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Quorum Quenching in Anaerobic Membrane Bioreactor for Fouling

Control

Jianbo Liu ^{1,3}, Chin Yee Eng ¹, Jia Shin Ho ^{2,4}, Tzyy Haur Chong ^{2,4}, Li Wang

¹, Panyue Zhang ³, Yan Zhou ^{1,4*}

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

2. Singapore Membrane Technology Centre, Nanyang Environment & Water Research Institute, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

3. College of Environmental Science and Engineering, Hunan University, Changsha 410082, China

4. School of Civil and Environmental Engineering, Nanyang Technological University, Singapore 639798, Singapore

Abstract

Quorum quenching (QQ) is an effective method to control membrane biofouling in aerobic membrane bioreactors (AeMBRs). However, it is not clear if QQ is feasible in an anaerobic membrane bioreactor (AnMBR). In this study, *Microbacterium*. sp that has QQ capability was embedded in alginate beads, known as QQ beads (QQB), and applied in a lab-scale AnMBR to investigate their potential in fouling control. With

the addition of QQB, the operating period of AnMBR-QQB reactor was prolonged by about 8 - 10 times at constant flux operation before reaching the pre-set maximum transmembrane pressure (TMP). The concentration of Acyl-homoserine lactones (AHLs) in the bulk liquid was significantly higher during the 'TMP jump' period compared to QQB and control phases, while AHLs in the membrane foulants were remarkably lower in QQB phase compared to control phase. Furthermore, a much lower level of soluble microbial production (SMP) was observed in QQB phases. Extracellular polymeric substance (EPS), protein in particular, was reduced by 39.73-80.58% in the cake layer of the membrane from QQB phases. Significant changes of organic functional groups were observed in cake layer from QQB membrane as compared with that from control membrane. At the end of operation, bio-polymer (BP), building blocks (BB) and low molecular weight (LMW) organic matters increased in the foulant from control phases but such increase was not observed in QQB phase. After long-term operation, revival of QQB is required due to the declined activity for AHLs degradation.

Keywords: Quorum quenching; AnMBR; Membrane fouling control; AHLs.

1. Introduction

Anaerobic membrane bioreactor (AnMBR) has been used in the treatment of various types of wastewater due to its advantages of low energy consumption, energy recovery and low sludge residual (Smith et al., 2012; Stuckey, 2012; Su et al., 2019). However, the broad application of AnMBR is limited by several key factors, of which

the membrane fouling is one of the most important issues (Ahmed et al., 2012; Meng et al., 2017). Different methods have been explored for membrane fouling mitigation in AnMBR, such as novel membrane materials (Jeong et al., 2018), chemical cleaning and new mode of operation (Lew et al., 2009). However, many of these methods show low efficiency for mitigation of biofilm formation. Among the various fouling mechanisms, biofouling probably can be controlled and/or reduced in-situ since it is caused by the biological activities of the microbial community. Since quorum sensing (QS) was discovered, it has been found that the communication between microbial communities mainly depends on several types of signaling molecules (Le-Clech et al., 2006; Kalia et al., 2019). When the concentration of signaling molecules reaches a certain threshold, mutual growth between microorganisms is promoted and extracellular polymeric substances (EPS) is produced (Shrout and Nerenberg, 2012). It has been reported that the level of signaling molecules, e.g. Acyl-homoserine lactones (AHLs), which is the most common type in aerobic membrane bioreactor (AeMBR) system (Gül et al., 2017), is directly linked to the EPS production and biofilm formation on the membrane (Jiang et al., 2013).

AHLs can be facilitated by gram-negative bacteria and initiate biofilm formation (Huang et al., 2016; Yeon et al., 2009). In the biological wastewater treatment systems, there are many bacterial species that can secrete AHLs (Lin et al., 2003), e.g. *Pseudomonas* sp., *Rhodobacter maris*, and *Methanosaeta harundinacea*. AHL-based quorum sensing has been used to enhance the effectiveness wastewater processes (Jiang et al., 2013; Siddiqui et al., 2015). For example, AHLs played a significant role

in promoting ammonia activity and ammonium oxidation process (Chen et al., 2018; Clippeleir et al., 2011). However, in the membrane bioreactors, AHL-inducer may lead to the biofilm formation on the membrane surface, which in turn reduces the membrane flux and poses a serious problem in membrane technology for wastewater treatment (Weerasekara et al., 2016; Kalia et al., 2019).

