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

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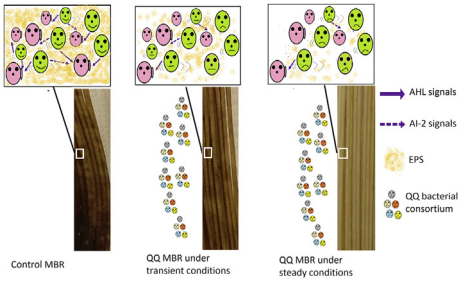
HIGHLIGHTS

- Impact of OLR on QQ based biofouling control was investigated.
- An increase in OLR resulted in higher EPS levels in the biocake and bulk sludge.
- S-EPS and LB-EPS were the major contributors to membrane fouling.
- Within EPS, polysaccharides contributed more to membrane fouling than proteins.
- Transient conditions reduced the efficacy of immobilized QQ bacteria.

GRAPHICAL ABSTRACT



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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%).

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

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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.

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

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), $MgSO_4 \cdot 7H_2O$ (5.7 mg/L), $FeCl_3$ (1.5 mg/L), $MnCl_2$ (1 mg/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.

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 °C; finally, alginate beads were dipped in polymeric solution for 15 s and stored in deionized water at 4 °C. The QQ strains were entrapped separately in polymeric beads to avoid non-homogenous