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In tandem effects of activated carbon and quorum quenching on fouling control and simultaneous removal of pharmaceutical compounds in membrane bioreactors

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Abstract

This study aimed to integrate the quorum quenching (QQ) strategy with powdered activated carbon (PAC) adsorption for fouling control and simultaneous removal of trace amounts of selected pharmaceutically active compounds (PhACs) in laboratory membrane bioreactors (MBRs). With the addition of QQ strains immobilized in PAC-alginate beads, a 4.6-folds delay in fouling was achieved. The QQ strains not only altered the sludge properties, but also influenced the microbial communities in the MBRs, thus leading to distinct quorum sensing signal molecule (acyl-homoserine lactones, AHLs) profiles. Despite the difference in AHL profiles, the total AHL concentrations were greatly reduced in bulk sludge, and completely quenched in biocake with QQ supplementation. PAC addition enabled high removals of all PhACs, and also resulted in a prominent increase in sludge floc size, which further enhanced sludge filterability. These QQ-entrapped PAC-alginate beads provide a novel MBR setting with less biofouling and high removal efficiency of PhACs.

Keywords: Fouling control, Membrane bioreactor (MBR), Pharmaceutically active compounds (PhACs), Powdered activated carbon (PAC), Quorum quenching (QQ)

1. Introduction

Membrane bioreactor (MBR) has gained increasing popularity in wastewater reclamation globally, owing to its prominent advantages of smaller footprint, better effluent quality and less sludge production as compared to conventional activated sludge processes [1,2]. However, the inherent membrane biofouling remains a major challenge to its wider

application, as the cost of biofouling control accounts for about 50% of its total energy consumption [2].

For the past few years, bacterial quorum quenching (QQ), which introduces QQ bacteria, either in liquid cultures [3,4] or immobilized in alginate beads [5], to MBRs, has been adopted to retard biofilm development on membrane via interrupting quorum sensing, the cell-to-cell communication among bacteria. Successful applications of QQ bacteria to biofouling control in laboratory [5–8] as well as pilot MBRs [9] have been reported. However, continuous suspension of immobilized QQ bacteria within the bioreactor caused the reduction in floc size [9,10]. This may further alter the sludge characteristics, including reduction in sludge settleability and dewaterability along with the release of more extracellular polymeric substances (EPS), thereby worsening membrane fouling. Moreover, pore blockage, which was identified as a governing factor leading to membrane fouling when QQ was applied, could not be tackled through QQ mechanism. Alternatively, addition of activated carbon (AC) to MBRs has shown some effects in adsorbing micromolecular organics thus reducing pore blockage and mitigating membrane biofouling [11,12]. PAC is also widely utilized for removing organic micro pollutants (OMP) including odor compounds, pharmaceuticals, and industrial chemicals from drinking waters and wastewater treatment plant (WWTP) effluents [13,14].

This study aimed to integrate QQ strategy with PAC addition to improve the overall efficacy of fouling control. Batch adsorption isotherm tests were conducted to determine the optimal PAC dosage in the integrated beads prior to tests in MBRs. As the biocake consists of diverse groups of bacteria communicating with a variety of quorum sensing

(QS) signal molecules, which could be very difficult to be quenched by a single QQ strain, a consortium of bacterial strains capable of quenching different signal molecules was used. The production of soluble microbial products (SMP) and extracellular polymeric substances (EPS), change of sludge floc size and sludge filterability, together with the quorum quenching activity were continuously monitored to gain deep understanding of the integrated fouling control strategy. In addition, simultaneous removal of OMP was also evaluated. Five pharmaceutically active compounds (PhACs), namely trimethoprim (TrMP), sulfamethoxazole (SMX), carbamazepine (CBZ), diclofenac (DCF) and triclosan (TCS), were selected as the target trace organic compounds based on their broad range of hydrophobicity.

2. Materials and Methods

2.1. Chemicals and QQ strains

Unlabelled SMX, CBZ, DCF and TCS were purchased from WAKO (Singapore), while TrMP was purchased from MP Biomedical (Singapore). Standards of deuterium-labeled TrMP-d₃, SMX-d₄, CBZ-d₁₀, DCF-d₄, and TCS-d₃ were purchased from C/D/N Isotopes Inc. (Quebec, Canada). 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).