Yeon et al. (2008) successfully used a quorum quenching (QQ) enzyme - acylase to control membrane fouling in an AeMBR. Kim et al. (2013) and Oh et al. (2012) discovered *Rhodococcus* sp. BH4 has quorum quenching effect, and applied the QQ technology in the full scale MBR plant to mitigate the membrane fouling. So far, many researchers have been working on identifying the quorum quenching effect of various bacteria, and optimizing the influence of QQ bacteria in AeMBR from the aspects of bacterial carrier, reactor operation mode and reactor design (Cheong et al., 2014, Mukherji et al., 2014; Ochiai et al., 2014; Ergön -Can et al., 2017).

Compared to AeMBR, AnMBR is a rising technology with lower energy consumption and resource recovery potential. However, it is not clear if QQ technology can also be applied in the AnMBR for fouling control. Ma et al. (2018) examined the diversity, distribution and function of AHL in an industrial anaerobic granular sludge and confirmed the role of four types AHL (N-hexanoyl-, N-octanoyl-, N-decanoyl- and N-dodecanoyl-DL-homoserine lactone, abbreviated as C6-HSL, C8-HSL, C10-HSL and C12-HSL, respectively) in promoting EPS production and granule formation (Feng et al., 2014). Thus, it is possible that quorum sensing mechanism also exists in the AnMBR.

In order to make sure QQ works under anaerobic conditions, anaerobic or facultative bacteria that are responsible for QQ should be selected. In addition to the well-known *Rhodococcus* sp. BH4, Kim et al. (2014) isolated 225 bacteria with QQ potency from their experimental-scale MBR and actual sewage plant, *Pseudomonas* sp., *Microbacterium* sp. and *Rhodococcus* sp. were found to have the AHL-degrading activities. Moreover, the AHL degradation function of genus *Microbacterium* was also confirmed by Wang et al. (2012). It is worth noting that *Microbacterium* sp. is a facultative anaerobe, which can be potentially applied under anaerobic conditions. However, it is not clear if *Microbacterium* sp. can maintain its quorum quenching function under anaerobic conditions.

The main objectives of this study are to (1) select effective QQ strain for the application under anaerobic conditions, (2) understand the working mechanisms of selected strain in controlling the formation of biofilm and prolonging the operation of AnMBR, (3) investigate the effect of QQ on the foulant compositions.

2. Materials and methods

2.1 QQ beads (QQB) preparation and QQ strain selection

Microbacterium sp., *Pseudomonas plecoglossicida*, *Pseudomonas putida* and *Enterobacter cloaca*, which were isolated by Kim et al., (2014) and Waheed et al., (2016), were individually entrapped in alginate beads, according to the method described by Waheed et al. (2017). Briefly, fresh QQ bacterial culture, grown in Luria Bertanni (LB) broth with an optical density of 1.00 (at 600 nm), was centrifuged at

