S., Park, S.-K., Kwon, H., Lee, S.H., Lee, K., Nahm, C.H., Jo, S.J., Oh, H.-S., Park, P.-K., Choo, K.-H., Lee, C.-H., Yi, T., 2016. Crossing the Border between Laboratory and Field: Bacterial Quorum Quenching for Anti-Biofouling Strategy in an MBR. Environ. Sci. Technol. 50, 1788–1795. doi:10.1021/acs.est.5b04795

Li, X.Y., Yang, S.F., 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Res. 41, 1022–1030. doi:10.1016/j.watres.2006.06.037

Lin, H., Zhang, M., Wang, F., Meng, F., Liao, B.-Q., Hong, H., Chen, J., Gao, W., 2014. A critical review of extracellular polymeric substances (EPSs) in membrane bioreactors: Characteristics, roles in membrane fouling and control strategies. J. Membr. Sci. 460. doi:10.1016/j.memsci.2014.02.034

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193, 265–275.

Maqbool, T., Khan, S.J., Waheed, H., Lee, C.-H., Hashmi, I., Iqbal, H., 2015. Membrane biofouling retardation and improved sludge characteristics using quorum quenching bacteria in submerged membrane bioreactor. J. Membr. Sci. 483, 75–83. doi:10.1016/j.memsci.2015.02.011

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F., 2009. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. Water Res. 43, 1489–1512. doi:10.1016/j.watres.2008.12.044

Metzger, U., Le-Clech, P., Stuetz, R.M., Frimmel, F.H., Chen, V., 2007. Characterisation of polymeric fouling in membrane bioreactors and the effect of different filtration modes. J. Membr. Sci. 301, 180–189. doi:10.1016/j.memsci.2007.06.016

Miura, Y., Watanabe, Y., Okabe, S., 2007. Membrane Biofouling in Pilot-Scale Membrane Bioreactors (MBRs) Treating Municipal Wastewater: Impact of Biofilm Formation. Environ. Sci. Technol. 41, 632–638. doi:10.1021/es0615371

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. Environ. Sci. Technol. 46, 4877–4884. doi:10.1021/es204312u

Ramesh, A., Lee, D.J., Lai, J.Y., 2007. Membrane biofouling by extracellular polymeric substances or soluble microbial products from membrane bioreactor sludge. Appl. Microbiol. Biotechnol. 74, 699–707. doi:10.1007/s00253-006-0706-x

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C., 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. Water Res. 40, 710–720. doi:10.1016/j.watres.2005.11.028

Shariati, F.P., Mehrnia, M.R., Sarrafzadeh, M.H., Rezaee, S., Grasmick, A., Heran, M., 2013. Fouling in a novel airlift oxidation ditch membrane bioreactor (AOXMBR) at different high organic loading rate. Sep. Purif. Technol. 105, 69–78. doi:10.1016/j.seppur.2012.12.008

Shrout, J.D., Nerenberg, R., 2012. Monitoring Bacterial Twitter: Does Quorum Sensing Determine the Behavior of Water and Wastewater Treatment Biofilms? Environ. Sci. Technol. 46, 1995–2005. doi:10.1021/es203933h

Taga, M.E., Xavier, K.B., 2011. Methods for analysis of bacterial autoinducer-2 production. Curr. Protoc. Microbiol. Chapter 1, Unit1C.1. doi:10.1002/9780471729259.mc01c01s23

Thompson, J.A., Oliveira, R.A., Djukovic, A., Ubeda, C., Xavier, K.B., 2015. Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota. Cell Rep. 10, 1861–1871. doi:10.1016/j.celrep.2015.02.049

Trussell, R.S., Merlo, R.P., Hermanowicz, S.W., Jenkins, D., 2006. The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. Water Res. 40, 2675–2683. doi:10.1016/j.watres.2006.04.020

Waheed, H., Hashmi, I., Khan, S.J., Kim, S.R., Arshad, M., Nasir, H., 2016. Microbial population dynamics and profiling of quorum sensing agents in membrane bioreactor. Int. Biodeterior. Biodegrad., Challenges in Environmental Science and Engieering – 2015 113, 66–73. doi:10.1016/j.ibiod.2015.12.014

Wang, X.-M., Li, X.-Y., Huang, X., 2007. Membrane fouling in a submerged membrane bioreactor (SMBR): Characterisation of the sludge cake and its high filtration resistance. Sep. Purif. Technol. 52, 439–445. doi:10.1016/j.seppur.2006.05.025

Wang, Z., Wu, Z., Tang, S., 2009. Extracellular polymeric substances (EPS) properties and their effects on membrane fouling in a submerged membrane bioreactor. Water Res. 43, 2504–2512. doi:10.1016/j.watres.2009.02.026

Yigit, N.O., Harman, I., Civelekoglu, G., Koseoglu, H., Cicek, N., Kitis, M., 2008. Membrane fouling in a pilot-scale submerged membrane bioreactor operated under various conditions. Desalination, Selected Papers Presented at the 4th International IWA Conference on Membranes for Water and Wastewater Treatment, 15-17 May 2007, Harrogate, UK. Guest Edited by Simon Judd; and Papers Presented at the International Workshop on Membranes and Solid-Liquid Separation Processes, 11 July 2007, INSA, Toulouse, France. Guest edited by Saravanamuthu Vigneswaran and Jaya Kandasamy 231, 124–132. doi:10.1016/j.desal.2007.11.041

Yu, C.-H., Fang, L.-C., Lateef, S.K., Wu, C.-H., Lin, C.-F., 2010. Enzymatic treatment for controlling irreversible membrane fouling in cross-flow humic acid-fed ultrafiltration. J. Hazard. Mater. 177, 1153–1158. doi:10.1016/j.jhazmat.2010.01.022

Zhang, J., Chua, H.C., Zhou, J., Fane, A.G., 2006. Factors affecting the membrane performance in submerged membrane bioreactors. J. Membr. Sci. 284, 54–66. doi:10.1016/j.memsci.2006.06.022

Graphical Abstract