Three acyl-homoserine lactones (AHLs)-degrading strains *Enterobacter cloaca* QQ13, *Microbacterium* sp. QQ1 and *Pseudomonas* sp. QQ3 isolated from activated sludge in the authors' laboratory, together with *Rhodococcus* sp. BH4 [6,7] and one autoinducer-2 (AI-2) degrading strain *Escherichia coli* Δ lsrR Δ luxS [15], were formed as a QQ consortium for this study [16]. The AHL-degrading strains were grown in Luria-Bertani (LB) broth at 30 °C for about 16 hours prior to immobilization in beads, while the AI-2-degrading strain was grown in LB broth containing 100 mg/L of streptomycin at 37 °C for ~ 16 hours.

2.2. Beads preparation

The QQ strains and PAC were immobilized in alginate beads with the method modified from previous reports [5,7,8]. Briefly, the PAC-alginate beads were prepared by mixing PAC (SAE2, Norit, the Netherlands) with Na-alginate solution (2% w/v) at a dosage of 1%, the optimized ratio based on batch adsorption tests as detailed in the Appendix A, and dripping into CaCl₂ solution (4 % w/v) through a nozzle at a rate of 1 mL/min using a peristaltic pump; after soaking in the CaCl₂ solution for 3 hours, half of the beads were dipped in polysulfone solution (8 % w/v in N-methyl-2-pyrrolidone) for 10 seconds before transferring to Milli-Q H₂O and stored at 4°C until use, while the remaining beads were transferred to Milli-Q H₂O directly. Beads without PAC were also prepared as control.

To prepare QQ-entrapped PAC beads, the QQ strains were harvested from the fresh-grown cultures and resuspended in a phosphate buffer solution (PBS); then, they were added (10% v/v) to the PAC-Na-alginate mixture (same method as above). The QQ strains were entrapped separately in the beads (2 mg QQ bacteria/g alginate) and added equally to MBRs to avoid non-homogenous mixing.

2.3. Lab scale MBR set-up and operation

A cylindrical acrylic reactor (working volume of 3.2 L), equipped with a hollow fiber ultrafiltration PVDF membrane module (ZeeWeed, GE, US) with a pore size of 0.04 μm and surface area of 0.047 m^2 (submerged), was operated as a conventional MBR (MBR-C), MBR with vacant PAC beads (MBR-PAC_V), and MBR with QQ-PAC beads (MBR-PAC_{QQ}) sequentially. For the latter two modes with beads inside, the membrane was backwashed with the effluent at the same flux as of filtration for 5 minutes every 2 hours. Each operation cycle was terminated when the trans-membrane pressure (TMP) values reached 30 kPa. Then, the membrane was removed from the reactor, soaked in sodium hypochlorite solution (2%) for one hour and rinsed thoroughly with tap water prior to next run, except for MBR-C where virgin membrane was used. A synthetic wastewater was used as the feed for MBR-C, while the five PhACs were supplemented to the feed at concentrations of 2 $\mu\text{g/L}$ for the others. The recipe of the synthetic wastewater, the properties of the PhACs, and the diagrams of the MBRs were detailed in Appendix B.

The sludge (collected from Ulu Pandan Water Reclamation Plant, Singapore) was acclimatized to the synthetic wastewater for one month before being used as the seed sludge. The mixed liquor suspended solids (MLSS) concentration was maintained in the typical range of MBR (5.7 ± 0.5 g/L) throughout the study. The solids retention time (SRT) and hydraulic retention time (HRT) were maintained at 30 d and 6 h, respectively, at an initial flux of 15 $\text{L/m}^2/\text{h}$ (LMH). The beads were added to the MBR at a volume ratio of 5%.

2.4. System characterization and analysis

Membrane resistance analysis - Membrane resistances were evaluated using a simple resistance in series model as detailed previously [16]. In MBR, the total hydraulic resistance R_t is the sum of cake layer resistance (R_c), pore blockage resistance (R_p) and intrinsic membrane resistance (R_m).