4000 rpm for 30 min and re-suspended in a phosphate buffer solution (PBS). 5 mL of bacterial suspension was mixed with sterile sodium alginate (2% w/v). Then the mixture dropped into CaCl₂ solution (4% w/v) through a nozzle at a rate of 1 mL/min using a peristaltic pump. Produced alginate beads were coated with polysulfone, and stored in deionized water at 4 °C. The QQ activity of the four types of beads was tested in four AnMBR that were prepared with sealed Schott glass bottles (working volume of 500 mL). The beads to working volume ratio was 2% (V/V). The four reactors were operated with beads and assigned as M-AnMBR, Ppl-AnMBR, Ppt-AnMBR and E-AnMBR with *Microbacterium* sp., *Pseudomonas plecoglossicida*, *Pseudomonas putida* and *Enterobacter cloacae*, respectively. A control reactor (C-AnMBR) with vacant beads was also prepared. The hollow fiber membrane with pore size of 0.1 µm and designed surface area of 0.007 m² was used in the MBRs. The membrane flux was fixed at 8 L/m²·h (LMH) and hydraulic retention time (HRT) of the reactors was 18.5 h. The seed sludge was collected from an anaerobic membrane bioreactor in the laboratory. The AnMBRs were acclimated to synthetic wastewater (same as parent reactor), including glucose (341.75 mg/L), Peptone (102.25 mg/L), meat extract (35.25 mg/L), NH₄Cl (25 mg/L), KH₂PO₄ (10.24 mg/L), CoCl₂ (0.305 mg/L), Na₂MoO₄·7H₂O (0.048 mg/L), FeCl₂·4H₂O (2.01 mg/L), MnCl₂·4 H₂O (0.096 mg/L), NiCl₂·6H₂O (0.058 mg/L), NaHCO₃ (832 mg/L). The mixed liquor suspended solids (MLSS) concentration of all bioreactors was 5000 ± 100 mg / L, with particle size of 51.8 ± 0.8 µm. The temperature of the reactors was maintained at 35 ± 1 °C in a water bath.

According to the preliminary results obtained (Fig. S1), *Microbacterium* sp. was selected for long-term study. Then, two new AnMBR were set up in parallel with a working volume of 1 L (Fig. 1). Same type of hollow fiber membrane was applied and the operating conditions and sludge properties were similar as the previous reactor set-up. It should be noted that the configuration of the two reactors may not be identical since the reactors and membrane modules were manually fabricated. Thus, each reactor was operated in the following sequence: (1) control, (2) vacant beads (VB) and (3) QQ beads (QQB) phases for a fair comparison. In phase 1, there was no bead added. In phase 2, empty beads were added at a ratio of 2% (V/V reactor). In phase 3, alginate beads with entrapped *Microbacterium* sp. were added at a ratio of 2% (V/V reactor). At a constant flux operation, when the membrane is fouled, the transmembrane pressure (TMP) will rise. In this study, the experiments stopped when TMP reached 30 kPa. The fouled membrane was then cleaned with 0.14% NaClO. After that, next phase of experiment was then carried out. The operating conditions of different phases were detailed in Table S1.

2.2 AHLs analysis

The AHL molecules were extracted from the permeate from AnMBR. Samples were taken on day 1, 6, 15, 29, 43 and 59. Solids samples from the fouling layer on the membrane were taken at the end of experiment. For the samples from AnMBR permeate, 50 mL of ethyl acetate (EA) was added to an equal volume of permeate sample, and the mixture was dehydrated with Na₂SO₄ followed by drying in a rotary

evaporator. The residual was then dissolved in 50 uL acetonitrile (ACN). For the samples from foulant layer, 110 mL of deionized water was used to rinse off the attached foulants from the surface of membrane, both solids and liquid was collected and centrifuged. The supernatant was discarded (no AHLs were detected in the supernatant). The remaining solids were washed twice with PBS buffer solution and sonicated for 15 minutes, 30 mL of EA was then added in the remaining washed solids. The following steps of drying and re-dissolution were same as previously described. The AHL composition in ACN samples was analyzed using Agilent 6460 (USA) QQQ triple quadrupole mass spectrometer (MS) equipped with an AJS electro-spray interface ([Waheed et al., 2017](#)).

2.3 QQ activity examination

The QQ activity, i.e. degradation performance of AHL, of the QQB was evaluated before (QQB-before) and after (QQB-after) the experiment. Initial AHLs concentration was designed at 8 µg/L. Oxygen free Tris-HCl solution (pH=7.0) was used to dissolve and dilute AHLs. QQ beads were incubated with AHLs in sealed serum bottles in a 35°C shaker at 130 rpm for 24 hours. Headspace of serum bottles was filled up with N₂ before sealed. Similar test with VB was used as control. Liquid samples were taken at 0, 1, 2, 4 and 24 hours for the AHLs concentration measurement.

