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Impact of quorum quenching bacteria on biofouling retardation in submerged membrane bioreactor (SMBR)

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Abstract. Membrane biofouling is a critical operational problem that hinders the rapid commercialization of MBRs. Quorum quenching (QQ) has been investigated widely to control membrane biofouling and is accepted as a promising anti-fouling strategy. Various QQ strategies based on bacterial and enzymatic agents have been identified and applied successfully. Whereas, this study aimed to compare indigenously isolated QQ strain i.e., *Enterobacter cloaca* with well reported *Rhodococcus sp*. BH4. Both bacterial species were immobilized in polymeric beads and introduced to two different MBRs keeping the overall beads to volume ratio as 1%. Efficiencies of these strains were monitored in terms of prolonging the membrane filtration cycle of MBR, release of extra-cellular polymeric substances, membrane resistivity measurements and mineralization of signal molecules and permeate quality. Indigenous strain (*Enterobacter cloaca*) was added to QQ-MBR_E while *Rhodococcus sp*. BH4 was introduced to QQ-MBR_R. QQ bacterial embedded beads showed enhanced filtration cycles up to 1.4 and 2.3 times for QQ-MBR_E and QQ-MBR_R respectively as compared to control MBR (C-MBR). Soluble EPS concentration of 52 mg/L was observed in C-MBR while significantly lower EPS concentration of 20 and 10 mg/L was witnessed in QQ-MBR_E and QQ-MBR_R, respectively. Therefore, substantial reduction in biofouling showed the effectiveness of indigenous strain.

Keywords: membrane bioreactor; biofouling control; quorum quenching; filtration cycle

1. Introduction

Over the past 25 years, membrane bioreactor (MBR) has gained increasing popularity in wastewater reclamation and reuse worldwide (Ramesh *et al*. 2006). MBR has several advantages over conventional activated sludge process. These include less sludge production, smaller footprints, better effluent quality with efficient nutrients removal (Lesjean *et al*. 2011). However, membrane fouling, due to deposition of undesired colloidal and organic particles over the membrane surface, is one of the major drawback that limits its application at large scale (Le-Clech *et al*. 2010, Urbanowska *et al*. 2016). Among various types of fouling, the inherent membrane biofouling remains a major challenge that severely declines flux, requires regular cleaning and results in increased operating and maintenance costs (Drews 2010).

Advanced molecular techniques have exposed the biofilm formation, a natural biological process, as the main constituent that results into ultimate membrane fouling causing loss of membrane flux and life span (Wang *et al*. 2005). Various methods, including different filtration modes, intermittent aeration and suction, modification in membrane module and addition of coagulant, have been investigated to mitigate biofouling (Deng *et al*. 2014, Fu *et al*. 2012, Lee *et al*. 2009, Maqbool *et al*, 2015, Wu *et al*. 2008, Urbanowska *et al*. 2016). All these techniques have limitations that they cannot stop the natural process of bacterial communication (i.e., quorum sensing) which has been considered as a backbone of biofouling (Jahangir *et al*. 2012). This suggests that retardation of biofilm formation could be a more direct solution to control biofouling than conventional approaches based on the physico-chemical principles. Therefore, it is suggested that disruption of signal molecules to retard bacterial communication is more reliable solution to control biofouling as compared to conventional physical and chemical cleaning methods (Yeon *et al*. 2009). Signal molecules may include Acyl-homoserine lactones (AHLs), auto inducers and oligopeptides. Furthermore, production of extracellular polymeric substances (EPS) is also considered as the key factor in causing membrane fouling which helps in the accumulation of microbial flocs and biofilm. To counteract quorum sensing, bacterial quorum quenching (QQ) mechanism has been developed for the control of membrane biofouling by diminishing the AHLs production and consequently reducing EPS production. A well reported QQ bacterial specie Rhodococcus BH4 has been applied successfully for the control of membrane biofouling. Studies have already proved that *Rhodococcus sp*. BH4 increased the life span of membrane many folds as compared to conventional MBR (Maqbool *et al*. 2015). However, *Rhodococcus sp*. BH4, releasing lactonase enzyme, can target few AHLs only. Targeting the diverse bacterial communication, it is important to have more

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options to disrupt QS mechanism than restricting to single QQ strain. Thus, various indigenously isolated bacterial species, besides *Rhodococcus sp*. BH4, have been investigated and their quorum quenching effect was verified in disrupting the bacterial signaling as well as EPS production.