PhACs and AHLs analysis - PhACs in aqueous samples from the MBR experiments were concentrated using the 6-mL 30- μ m Oasis[®] HLB solid-phase extraction (SPE) cartridges (Waters, Singapore) as described previously [17]. The amount of PhACs adsorbed onto sludge or beads were extracted from 1 mL of mixed liquor sample or 10 randomly-selected beads using liquid-liquid extraction. The beads were grinded with a pestle before extraction. After pre-treatment, PhAC was analyzed with liquid chromatography mass spectrometer (LC/MS/MS). AHLs and AI-2 were extracted or derivatized and then analyzed with LC/MS/MS as detailed previously [16].

EPS extraction and analysis - The heat extraction method [18] was used for extraction of soluble EPS, loosely bound (LB) EPS, and tightly bound (TB) EPS; the protein (PN) and polysaccharide (PS) content in each fraction were analyzed as detailed previously [16].

SMP analysis - Size-exclusion chromatography, in combination with organic carbon and nitrogen detections (LC-OCD-OND) (DOC-LABOR, Germany) was used to characterize and quantify soluble microbial products (SMP) in the mixed liquor of all MBRs. Briefly, organic compounds, i.e. biopolymers, hydrophilic dissolved organic carbon (DOC), hydrophobic DOC, high molecular weight (HMW) protein, building blocks, low molecular weight (LMW) neutrals, LMW acids and HMW polysaccharide, were identified,

with details described elsewhere [19]. The software ChromCALC (DOC-LABOR, Karlsruhe, Germany) from the manufacturer was used for data acquisition and processing [20].

The concentrations of total protein and polysaccharide were determined by the modified Lowry method and phenol-sulphuric acid method [21]. The concentrations of LMW protein and LMW polysaccharide were determined by subtracting HMW protein and HMW polysaccharide from the total protein and polysaccharide concentrations tested by spectrometer method, respectively.

Performance and sludge characterization - 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 [22]. The dissolved oxygen (DO), specific oxygen uptake rate (SOUR), floc size distribution, and capillary suction time (CST) were monitored using previously reported methods [16]. Sludge flocs were also imaged occasionally using a fluorescent microscope (Nikon-ECLIPSE, Japan). Zeta potential and electrical conductivity (EC) of mixed liquor was measured through a zetasizer (MRK570-01, Malvern, UK).

RNA Extraction and microbial community analysis - Duplicate samples of bulk sludge, biocake and beads from MBRs were frozen immediately at -80°C when the operation was terminated. Genomic DNA and RNA were extracted from the frozen samples using the method of Towe et al. [23]; the mixture was treated with RQ1 RNase-Free DNase (Promega, USA) according to the manufacturer's instructions to obtain RNA. The cDNA was synthesized from the RNA using the GoScript Reverse Transcription System

(Promega, USA) in a total volume of 40 µl as per the manufacturer's protocol. The cDNA was used for sequencing on an Illumina Miseq platform at the Research Technology Support Facility, Michigan State University, US. Dual indexed Illumina compatible libraries were prepared using primers described by Takahashi et al. [24] which amplifies the V3-V4 region of the 16S rRNA of a wide range of bacterial and archaeal species. Following polymerase chain reactions (PCR), the reaction products were normalized using Invitrogen Sequal Prep DNA normalization plates and recovered DNA was pooled. The final pool was cleaned up using AmpureXP. Library pools were validated and quantified using a combination of Qubit dsDNA assay, Caliper LabChipGX and Kapa Illumina Library Quantification qPCR kit. Each pool was loaded on an Illumina MiSeq standard v2 flow cell and sequencing was performed in a 2x250bp paired end format using a v2 500 cycle reagent cartridge.