2.4 Fouling resistance

The filtration resistance was calculated using a resistance in series model ([Waheed et](#)

al., 2017):

$$R_t = \Delta P / J\mu = R_m + R_r + R_{ir}$$

where R_t is the total resistance (1/m), ΔP is the TMP (Pa), J is the flux ($\text{m}^3/\text{m}^2/\text{s}$), and μ is the permeate dynamic viscosity (Pa.s), R_m , R_r , and R_{ir} represents the membrane, reversible and irreversible resistance, respectively.

All the resistances were determined from filtration test with deionized water at fixed flux of 8 L/m²h. Briefly, R_t was measured before the foulant was removed from the membrane after the experiment; R_m was measured for new membrane (in the first test of each phase) or chemically-cleaned membrane (in the repeated test of each phase); $R_{ir} + R_m$ was measured after the foulant was removed from the membrane surface by rinsing with deionized water (as above in the AHLs analysis); R_r was measured by subtracting $R_{ir} + R_m$ from R_t .

2.5 Chemical analysis

Liquid samples were collected every alternate day from the influent and permeate of AnMBR for chemical oxygen demand (COD) and total organic carbon (TOC) analysis. Sludge samples were collected weekly for mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) analysis according to standard methods (APHA, 2005). Methane (CH₄) was analyzed by gas chromatography (GC) and quantified by a gas flow meter. In addition, the sludge samples were also collected on day 6, 14, 21, 31, 41, 51 and 59 for EPS extraction and analysis according to Waheed et al., 2017.

2.6 Membrane autopsy

An area of 0.7 cm² fouled membrane was sheared and dried at 60 °C. Dried membrane was analyzed with attenuated total reflection Fourier transform infrared spectrometry (ATR-FTIR) and visualized with scanning electron microscope (SEM). Liquid chromatography-organic carbon detector (LC-OCD) analyzer (LC-OCD Model 8, DOC-LABOR, Germany), a size-exclusion chromatography system coupled with organic carbon detection and organic nitrogen detection, was used for analysis of dissolved organic carbon (DOC) in the foulants according to their molecular weight into 5 main fractions, namely the biopolymers (BP), humic substances (HS), building blocks (BB), low molecular weight neutrals (LMW-N) and low molecular weight acids (LMW-A) (Huber et al., 2011). An area of 0.007 cm² fouled membrane was soaked in 0.85% NaCl solution for 1 day. Then, the solution was filtered through a 0.45 µm nylon syringe filter (Tisch Scientific, US) and the filtrate was diluted to TOC level below 5 mg/L before DOC analysis.

3. Results and discussion

3.1 Strain selection for anaerobic condition

The performance of the five reactors in terms of TMP profile, COD removal efficiency and methane composition is displayed in Fig. S1. The TMP of C-AnMBR, M-AnMBR, Ppt-AnMBR, Ppl-AnMBR and E-AnMBR rose to 30 kPa in about 113.7, 350.9, 114.1, 34.1 and 39.8 hours, respectively. Comparing the operating duration, COD removal efficiency and methane composition, the M-AnMBR out-performed

other reactors. Thus, *Microbacterium* sp. was selected in long-term study.

3.2 AnMBR performance with selected QQB

3.2.1 Transmembrane pressure (TMP) profile

As the performance of the two parallel reactors was equally representative, the result of one reactor is focused herein, the performance of the other reactor can be found in the supporting document (Fig. S4-S9). The TMP profile during the three phases of control, VB and QQB are described in Fig. 2 and Figure S4. It is clear that the presence of QQB can extend the operating duration by 8 -10 times compared to control. The vacant beads on the other hand were not helpful to extend the operation. The slight improvement in VB phase was likely due to the scouring effect from the beads (Kim et al., 2013). The results confirm the positive effect of QQB strategy to prolong the AnaMBR operation to delay the ‘TMP jump’ phenomenon. It should be noted that the TMP experienced a quick rise to 30 kPa within a very short period once TMP began to rise. The ‘TMP jump’ was also observed by the Lee et al., (2016), where AeMBR were operated with VB and QQ beads. The emergence of the TMP jump phenomenon could be attributed to the local flux effect and sudden changes in the biofilm or biocake structure (Zhang et al., 2006).