In this study, a comparison of well reported *Rhodococcus sp*. BH4 with indigenous strain *Enterobacter cloaca* was conducted. The objective of the study was to analyze the role of indigenously isolated QQ strain in biofouling control and mode of AHLs mineralization. Whereas, effectiveness was evaluated by correlating the EPS production/reduction rate, release of AHLs and development of bio-cake over the membrane surface with time. Moreover, influence of both QQ strains, belonging to different genera, on sludge morphology was also evaluated.

2. Material and methods

2.1 Experimental set up

Three MBRs, having effective volume of 5 L, were installed at IESE-NUST (Fig. 1). Each hollow fibre membrane module (Mitsubishi Rayon, Japan), made up of polyvinyl difluoride (PVDF), having pore size of 0.05 µm and surface area of 0.07 m^2. MBRs were operated with optimized filtration and relaxation mode i.e., 8 min filtration with aeration and 2 min relaxation without aeration (Maqbool *et al*. 2014) using peristaltic pumps (Master flex, U.S.A.).

Air was supplied with the help of air compressor (HAILEA ACO-208, China) at a flow rate of 8 L/min for coarse bubbling throughout the MBR to maintain dissolved oxygen (DO) concentration of 2 to 4 mg/L for microbial growth, to create turbulence for membrane scouring and to avoid dead zones at the bottom of bioreactor. To maintain the mixed liquor suspended solids (MLSS) concentration of 6-8 g/L, excess sludge was wasted by keeping sludge retention time (SRT) of 20 days. Whereas, hydraulic retention time (HRT) of 4 h was maintained at an operational flux of 20 L/m^2/h.

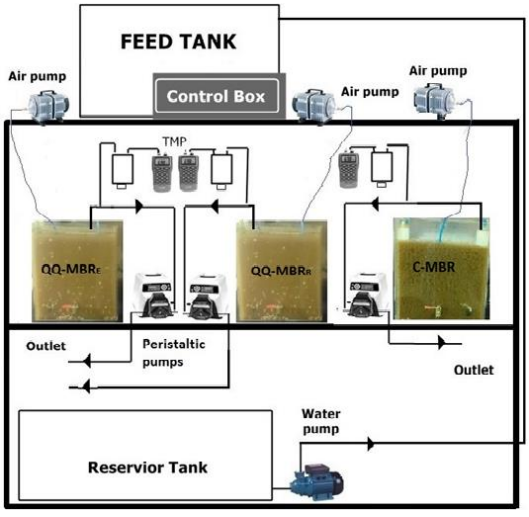

Fig. 1 Process flow diagram of bench scale MBRs setup

The composition of synthetic feed was as follows: 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), $MnCl_2$ (1 mg/L) and $NaHCO_3$ (120 mg/L) to keep pH 7.0-7.5. Seed sludge was taken from a semi-pilot scale MBR (30 L capacity) operated under steady state condition.

2.2 Bacterial immobilization

Selected bacterial species i.e., *Enterobacter cloacae* (indigenously isolated) and *Rhodococcus sp.* BH4 (previously reported) were immobilized using alginate-$CaCl_2$ mixture and further coated with Polysulfone as per method described by Kim *et al*. (2015) with some modifications. Briefly, fresh culture of QQ bacteria, was centrifuged at 4000 rpm for 30 min and re-suspended in autoclaved water. 5 ml of bacterial suspension was mixed with sterile sodium alginate (2% w/v) and final suspension was dropped into $CaCl_2$ solution (4% w/v) through a nozzle at a rate of 1 ml/min. For polymeric coating, pellets of Polysulfone were dissolved in N-methyl-2-pyrrolidone (8% w/v) at 60°C. Finally, the alginate beads were dipped in polymeric solution for 15 s and stored in deionized water at 4°C. QQ bacterial content of alginate-polymeric beads was 2 mg QQ bacteria/g alginate solution.

