Combined Impact of Quorum Quenching and Backwashing on Biofouling Control in a Semi-Pilot Scale MBR Treating Real Wastewater

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Summary: This study demonstrates the combined effect of quorum quenching (QQ) and backwashing on biofouling control in MBR treating real wastewater. The quorum quenching mechanism is an emerging biological technique using *Rhodococcus* sp. entrapped in polymer coated sodium alginate beads whereas, backwashing is a distinguished physical technique for biofouling control. Two parallel semi-pilot scale MBRs *i.e.*, QQ-MBR (quorum quenching MBR) with cell-entrapping beads (CEBs) and C-MBR (conventional MBR) with vacant CEBs at 0.5% effective volume of the bioreactor, were monitored for comparative performance evaluation. In the first phase, both the MBRs were operated without backwashing having operational cycle of eight min filtration and two min relaxation and in the second phase; MBRs were operated with backwashing having operation cycle of eight min filtration, one min relaxation and one min backwashing. QQ-MBR with backwashing exhibited greater biofouling control capability and elongated filtration duration with respect to QQ-MBR without backwashing. Comparatively less soluble EPS concentrations were detected in QQ-MBR as compare to C-MBR in both modes of operation while backwashing contributed to retard the rapid increase in trans-membrane pressure (TMP) also known as TMP jump. Study reveals the novelty of successful application of combined influence of permeate backflushing technique and QQ (anti-biofouling) strategy in MBR and potential use for full scale applications.

Keywords: Membrane bioreactor, Quorum quenching, Backwashing, Soluble EPS, Real wastewater.

Introduction

Membrane bioreactors (MBRs) have become a favored technological innovation for wastewater treatment because it provides a higher quality permeate and better solid-liquid separation [1-3]. The biomass concentration in a MBR plant treating domestic wastewater is normally 3-4 times higher as compared to conventional activated sludge (CAS) process [4]. For this reason, biofouling, due to extra-cellular polymeric substances (EPS) and microbial cells, on the surface of membrane is a persistent problem in the widespread application of MBR technology. It has been discovered that bacteria present in wastewater mostly depend on N-acyl homo-serine lactones (AHLs) facilitated quorum sensing via cell to cell communication to match their activities by releasing soluble EPS into the environment and causing biofilm formation [1].

Previous studies have proven the significance of backwashing and QQ techniques for reducing biofouling separately. Recently, use of bacterial quorum quenching (disruption of quorum sensing), has successfully been reported to control the biofilm formation by mineralizing the AHLs in membrane bioreactor systems [5-7]. This discovery opens up new horizons and becomes a novel strategy as anti-biofouling technique in membrane systems [8]. For quorum quenching, bacteria could be applied as free/suspended cells, however lack of cell separation, competition with other bacterial species and loss of stability are the major problems associated with this process. Potential solution to this problem could be bacterial immobilization as it stops cell washout, protects against competition, allows reuse and improves stability [9, 10]. Immobilization of quorum quenching bacteria into calcium alginate beads has been reported earlier [11, 12]. Maqbool *et al.* [12] immobilized the quorum quenching bacteria (*Rhodococcus* sp. BH4) on free moving calcium alginate beads in MBR and reported the effective control of biofouling through quorum quenching. However, calcium alginate being a natural polysaccharide is susceptible to biodegradation and low mechanical stability. This makes calcium alginate matrix unsuitable for real field applications under harsh environment [13]. Researchers have made some efforts to improve the stability of alginate beads by applying polyelectrolytes *i.e.*, poly-lysine [14], chitosan [15, 16] and poly-vinylamine [17]. However, coating of these materials on alginate core suffers from high costs and complicated production steps which are the limitations for its practical application to real wastewater treatment. Kim *et al.* [18] reported use of polymers such as poly-sulfone

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(PSF), poly-ethersulfone (PES) and poly-vinylidene fluoride (PVDF) to strengthen beads and found that poly-sulfone (PSF) coated beads were more stable in a harsh hydrodynamic environment while no significant change in QQ activity was observed.