3.2.2 COD removal efficiency

The mean COD removal efficiency (Fig. 3) of 97.98, 98.84 and 98.75% was achieved during the control, VB and QQB phases, respectively, which reflects an excellent

permeate quality (average value of 17.2 ± 6.8 mg/L), and suggests that the addition of QQ bacteria did not affect the AnMBR performance. The results were also confirmed by the performance of reactor 2 (Figure S5).

3.2.3 Methane production

The methane production performance in the entire experiment was investigated (Fig. 4 and S6). During the control and VB phases, methane yield was around 252.48 - 338.80 mL/g·COD. Interestingly, a significant improvement occurred when QQBs were added in the reactors (336.63 mL/g·COD). That was mainly attributed to the addition of QQ bacteria that changed the microbial community structure, which may be more conducive to the function of methanogens (Xu et al., 2017). Surprisingly, the abundance of all methanogens in control phase was in fact higher than that in the QQB phase (Fig. S2). The reason for the increase in methane yield in the QQB phase is probably because methanogens may have higher activity in the QQB phase (Liu et al., 2017a; Liu et al., 2017b). Smith et al. (2011) also observed that methanogens with low relative abundance could present high relative activity.

3.3 AHLs profiles

In this study, AHLs types and concentration were closely monitored with the absence and presence of *Microbacterium* sp. beads. The results are shown in Fig. 5 and S7. Both types and concentrations of AHLs in the bulk liquid phase of AnMBR varied in the operation stage as well as the fouled stage (Fig. 5a and S7a). The most abundant AHL before TMP jump during the control and QQB phases was N-heptanoyl-

DL-homoserine lactone (C7-HSL) in both reactors, with an average concentration of 130.95 (147.97 ng/g-VSS in reactor 2) and 177.20 (153.30 ng/g-VSS in reactor 2) ng/g-VSS, respectively. ~~Its concentration accounted for more than 90% of all AHLs, which indicates that the long chain signal molecules secreted by quorum sensing bacteria in the sludge system are more easily degraded. This observation is consistent with the study in aerobic membrane bioreactor (Tan et al., 2015; Yu et al., 2016).~~

Other compounds such as N- butyryl-DL-homoserine lactone (C4-HSL) and N-(3-oxohexanoyl)-L-homoserine lactone (3OC6-HSL) appeared at extremely low concentrations, with an average of 13.97 (16.80 ng/g-VSS in reactor 2) and 3.57 (0.49 ng/g-VSS in reactor 2) ng/g-VSS, respectively. However, when TMP jump occurred during the control phase, the concentration of C7-HSL dropped to 0.00 ng/g.VSS. At the same time, the concentration of N-octanoyl-, N-dodecanoyl-, and N-tetradecanoyl-DL-homoserine lactone (C8-, C12- and C14-HSL) and N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) in the permeate increased. In addition, C8-HSL increased significantly during the QQB phase, much higher than those during the control phase.

The concentration of AHLs in the fouling layer is also an important indicator that can directly reflect the relationship between the AHLs and the fouling extent. The AHLs types and corresponding concentration in the fouling layer for the control, VB and QQB phases are shown in the Fig. 5b and S7b. C4-, C8-, and C14-HSL were mainly detected, and a very small amount of N-(3-oxotetradecanoyl)-L-homoserine lactone (3OC14-HSL) was also present in reactor 1. C4- and C8-HSL were also