2.3 Extraction and detection of signal molecules

AHLs were extracted and detected from activated sludge of MBR as per method described by Waheed *et al*. (2015). For the extraction, 20 mL of activated sludge sample centrifuged at 4000 rpm for 20 min to remove large flocs and supernatant was mixed with an equal volume of ethyl acetate at 120 rpm for 2 h. Separating funnel was used to remove the organic layer. Colloidal removal was achieved by centrifugation at 4000 rpm for 10 min, supernatant was dried using rotary evaporator at 30°C and residue was dissolved in 200 µL of methanol. 1 mg/mL standard solution of N-octanoyl homoserine lactone (C8HSL) (Sigma-Aldrich) was prepared by dissolving C8HSL in methanol. Sample solution was prepared by mixing 20 µL of methanol having 0.1% formic acid. Analysis was performed using water/methanol composition of 35:65 as a mobile phase and the UV detector was set at 210 nm. Column C18 was used for the high performance liquid chromatography (HPLC) system (Water, Breeze system, U.S.A). AHL standard/extract was injected at a flow rate of 0.8 mL/min.

2.4 Extraction and quantification of EPS

Activated sludge sample (50 mL) from the MBRs was centrifuged for the removal of supernatant comprising of soluble EPS at 4000 rpm for 15 min at 4°C (K2015R, Pro-Research, Britain). The remaining mixed liquor pellets containing loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), were extracted using a modified extracted method (Zhang *et al*. 2006). Protein (PN) concentration was measured by Lowry method using the Folin-ciocalteu

phenolic reagent which measures the copper ions reacting with peptide bond as the aromatic protein oxidize in alkaline solution (Lowry *et al*. 1951, Kunacheve and Stuckey 2014) and absorption was taken at 750nm. Bovine serum albumin (Sigma-Aldrich) was used for the preparation of PN standard curve. For the quantification of polysaccharide (PS), Dubois method was employed in which sulfuric acid and phenol were used. Phenol and sulfuric acid addition turned the solution to yellow and absorption was taken at 490 nm. Standard glucose was used for the determination of PS in the sample (Dubois *et al*. 1956).

2.5 Resistance analysis

Total hydraulic resistance (R_t) comprises of three types of resistances, intrinsic membrane resistance (R_m), pore blockage resistance (R_p) and cake layer resistance (R_c).

$$R_t = R_c + R_m + R_p \quad (1)$$

R_m is the resistance of the membrane only, R_p is the resistance by the blocked pores on the membrane surface whereas R_c is the resistance of the cake layer over membrane surface that is formed during the operation of MBR. The membrane fouling resistance (R_t) was calculated using Darcy's Law as follows

$$R_t = \Delta P / J\mu \quad (2)$$

where ΔP is rise in TMP (Pa), J is operated flux ($m^3/m^2/s$) and μ is dynamic viscosity (Pa.s) of permeate.

2.6 Other parameters

Effluent quality and mixed liquor properties were examined regularly to evaluate the efficiency of MBRs, using Standard Methods (APHA *et al*. 2012). Chemical oxygen method (COD) was measured by using closed reflux method whereas ammonium (NH^{+4}-N), nitrate (NO_3^- N) and phosphate (PO_4^{-3}) were measured by using spectrophotometer (DR2010, HACH, U.S.A.). MLSS, Mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) were measured according to Standard Methods (APHA *et al*. 2012). The pH/DO multi-meter (pH/DO 300 series, Oakton, U.S.A) was used for pH and dissolved oxygen (DO) measurement.

Specific cake resistance (SCR) was determined as described in Maqbool *et al*. (2015). Whereas, sludge dewaterability was evaluated in terms of capillary suction time (CST) using CST apparatus (Triton, Canada).

3. Results and discussion

3.1 Effect of QQ on membrane fouling tendencies

Transmembrane pressure (TMP) is an important indicator to determine membrane permeability as the extent of membrane fouling is directly linked with the TMP build up. Dominant stages in overall TMP profile including initial rise due to direct absorption of solute particles on the membrane surface (Stage 1), slow TMP build up because of accumulation of EPS on membrane surface (Stage 2) and a sharp rise (Stage 3) were thereof, critically monitored for all MBR systems (Zhang *et al*. 2006). With the 8 min filtration and 2 min relaxation mode for the MBRs, significant difference in TMP build-up was observed.