Furthermore, one of the standard operating strategies to mitigate fouling incorporated in most of the MBR systems around the world is backwashing through permeate. It is also one of the physical cleaning techniques that can recover membrane permeation more efficiently. It can easily detach and loosen the sludge cake from the surface of membrane that was believed to be removed by air bubbles or cross flow. Thus, recurrent backflushing can offer an opportunity for macro-molecular components to pass in the membrane pores and cause irreversible fouling [19]. Previous studies suggest that TMP increase can be categorized into three stages. First one is an initial stage of short-term TMP rising due to soluble microbial products (SMP) deposition which ultimately leads to pore blocking. The second stage of long-term TMP raising either linearly or moderate exponentially due to the development of cake formed by either the SMP or suspended solids. Whereas, a third stage of sudden TMP raising also known as the TMP jump due to inhomogeneous fouling [20]. Membrane fouling control strategies including physical/chemical or biological mechanisms focus on prolonging the second stage of filtration by retarding the biofilm development and cake formation, and diminishing the third stage (TMP jump) by minimizing cake densification.

In the present study, two techniques of biofouling control, cyclic backwashing and QQ with *Rhodococcus* sp. entrapped into calcium alginate core and coated with poly-sulfone layer were simultaneously applied to evaluate their combined influence on MBR filtration performance.

Experimental

MBR operation

To evaluate the effect of polymer coated cell entrapping beads (CEBs) on biofouling control and sludge characteristics, two semi-pilot scale MBRs having same effective volume of 35 L *i.e.*, quorum quenching MBR (QQ-MBR) with CEBs (*Rhodococcus* sp.) and conventional MBR (C-MBR) with vacant beads along with backwashing mechanism were operated in parallel (Fig. 1). A semi pilot scale MBR is a small system built in the lab to generate information about the behavior of the full scale MBR plant having treatment capacity of 50m^3/day in actual physical environment inside NUST campus. Semi pilot scale is relative term in a sense that this plant is typically smaller than that of full scale MBR plant. However, similar automation facilities provided to compare the results. Further, typical MBR sludge concentration (MLSS = 8 to 10 g/L) was maintained throughout the study period.

The study was divided into two phases; under the first phase, the MBRs were operated without backwashing having operation cycle of eight min filtration and two min relaxation and under the second phase, the MBRs were operated with backwashing having operation cycle of eight min filtration, one min relaxation and one min backwashing. Hollow fiber membrane modules having 0.1 μm pore size and 0.7m^2 surface area (Memstar, China) were used in the MBR systems. Real wastewater generated from the university residential area (NUST, Islamabad, Pakistan) was fed to the reactors. The wastewater was collected and stored in an overhead tank after screening (Ø: 1mm). The real raw wastewater characteristics are reported in Table-1.

Table-1: Real wastewater characteristics.

Parameter	Unit	Values
BOD	(mg/L)	185.86 (10) ± 28.28
COD	(mg/L)	269.37 (30) ± 40.99
PO_4^{-3}-P	(mg/L)	14.89 (30) ± 2.16
NH_4^+-N	(mg/L)	29.67 (30) ± 6.66
pH	-	7.73 (30) ± 0.34

Permeate flux was set at 16.5 L/m^2/h and transmembrane pressure was recorded after every two minutes using data logging manometer. The operating conditions are given in Table-2.

Table-2: Operating conditions of MBRs

Operating conditions	
Working volume	35L
Permeate flux	16.5 LMH
HRT	3 hrs.
SRT	40 days
MLSS	8-10 g/L
F/M	0.2± 0.03
Beads concentration	0.5% of working volume
Operation cycle without backwashing (Phase I)	8 min Filtration, 2 min relaxation
Operation cycle with backwashing (Phase II)	8 min Filtration, 1 min relaxation, 1 min backwashing
Backpulse Flux in phase II (1.5 of Permeate flux)	24.75 LMH