dominant in reactor 2. It shall be noted that the concentration of AHLs in the foulants for the QQB phase was much lower than that in the control and VB phases, especially C4- and C14-HSL (23.02 v.s. 1.99 ng/g-VSS and 201.77 v.s. 0.81 ng/g VSS) in reactor 1. Meanwhile, C8-HSL was also only reduced by 35.5% with the presence of QQB. C7-HSL was the major detected QS signaling molecule and maintained at a high level in the permeate before TMP jump during both control and QQB phases. However, the AHLs in the foulant extracted from the membrane surface appeared to have different profile. First, no or very low level of C7-HSL was detected in all the foulants in both reactors. The AHL content in the foulant for QQB phase was significantly less than that for control and VB phase. This is consistent with the study by [Kim et al. \(2013\)](#) where the AHL concentrations in the biofilm obtained from cell entrapping beads were significantly less than in the biofilm on vacant beads. It could be deduced that the main reason that *Microbacterium* sp. can effectively reduce the biofilm on the membrane surface, thus delaying the TMP jump, was due to the degradation of AHLs by QQB in the fouling layer. However, C8-HSL was not successfully suppressed in the fouling layer by QQB.

3.4 EPS and SMP profiles

In this study, the profiles of PS and PN in EPS and SMP were investigated in different phases (Fig. 6 and S8). The overall trends of the two reactors were similar. The EPS profile of reactor 1 is discussed in details. A 35.42% lower concentration of polysaccharide in loosely bond EPS (LB-PS) was observed during the QQB phase

300 compared to the control phase. When the TMP jump occurred, the concentration of
301 LB-PS increased slightly, but still significantly lower than that in the control and VB
302 phase (36.76 vs. 43.74 and 46.27 mg/g·VSS). Similar trend of polysaccharide in SMP
303 (SMP-PS) was observed. The overall change was not significant. Protein in loosely
304 bound EPS (LB-PN) and polysaccharides in tightly bound EPS (TB-PS), were at low
305 concentration and there was no significant change during the three phases (0.41 - 0.70
306 mg/g·VSS). It is noteworthy that the content of protein in SMP (SMP-PN) and tightly
307 bound EPS (TB-PN) did not show a clear trend. The maximum concentration of
308 SMP-PN was observed at the beginning of the QQB phase. When the TMP jump
309 occurred, the SMP-PN concentration decreased again. The total polysaccharides and
310 proteins (PS+PN) displayed a similar trend of change with that of LB-PS. The results
311 are in agreement with previous studies that the amount of EPS and SMP would
312 increase with the increase of membrane biofouling potential (Aquino et al., 2010;
313 Chen et al., 2017).

314 The polysaccharide and protein content in loosely bound EPS and tightly bound
315 EPS extracted from the membrane foulant is described in Fig. 6b. The amount of
316 polysaccharide was relatively consistent and low in both LB - and TB-EPS for all
317 phases. Compared to the control phase, TB-PN and LB-PN were remarkably lower in
318 the foulant from the QQB phase. This conclusion is in good agreement with the
319 concentration of AHL in membrane foulant (Fig. 5b) (Davies et al., 1998; Yeon et al.,
320 2008). It was suggested by other researchers that the concentration of the quorum
321 sensing signal molecules in the sludge system would affect the secretion of SMP and

EPS, and thus affect the membrane biofilm formation (Flemming et al., 1997; Le-Clech et al., 2006). Further, the secretion of EPS can affect the amount of biomass attached to the membrane surface and cause a sharp increase in TMP (Wang et al., 2009; Iqbal et al., 2018).

In the bulk liquid and membrane foulant, the total EPS content was reduced by 15.19% and 75.32% in QQB phase compared to control phase, respectively. Thus, the decline of EPS content in fouling layer should be the main reason for mitigating membrane biofouling. Among the EPS components in foulant, the amount of protein decreased significantly, 80.98% and 82.83% reduction, for QQB phase compared to control and VB phase, respectively. This is consistent with the report in Weerasekara et al. (2014), which showed the protein content was the main factor affecting membrane biofouling. In short, *Microbacterium* sp. could reduce AHL levels in foulant (Fig. 5), which in turn suppressed the EPS production and biofilm formation, thus prolonged the MBR operation.