Membrane fouling propensity of all MBRs is shown in Figure 2. Where, QQ-MBR_R exhibited longer steady state TMP trend along with steeper jump as compared to QQ-MBR_E. In control MBR (C-MBR), the value of 30 kPa was reached within 12-13 days of MBR operation, which could be attributed to accumulation and deposition of EPS and other biological secreted products on the membrane surface. Whereas, MBR having *Enterobacter* and *Rhodococcus sp*. entrapped beads fouled in 17 and 27 days respectively. Overall, the average fouling rate (ΔTMP/Δt) for MBR-C, QQ-MBR_E and QQ-MBR_R was 2.5, 1.8 and 1.1 kPa/d, respectively. Therefore, it can be anticipated that, pore blockage or direct deposition of solute particles could be the cause of fouling in QQ MBRs. These results depict the superior QQ activity of *Rhodococcus sp.* BH4 as compared to *Enterobacter sp*. and its ability to delay the biofilm formation, thereby prolonging the membrane filtration cycle in MBR (Oh *et al*. 2011, Cheong *et al*. 2014).

3.2 Effect of QQ on membrane resistance

Resistance in series model was used to study the influence of QQ mechanism on membrane resistances. In C-MBR operation, the contribution of R_c was found to be 50% which could be linked to the formation of matured colonies on membrane surface, thereby affected membrane filterability and caused reduction in filtration cycle (Table 1). Major contribution in R_t was of cake layer formation and pore blockage in C-MBR and QQ MBRs respectively.

As the cake layer consists of soluble microbial products (SMP), EPS, organic and inorganic substances (Flemming *et al*. 2001), therefore its formation on membrane surface possesses serious concerns. Moreover, few patches of bio-cake in QQ-MBRs were observed on the membrane surface confirming the disruption of bacterial communication and reduction in EPS production. Further, pore clogging resistance (R_p) was high in QQ-MBR_R and QQ-MBR_E (53.8 and 40% respectively) due to delay in cake layer build up and colloidal particles deposition on the membrane and inside the pores. A fact of faster blockage of pores was also due to the smaller pore size (0.05um) which may contribute to rapid pore blockage (Hwang *et al*. 2008).

Table 1 Membrane resistance analysis

Resistance (10^{12} 1/m)	C-MBR	QQ-MBR_R	QQ-MBR_E
Total hydraulic resistance, (R_t)	0.8 ± 0.2	0.65 ± 0.2	0.75 ± 0.3
Cake layer resistance, (R_c)	0.4 ± 0.1	0.15 ± 0.1	0.25 ± 0.1
Pore blocking resistance, (R_p)	0.3 ± 0.1	0.35 ± 0.1	0.3 ± 0.1
Intrinsic membrane resistance, (R_m)	0.1 ± 0.1	0.15 ± 0.1	0.2 ± 0.1
R_c/R_t (%)	50	23	33.3
R_p/R_t (%)	37.5	53.8	40