The sequencing data was analyzed using the QIIME 1 pipeline [25]. The paired end data was joined using the join_paired_ends script at the settings of 50 bp of min_overlap and 15 % of perc_max_diff, and then quality filtered using the default strategy in QIIME [26]. The sequences were clustered into operational taxonomic units (OTUs) using the open-reference OUT picking protocol [27]. The diversity was analyzed using the core-diversity-analyses script and the results were visualized in principal coordinate analysis (PCoA) plots with the tool of Emperor [28].

3. Results and Discussion

In this study, five independent sets of batch-scale experiments with different bead compositions and bead loads (i.e. beads to reactor's working volume ratio) were conducted

to optimize the adsorption of PhACs. The results show that the same adsorption of PhACs was achieved irrespective of an increase in PAC dose from 1% (set 1) to 2% (set 2); the increase in bead volume ratio from 1 to 5% resulted in a significant increase in adsorption of PhACs, but beyond 5% no further increase was observed. Therefore, a PAC dose of 1% and a bead dose of 5% were selected for the MBR studies. More details are provided in the Appendix A. Addition of PAC to the alginate beads slightly increased their mechanical strength, but no apparent changes in size and shape were observed with the addition of QQ strains, which was also observed by [5].

3.1. Effect of PAC and QQ on MBR performance

Fig. 1 shows the time-course of TMP in MBR-C, MBR-PAC_V and MBR-PAC_{QQ}. The virgin membrane in MBR-C was fouled after 4 days of operation. An increase in filtration time by 100% was achieved in MBR-PAC_V through the combination of PAC and backwash. With the addition of QQ, the filtration time was further increased by 450% in MBR-PAC_{QQ}, this clearly shows that integration of QQ strategy along with PAC impeded the membrane fouling to a maximum extent.

To elucidate the impacts of PAC and QQ on membrane fouling control, membrane resistance analysis was performed after each MBR run. A slight rise in the intrinsic membrane resistance (R_m) was observed after every chemical cleaning (Table 1). For MBR-C, the cake resistance (R_c) contributed to 86% of the total resistance (R_t), indicating the strong cake layer formation on membrane surface within the 4 days of operation. The contribution of R_c decreased to 50% in MBR-PAC_V; addition of QQ bacteria to PAC beads further reduced the relative contribution of R_c by another 50% as compared to MBR-PAC_V.

The superior reduction in cake layer formation by PAC_{QQ} beads was more prominent when the average increasing rates of resistances were compared; the average increasing rate of R_c in MBR-PAC_{QQ} was 1/5 of that in MBR-PAC_V, and only 1/46 of that in MBR-C. The average increasing rate of R_p in MBR-PAC_V was the largest among the three reactors. The mechanical scouring effects of PAC_V beads might have resulted in a higher release of EPS from sludge flocs as compared to MBR-C, and the PAC adsorption alone could not counteract this effect; therefore, more soluble products led to the higher increasing rate of R_p in MBR-PAC_V. Instead, in MBR-PAC_{QQ}, the PAC adsorption together with QQ effectively reduced the release of soluble products, and resulted in an average increasing rate of R_p similar to that in MBR-C. This clearly shows the effective control of both cake layer formation and pore blockage by the combination of QQ and PAC.

Various fouling control mechanisms of PAC addition have been proposed in the literature. Loulergue et al. [29] suggested that PAC particles could alter the structure of cake layer, making it thicker but more permeable; while Remy et al. [12] showed that PAC could make cake layer less compact. At low dosages, PAC was believed to mitigate biofouling by enhancing the strength of sludge flocs and decreasing foulant release [12]. In the present study, PAC particles were entrapped in alginate beads, which could greatly enhance the mechanical scouring effects while keeping the adsorption of foulants.