3.5 Fouling resistance evaluation

The ratios of R_c , R_{ir} and R_m to the R_t in each phase was compared and described in Fig. 7 and S9. It should be noted that fouling and membrane resistance is closely related to MBR operation length and fouling extent. Given the operation period and performance of the two reactors were not identical. The results of one of the reactors are present here. R_{ir}/R_t increased by 1.36 folds after 59 days operation compared to control phase in reactor 1. This suggests that more molecules or small particles

blocked the membrane pores with multiple times usage of the membrane. [Shen et al. \(2015\)](#) suggested that the small particles were difficult to be obliterated once they attached the membrane. At the same time, it is noted that the rise of R_{ir}/R_t was the main factor for membrane fouling in the QQB phase ([Maqbool et al., 2015](#)). In addition, R_c/R_t slightly decreased by 2.53%, indicating that less biofilm accumulated on the membrane surface, which was also consistent with the above results and discussion on EPS and AHL content in the biocake. R_m/R_t reduced from 61.86% to 56.35% during the operation.

3.6 Foulant analysis

From the results above, it can be concluded that QQ bacteria can effectively control the production of protein in EPS, thereby reducing EPS/biofilm formation on the membrane. Therefore, QQ bacteria had a corresponding influence on the organic matter formation on the biocake. ATR-FTIR and LC-OCD were employed to further identify the role of QQB on alteration of organic matter composition.

Compared with the new membrane, there were prominent increase in several peaks for the fouled membranes from the control and QQB phase, such as the bands at 3251.96, 2922.16 and 1633.71 cm^{-1} , corresponding to aliphatic hydroxyl, methylene and amide groups, respectively (Fig. 8) ([Weerasekara et al., 2014](#)). The broad and intense peak for control phase at 3251.96 cm^{-1} refers to the N-H and O-H stretching vibrations in polysaccharides and proteins, this could be attributed to the larger amount of polysaccharide and proteins formed on the control membrane. Furthermore,

the control phase has more intense peaks at 1539 and 1633 cm^{-1} as compared to QQB phase. This could be associated with the amide group in protein which formed on the control membrane. This is consistent with the EPS results detailed in Section 3.4. The other bands at 1394.53, 1180.44, 875.68, 840.96 and 487.99 cm^{-1} were detected in both new membrane and fouled membrane from QQB phase. Obvious difference was observed in the spectra (500-1300 cm^{-1}) between the new membrane and fouled membrane from control phase, which was attributed to a series of reactions occurred at organic bonds with chemical bonds on the membrane surface (Corker et al., 1996; Kim et al., 2008). Further, the intensity of spectra for the fouled membrane from control phase was significantly stronger than QQB phase, indicating that less foulant was deposited on the membrane. This again confirms that QQB could reduce the biofilm formation.

The fouled membranes were visualized using SEM (Fig. S3). It can be seen that the surface of the fouled membranes from control and QQB phase was covered with a layer of dense organic matter. However, the images did not show a clear difference between the control and QQB membrane surface.

The organic components of foulant were characterized by LC-OCD (Table 1). The total DOC concentration of the foulants from the control and QQB phase were comparable (1488 $\mu\text{g/L}$ and 1491 $\mu\text{g/L}$, respectively). However, the compositions of hydrophobic organic carbon (HOC) and chromatographic dissolved organic carbon (CDOC) were distinguishingly different. Compared to the foulant from the control phase, the CDOC substances in the foulant from the QQB phase was greatly reduced

from 1434 $\mu\text{g/L}$ to 921 $\mu\text{g/L}$, in particular the biopolymer and building blocks decreased by 3-fold, while the HOC fraction was 10 times higher. The results suggest that CDOC substances are the major contributor to TMP jump, which agrees with the work by Chen et al. (2017). In addition, LMW-N has a very important role in membrane fouling (Aryal et al., 2009). It is found that the concentration of LMW-N was slightly higher in the foulant from control phase. The study reveals a direct impact of the QQ activity on reducing the BP and BB fractions, and consequently mitigating the membrane biofouling.