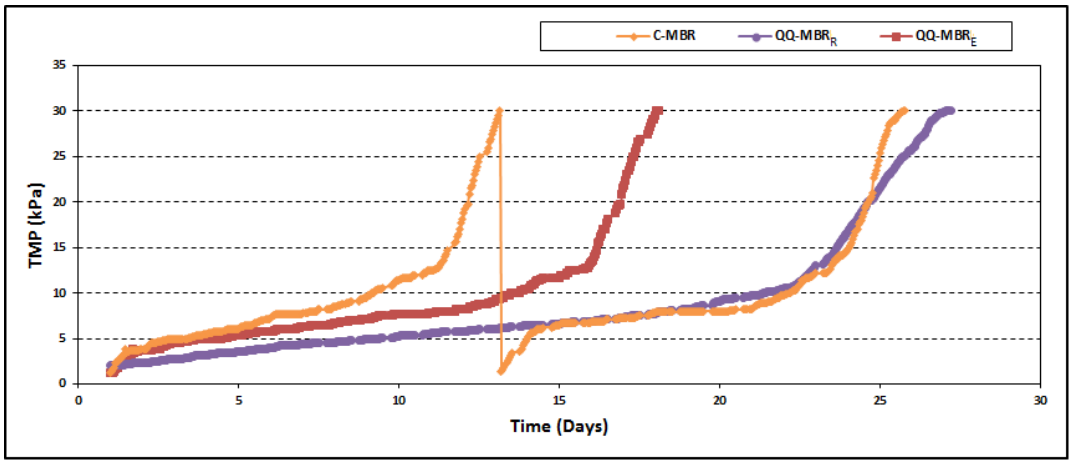

Fig. 2 TMP profiles of MBRs

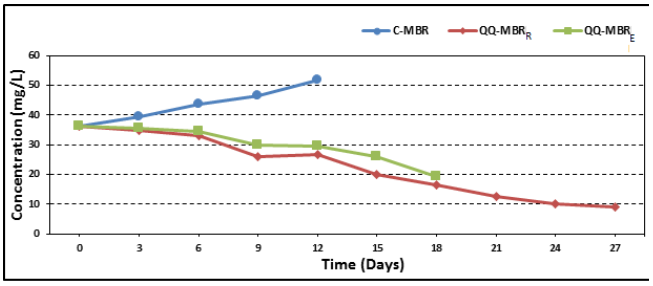

Fig. 3 Soluble EPS production in all MBRs

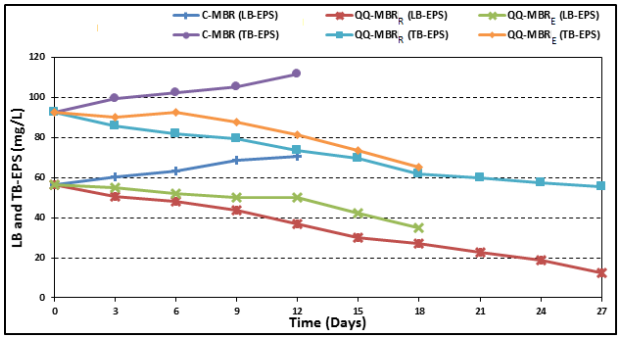

Fig. 4 Bound EPS production in all MBRs

3.3 Effect of QQ on EPS production

EPS has been extensively investigated as its accumulation on membrane surface is directly related to biofouling. In the present study, EPS content was investigated in terms of Soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). The initial concentration of EPS in sludge was similar in all MBRs as all MBRs were fed with sludge acclimatized in similar conditions. Figure 3 indicates that decrease in soluble EPS concentration was found to be more rapid in QQ-MBR$_R$ than QQ-MBR$_E$. This authenticates the quenching efficiency of *Rhodococcus sp.* BH4 with respect to *Enterobacter sp*.

Since the correlation between sludge hydrophobicity and attachment of microbial flocs over the membrane surface has been developed previously (Le-Clech *et al*. 2006). Therefore, higher PN or PS concentration in control MBRs may increase the hydrophobicity of mixed liquor which might cause rapid membrane fouling. Whereas, reduction in PN and PS concentration was witnessed in MBRs with QQ strains resulting in fouling retardation. Previous study (Waheed *et al*. 2017) also witnessed the contribution of PS related substances in membrane fouling.

In C-MBR, concentration of soluble EPS increased from 37 to 52 mg/L showing that hydrophobicity of activated sludge or mixed liquor increased which facilitated the attachment of flocs on the membrane surface developing a dense cake layer and causing rapid buildup in TMP. Immobilized QQ bacteria, having the ability to reduce cell to cell communication by deactivating signal molecules (Waheed *et al*. 2015) significantly decreased S-EPS concentration to 20 and 10 mg/L in QQ-MBR$_E$ and QQ-MBR$_R$, respectively.

Irrespective of gradual decrease in both LB-EPS and TB-EPS in QQ-MBR$_E$, bound EPS in QQ-MBR$_E$ was found to be higher than QQ-MBR$_R$ as indicated in Fig. 4. Moreover, concentration of TB-EPS was found to be greater than LB-EPS in all MBRs. These results infer that although *Enterobacter sp*. was found to be efficient in reducing EPS level, however, its efficiency was less as compared to *Rhodococcus sp.* BH4. Therefore, among indigenous versus known (Rhodococcus) QQ-bacteria, QQ activity varies based upon their AHL degradation capabilities, which eventually exhibits different biofouling retardation tendencies in QQ-MBRs; however, their performance in terms of permeability always remained better than that of C-MBR as reported in this study.