3.2. QS signal molecules

It is widely accepted that QQ mitigates biofouling in MBRs via quenching the QS signal molecules; however, very few studies have characterized the QS signal molecule profiles in MBRs and monitored their changes with QQ addition. In the present study, the

AHL concentrations in bulk sludge and biocake of all three MBRs were monitored when the MBRs were fouled. It is noted that the composition of AHLs in bulk sludge (Fig. 2A) were completely different among the three reactors. C6-HSL, the dominant AHL in both MBR-C and MBR-PAC_{QQ}, was not detected in MBR-PAC_V. 3OC12-HSL, an important one in both MBR-PAC_V and MBR-PAC_{QQ}, was not detected in MBR-C. Most of the AHLs in MBR-PAC_V including C4-HSL, C10-HSL and C14-HSL were not detected in other 2 MBRs. Furthermore, the biocake showed an AHL profile (Fig. 2B) different than that in the bulk sludge of the same reactor. C8-HSL, the most widely detected AHL in MBRs [30], was an important AHL species in biocake of both MBR-C and MBR-PAC_V, however, it was at very low concentrations in sludge. No AHL was detected in the biocake of MBR-PAC_{QQ}. The AHL concentrations in sludge were in the range of 0.5-1 ng/mg VSS for C6- and 3OC8-HSL in MBR-C, 0.25-0.5 ng/mg VSS for C4-, C10-, 3OC12-, and C14-HSL in MBR-PAC_V, and 0.2-0.4 ng/mg for C6- and 3OC12-HSL in MBR-PAC_{QQ}. All of these are slightly higher than those detected in aerobic granules [31], but are two orders of magnitude higher than other detected AHLs (Table C.1. in Appendix C). The differences in microbial communities between the MBRs (detailed in section 3.6) might have resulted in different AHL-mediated quorum sensing molecules. The difference in growth stages might also contribute to the difference in AHL profiles, as indicated by other studies [31].

Despite the distinct AHL profiles, the total AHL concentrations in the sludge of MBR-C and MBR-PAC_V were the same (~1.5 ng/mg VSS), which were 2.5 times as many as that in MBR-PAC_{QQ} (~0.6 ng/mg VSS). The total AHL concentrations in biocake of MBR-C and MBR-PAC_V were 3900 ng/m² and 6000 ng/m², respectively; while no AHLs could be detected in biocake of MBR-PAC_{QQ}. These results suggest that QQ partially

reduced AHLs in bulk sludge, but majorly depleted AHLs, especially the C8-HSL, in biocake, which is consistent with the previous findings [6,7,9].

In contrast to the significant reduction in AHLs, the AI-2 levels in MBR-PAC_{QQ} were almost the same as those in other MBRs when it was fouled, although it was reduced by more than 40% on day 4 (Fig. C.1 in Appendix C). The low survival of AI-2 degrading strain *E. coli ΔlsrRAluxS* in MBR-PAC_{QQ} (detailed in section 3.6) could be the major cause for this low efficiency in quenching AI-2.

3.3. Effects of PAC and QQ on SMP production

Generation of SMP in MBRs is a serious concern, as they contribute directly to biofouling [32], and effluent COD if permeate of MBR is recycled for various applications [33]. In the present study, the initial and final levels of SMPs in all MBRs were determined as the dissolved organic carbon (DOC), among them the hydrophilic (HI) DOC was fractionated into biopolymers, building blocks, low molecular weight (LMW) acids and neutrals via LC-OCD-OND. As shown in Fig. 3A, PAC addition did not counteract the build-up of SMPs in MBR-PAC_V and MBR-PAC_{QQ}, which indicates that PAC might have adsorbed certain DOC initially but up to saturation. Nevertheless, the strong affinity of aromatic compounds to PAC have resulted in a significant reduction of 74 and 36% in the aromaticity of SMPs in MBR-PAC_V and MBR-PAC_{QQ} (Fig. C.2 in Appendix C), respectively.

Fig. 3A also shows that, the DOC composition in MBR-PAC_{QQ} was significantly different from the other two when they were fouled. In MBR-PAC_{QQ}, DOC was primarily composed of LMW neutrals and hydrophobic (HB) DOC, which contributed to 76% and 20% of the total DOC content, respectively; whereas in the others, the sum of LMW

neutrals and HB DOC were less than 50%. Furthermore, the concentrations of building blocks, biopolymers and LMW acids decreased significantly in MBR-PAC_{QQ}, while they all increased in the other two MBRs. The decrease in high MW organic compounds like biopolymers and building blocks in MBR-PAC_{QQ} could be due to the decomposition of these molecules to low MW organics, as observed in an earlier study [34].