3.7 QQB activity

In order to have long-term stable operation, it is critical that the QQB can maintain the high activity of AHLs degradation (Kim et al., 2013). In this study, the degradation activity of QQB before and after usage in the AnMBR (labeled as QQB-before and QQB-after, respectively) was investigated using AHL standard solution. In general, long-chain AHLs such as 3OC6-HSL, 3OC8-HSL, 3OC12-HSL and 3OC14-HSL could be rapidly degraded to below the detection limit within 4 hours by both QQB-after and QQB-before, while it took longer to reduce the remaining AHLs (Fig. 9). According to the results from control reactor, the absorption of AHLs on the beads or hydrolysis of AHL may also occur (Decho et al., 2011). Assuming the trends of absorption and hydrolysis of AHLs were similar in all the reactors, the results suggest that there was a decline in the degradation activity of the QQB-after compared to fresh QQB. Kim et al. (2013) revealed that the QQ activity of beads entrapped

Rhodococcus sp. BH4 increased by about 3% after 30 d of lab-scale MBR operation. However, Lee et al. (2016) found that the activity of QQ beads had a 10-20% reduction after 100 days of operation compared to the initial status. It seems the activity of QQB would be related to many factors, e.g. operation period, activity of QQ bacteria, the interaction between the beads with local microbial community and wastewater properties, etc. Further study is required to investigate the optimization and revival of the beads' activity.

Conclusions

This study demonstrated that *Microbaterium* sp. embedded beads (QQB) can effectively control membrane biofouling in AnMBR. The operating period was significantly prolonged due to the degradation of AHLs by the QQB and subsequent suppression of EPS and SMP production. Furthermore, the following conclusions can be derived from this study:

1. AHLs concentration was reduced in QQB phase, and this is an important factor for the reduction of membrane biofouling.
2. EPS and SMP contents in the reactor and membrane foulant were closely related to the AHL concentrations.
3. Remarkable difference was found in the organic fractions and compositions in the membrane foulant obtained from the control and QQB phases.
4. CDOC substances contributed more to membrane fouling under anaerobic conditions.

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Fig. 1 The schematic diagram of the parallel AnMBR set-up

598

599 **Fig. 2** TMP profiles of control (day 0 to day 6), VB (day 6 to day 14) and QQB (day

600 14 to day 59) phases.

Fig. 3 COD removal performance during the control, VB and QQB phases. Due to the power failure on day 6, a COD concentration of 105.8 mg/L was observed in the permeate, but it was reduced to 18.2 mg/L soon after the power was resumed.

605

606 **Fig. 4** Methane yields during the phases of control, VB and QQB.

Fig. 5 AHLs concentration in AnaMBR permeate during the control, VB and QQB phases (a) and in membrane foulant from each phase (b).

Fig. 6 PS and PN concentration in SMP and EPS in the bulk liquid (a) and membrane foulant (b). PS: polysaccharide; PN: protein; SMP: soluble microbial product; LB: loosely bound; TB: tightly bound.

Fig. 7 Membrane resistance (%) analysis, where R_t , R_c , R_{ir} and R_m represents the total hydraulic resistance, cake layer resistance, irreversible resistance and intrinsic membrane resistance, respectively.

Fig. 8 ATR-FTIR spectra of the new membrane, fouled membrane from control and QQB phases.

Fig. 9 The activity to degrade AHLs of QQ beads before operation (QQ-before) and QQ beads after operation (QQ-after).

Table 1 LC-OCD analysis of organic fractions in the foulants from control and QQB phase.

DOC HOC CDOC BP BB LMW-N LMW-A							
μg/L							
% DOC							
Control	1488	54	1434	387	310	735	2
	100%	3.6%	96.4%	26.0%	20.8%	49.4%	0.0%
QQB	1491	570	921	102	146	671	3
	100%	38.2%	61.8%	6.8%	9.8%	45.0%	0.2%

Notes: DOC, HOC, CDOC, BP, BB, LMW-N and LMW-A represents dissolved organic carbon, hydrophobic organic carbon, chromatographic dissolved organic carbon, bio-polymer, building block, low molecular weight neutrals and low molecular weight acids, respectively.