3.4 Evaluation of compressibility and dewaterability

Filterability of sludge can be indicated by specific cake resistance (SCR) in the form of resistance offered by the cake layer developed over membrane surface in batch dead-end system. Whereas, capillary suction time (CST) was calculated to evaluate dewaterability of sludge. As indicated in Fig. 5, CST for QQ MBRs and C-MBR was found to be 15 and 25 s respectively. Better sludge dewaterability in QQ MBRs could be due to development of large flocs, as longer

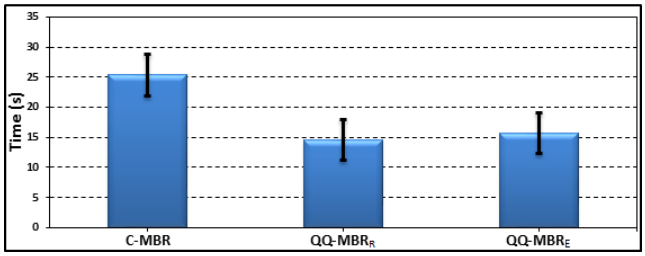

Fig. 5 Capillary Suction Time for all MBRs

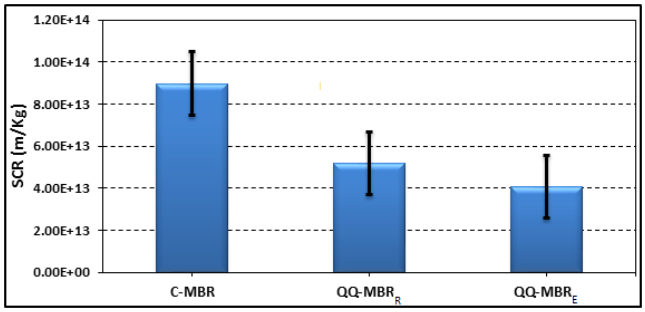

Fig. 6 Specific Cake Resistance (SCR) in all MBRs

filtration cycle may have enhanced floc formation. Whereas, smaller particle size or immature floc formation in C-MBR could be due to the shorter contact time (i.e., 12 d), thereby affected sludge filterability.

A direct relation between SCR and EPS production was reported by Cho *et al*. (2004). As shown in Fig. 6, SCR of QQ-MBR$_R$ was lower than that of QQ-MBR$_E$ exhibiting increased filterability of sludge in the presence of *Rhodococcus sp.* These results could further be linked with the R_c as indicated in Table 1. Overall, longer filtration cycle in tandem with less cake layer formation due to quenching mechanism enhanced sludge morphology.

3.5 Effect of QQ on organics and nutrients removal

Monitoring of MBR permeate quality in terms of nutrient (PO_4^{-3}-P, NH_4^+-N and NO_3^--N) removal was carried out regularly (Table 2). No significant difference in performance efficiency of C-MBR and QQ-MBRs authenticates that quorum quenching does not affect the nutrients removal.

Moreover, influent COD concentration in MBRs was consistently maintained as 500 mg/L. Average effluent concentration of COD in C-MBR was 98.2 ± 0.2 whereas with the addition of QQ embedded CEBs in QQ-MBRs, the effluent concentration of COD was 98 ± 0.1 (Table 2). Thereby, no adverse effects of QQ on the organic removal efficiency was observed.

4. Conclusion

QQ mechanism prolonged the filtration cycle significantly as time reached to 30 kPa for C-MBR, QQ-MBR$_R$ and QQ -MBR$_E$ was 12, 17 and 27 days respectively. Lower production of soluble EPS decreased the TMP propensity in QQ MBRs and prolonged membrane biofouling. Furthermore, role of indigenously isolated

Table 2 COD and Nutrients removal efficiency (%) in all MBRs

Parameters	C-MBR	QQ-MBR$_R$	QQ-MBR$_E$
COD	98.2 ± 1.2	98.5 ± 1.5	99 ± 0.5
NH_4^+-N	52 ± 1.5	51 ± 1.2	51 ± 0.7
NO_3^--N	92.5 ± 2.1	92 ± 1.4	92.5 ± 2.2
PO_4^{-3}-P	48 ± 2.5	49 ± 0.5	50 ± 0.2

QQ strain in biofouling control was also confirmed during the study.No adverse effect of QQ mechanism was observed on the organic and nutrients removal efficiencies as compared to conventional MBR. Thereby, this study further authenticates that QQ bacteria enhanced the membrane permeability and prolonged the filtration cycle without affecting the MBR effective treatment performance.

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