The biopolymer in SMPs was further characterized as polysaccharides and proteins (HMW+LMW). At the end of operation, the concentration of total protein and polysaccharides in MBR-C, MBR-PAC_V and MBR-PAC_{QQ} was found to be 27, 51 and 5 mg/L, respectively (Fig. 3B). The fraction of polysaccharides was reduced to <19% in MBR-PAC_{QQ}, while it was remained around 50% in the others. These results are in agreement with the reduction in soluble EPS of previous reports [6,7,9].

3.4. Altered sludge properties

It is widely known that QQ did not have any adverse effects on the performance of MBRs, however, the impacts of QQ on sludge flocs were barely studied. The present study (Table 2) clearly shows that QQ had increased the SOUR, SVI, electrical charges (zeta potential) and mean floc sizes, and decreased the CST values and bound EPS amounts. The SOUR of MBR-PAC_{QQ} was doubled as compared to those of MBR-C and MBR-PAC_V. This shows that QQ phenomenon may have a direct impact on metabolic activity of activated sludge.

The floc size in MBR-C did not change significantly, but it was increased by 59% and 128% in MBR-PAC_V and MBR-PAC_{QQ}, respectively, by the end of operation. This increase in sludge floc sizes was also evident in the microscopic images (Fig. 4). The increase can be attributed to PAC in the QQ beads, as it contradicts the commonly observed

reduction in sludge floc size in QQ-supplemented MBRs [9,10,16]. The same increase in sludge floc size due to PAC addition was also observed in previous studies [35,36]. The larger flocs in MBR-PAC_{QQ} might have contributed to better dewaterability, as evidenced by the lowest CST values among all MBRs (Table 2).

The zeta potential of sludge in MBR-C and MBR-PAC_V remained unchanged during the operation, while its absolute value increased by more than 50% in MBR-PAC_{QQ}. Previous studies have shown that the surface charge density increases with the decrease in EPS amount [37]; hence, it is inferred that the lowest amount of bound EPS in MBR-PAC_{QQ} (Table 2) rendered the highest negative charges on sludge flocs.

3.5. MBR performance and micro pollutants removal

Addition of PAC or QQ did not have any adverse or beneficial effects on COD removal efficiencies, as they were 93.0 ± 2.5 , 95 ± 4 and $92.1 \pm 1.5\%$ for MBR-C, MBR-PAC_V and MBR-PAC_{QQ}, respectively. No statistically significant differences in the PhAC removal efficiencies (Fig. 5A) between MBR-PAC_V and MBR-PAC_{QQ} were observed ($p > 0.05$ for all), which suggests that the removal efficiencies of all PhACs were not affected by QQ addition. However, the fate of SMX, DCF and TCS in the two MBRs were clearly different as shown in Fig. 5B. The contribution of biodegradation to the fate of these three PhACs increased with the addition of QQ strains. This was especially prominent for DCF, which was removed in PAC_V via biodegradation (33%) and adsorption to sludge (42%), but majorly removed via biodegradation (86%) in PAC_{QQ}. However, the contribution of biodegradation to CBZ decreased from 66% in MBR-PAC_V to 44% in MBR-PAC_{QQ}; its portion was largely replaced by adsorption to beads. The increase in adsorption to beads was also observed for other PhACs except for SMX (Table C.2 in

Appendix C). Considering the much longer operation time of MBR-PAC_{QQ}, it can be inferred that the PAC_V beads were not saturated with PhACs after 8 days of operation. The increase in biodegradation could also be because of the longer operation time in MBR-PAC_{QQ}, which allowed the sludge to acclimatize to PhACs.

3.6. Variations in microbial community

The Illumina MiSeq high-throughput sequencing was used herein to analyze the microbial communities for better understanding of the effects of QQ or PAC in the MBRs. The PCoA plot (Fig. 6A) shows that there were significant differences in microbial communities between the biocake of the three MBRs, and between the sludge of MBR-PAC_V and MBR-PAC_{QQ}. Members of the phylum Proteobacteria was the most dominant group in all communities (Fig. 6B), with the OUT counts ranging from 34 to 55%; it was followed by Actinobacteria (19-36%) in all sludge or bioackae communities. Members of the candidate phylum TM7 was a dominant group (26-30%) in the biocake of MBR-C, but it was barely present in other communities, and was largely replaced by the members of Cyanobacteria (11-22%) in MBR-PAC_V and by Bacteroidetes (24-25%) in the bioackae of MBR-PAC_{QQ}. The hydrodynamic forces generated by the beads could be the major factor leading to the difference in microbial communities between MBR-C and MBR-PAC_V, as similar influences on microbial communities resulted from shear forces were also observed in reverse-osmosis membranes [38]. PAC could also have contributed to the shift in microbial community. The difference in microbial communities between MBR-PAC_V and MBR-PAC_{QQ} could be attributed to the QQ activity, which was also observed by other

researchers [39]. These differences in microbial communities between MBRs have resulted in the distinct AHL profiles (Fig. 2) in MBRs.

It is noteworthy that the microbial community of the biocake of MBR-PAC_{QQ} was distinct from that of the sludge in the same reactor; the abundance of Bacteroidetes was greatly reduced to 3-5% in the sludge, while the composition of Proteobacteria and Actinobacteria was increased to 48-50% and 32-36%, respectively, in the sludge, from 39-41% and 19-21% in the biocake. This difference in microbial communities between biocake and bulk sludge in MBRs was widely observed in full-scale MBRs, where the fluctuations in HRT, food-to-microorganism ratio (F/M) or MLSS created selective pressures on biocake formation [40]. However, in laboratory studies (internal communications), the microbial communities in biocake were very similar to those in bulk sludge. *Spirosoma* and an unidentified genus of the family Comamonadaceae were the two dominant genera in biocake, which accounted for ~22% and ~14% of the total OUT counts, respectively; while two unidentified genera of the family Methylocystaceae and Streptomycetaceae were the dominant genera in the bulk sludge, with the abundance of ~17% and ~10%, respectively. Members of *Spirosoma* were observed to grow only as biofilms in a fresh river under defined successional stages [41]. Bacteroidetes was also an important portion in biocake of an anaerobic MBR under ultrasonic treatment, which increased the production of proteinaceous EPS [42]. Hence, with major inhibition of polysaccharide production in EPS, the QQ activity might have disturbed the balance of microbial community in MBR-PAC_{QQ} biocake and favored the growth of *Spirosoma*.

Fig. 6 also show that very distinct microbial communities formed in PAC_V and PAC_{QQ} beads; Proteobacteria was still the most dominant phylum (46-55%) in beads, but

methanogens of Euryarchaeota replaced a large portion of Actinobacteria and formed the second (15-21%) and third (13-15%) most dominant phylum in PAC_V and PAC_{QQ} beads, respectively. The genus *Methanosaeta* was almost the sole (>99.5%) member of Euryarchaeota in both beads. In addition, members of Firmicutes increased to 8-13% and 5-7% in PAC_V and PAC_{QQ} beads, respectively. Both *Methanosaeta* and *Firmicutes* were widely present in methanogenic reactors [43,44]. These results suggest that the oxygen diffusing into the alginate beads were completely consumed by the outer layer aerobic biofilm on the alginate beads, thus resulting in reducing conditions inside the beads and facilitating the growth of methanogens. The methane produced by methanogens in MBRs might have been utilized by members of Methylocystaceae, a family comprises of many methane-oxidizing bacteria [45] in the MBRs.

Members of the same genera as the QQ strains except for the AI-2 degrading *E.coli* strain were all present in PAC_{QQ} beads, however, their abundance is very low (< 0.5% of total OUT counts). The presence of these microbes does not guarantee the survival and activity of QQ strains, since these genera except for *Microbacterium* were also present (< 0.5% of total OUT counts) in PAC_V beads. This together with the presence of methanogens in beads suggests that the microbial communities in beads were primarily influenced by the sludge in reactors. The absence of *Escherichia* in microbial community of PAC_{QQ} beads explains the increasing AI-2 level in MBR-PAC_{QQ} reactor (Fig. C.1 in Appendix C).

4. Conclusions

The MBR filtration cycle was increased by 450% with QQ-entrapped PAC beads. The QQ activity contributed primarily to the distinct microbial community structures and

AHL profiles in both bulk sludge and biocake, largely reduced the total AHL levels in bulk sludge, completely depleted AHLs in biocake, and significantly decreased the amounts of building blocks, biopolymer and hydrophobic organics in SMPs. The PAC facilitated high removal of all PhACs, and resulted in a remarkable increase in sludge floc size, which further enhanced sludge dewaterability. In short, QQ-entrapped PAC beads provide a novel approach to mitigating biofouling and improving PhACs removal efficiency.

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Figure Captions:

Fig. 1. Trans-membrane pressure (TMP) trends of MBRs.

Fig. 2. The levels of AHLs in A) bulk sludge, and B) biocake in all MBRs.

Fig. 3. Composition of SMPs measured as A) Dissolved organic carbon (DOC) and B) protein (PN) and polysaccharide (PS) in the mixed liquor when MBRs were started and completely fouled.

Fig. 4. Microscopic images of sludge flocs in A) MBR-C, B) MBR-PAC_V and C) MBR-PAC_{QQ}

Fig. 5 A) The average removal efficiencies and B) fate of PhACs in MBR-PAC_V and MBR-PAC_{QQ} (n = 5 for MBR-PAC_V, n = 6 for MBR-PAC_{QQ}). The error bars represent one standard deviation.

Fig. 6 A). Principle coordinate analysis (PCoA) plot of weighted UniFrac distance comparing variation in MBR communities. T1-T12 are the sample names. B) Relative abundance of major bacterial/archaeal phyla (>1%) in the communities of biocake (BC), sludge and beads in the MBRs. Each bar shows the average of duplicate samples except for 'Sludge-MBR-PAC_V', which is the result of only one sample

Table 1. Filtration resistances, their average increasing rates and their relative contributions measured with resistance-in-series model

	Absolute value ($\times 10^{11} \text{ m}^{-1}$)			Relative contribution (%)			Average increasing rate ($\times 10^{11} \text{ m}^{-1} \text{ day}^{-1}$)		
	MBR- C	MBR- PAC _V	MBR- PAC _{QQ}	MBR- C	MBR- PAC _V	MBR- PAC _{QQ}	MBR- C	MBR- PAC _V	MBR- PAC _{QQ}
R _m	2.6	2.7	3.1	12	32	43	0.65	0.34	0.17
R _p	0.4	1.4	2.3	2	17	32	0.10	0.18	0.12
R _c	18.1	4.2	1.8	86	50	25	4.53	0.53	0.10
R _t	21.2	8.3	7.2	100	100	100	5.30	1.04	0.39

Table 2. Sludge characterization

Parameters	MBR-C	MBR-PAC _V	MBR-PAC _{QQ}
DO (mg/L)	4-5	4-5	4-5
MLSS conc. (mg/L)	5000-6000	5000-5700	5000-6500
SOUR (mg/hr/g)	59.0 ± 4.5	60.6 ± 2.4	96.7 ± 3.7
SVI (mL/g)	20 ± 3	42 ± 4	76 ± 3
CST (sec)	21.8 ± 0.5	18.0 ± 1.5	8.0 ± 0.3
Mean floc size (μm)	50 ± 3 (0 d)	32 ± 6 (0 d)	72 ± 4 (0 d)
	44 ± 7 (4 d)	51 ± 4 (8 d)	164 ± 11 (19 d)
Zeta potential (mV)	- (7.4 ± 1.1)	- (13.9 ± 1.1)	- (11.7 ± 1.4) (before day 10) - (18.2 ± 2.1) (after day 10)
Bound EPS* (mg/L)	38 ± 2 (0 d)	50 ± 2 (0 d)	38 ± 2 (0 d)
	67 ± 7 (4 d)	74 ± 4 (8 d)	50 ± 3 (19 d)

* The total amount of polysaccharides and proteins

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