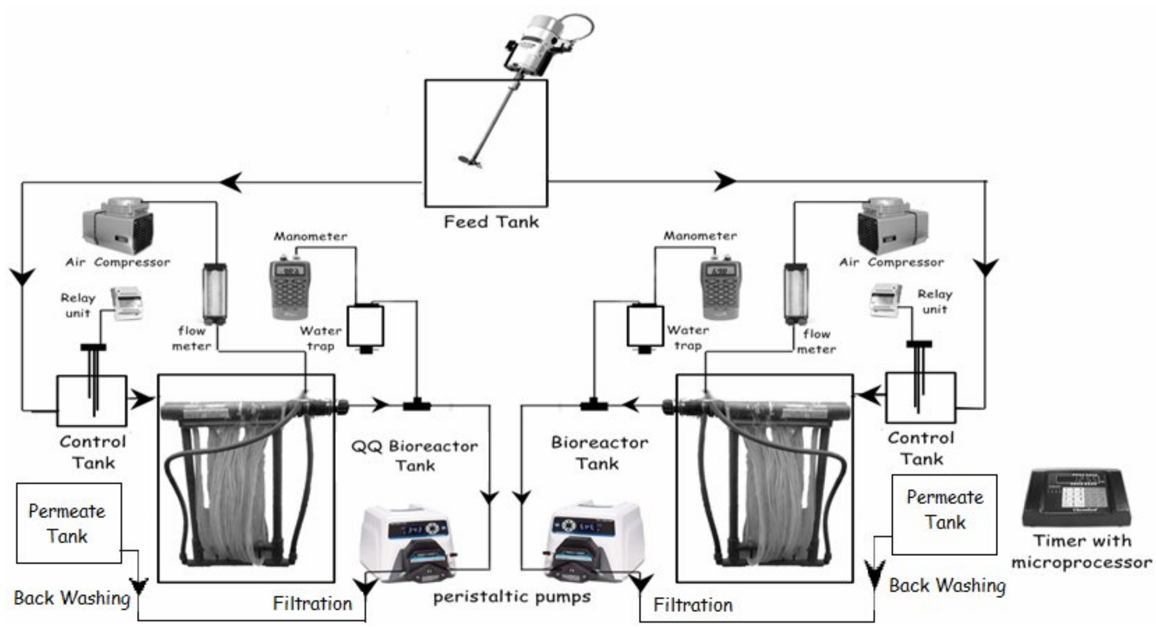


Fig. 1: Schematic of semi-pilot scale C-MBR and QQ-MBR systems with backwashing.

Beads material

Sodium alginate solution (5%) was prepared in sterilized distilled water. *Rhodooccus* sp. was grown on LB agar and cell pellets were mixed with sodium alginate solution to obtain uniform suspension. This suspension was augmented with $CaCl_2$ solution (4%) to obtain uniform-sized alginate beads. Coating of polymer layer of poly-sulfone was performed by phase inversion process mentioned earlier by Kim *et al.* [18]. The average size of beads was 3.73 mm and it covered 0.5% of reactor effective volume.

Extraction and quantification of EPS

Extraction of extra-cellular polymeric substances (EPS) from MBR sludge was carried out using cation-exchange resins (CER) (Dowex-USA) [21]. 50 mL sludge samples collected from both reactors were centrifuged at 4000 rpm (4°C) by centrifuge (Model: K2015R–Pro–Research-Britain) for 15 min to isolated supernatant from mixed liquor for soluble EPS. For bound EPS, extracted sludge pellets are mixed in phosphate buffer, stirred for one hour, centrifuged (4°C) for 15 minutes and then supernatant was removed. Further, sludge pellets were mixed in CER and buffer solution was mixed to make 50 mL and stirred for one hour and supernatant was preserved for bound EPS.

Lowry method was used to determine protein (PN) concentration by using Folin–ciocalteu phenolic reagent while absorption was measured at 750 nm by spectrophotometer (T60-UV, PG-Instrument, Britain) [22, 23]. For quantification of polysaccharides (PS) concentration, Dubois method was adopted. The standard curve of glucose was used to determine the PS concentrations [24]. The concentration of polysaccharides and proteins in QQ-MBR and C-MBR were measured on weekly basis during MBR operation under both the operational phases.

Filtration resistance measurements

For evaluation of fouling potentials of both MBRs, Darcy's Law and resistance-in-series (RIS) method adopted.

$$R_t = \frac{\Delta P}{\mu . J}$$

where

R	Hydraulic resistance (1/m)
J	Operational flux ($m^3/m^2/s$)
ΔP	TMP rise (Pa)
μ	Permeate dynamic viscosity (Pa s)

$$R_t = Rc + Rp + Rm$$

R_t, *i.e.* total hydraulic resistance is sum of three resistances, biocake resistance (R_c), resistance due to pore blockage (R_p) and intrinsic resistance

(R_m) of membrane. R_t was measured at the end of each operation cycle; to measure R_c, sludge cake from the surface of membrane was removed using sponge and then it was submerged in deionized (D.I.) water followed by TMP and flux measurement. R_c value was obtained by the subtraction of (R_m + R_p) from R_t, while R_m was determined after membrane's chemical cleaning [25]. Contribution to total hydraulic resistance by each component was equated in each MBR under both operational phases.

Water quality and sludge analysis

Dewaterability of sludge was measured through capillary suction-time (CST) conferring to standard methods [26, 27]. Nutrient removal, DO, COD, BOD and pH for influent & effluent along with sludge parameters *i.e.* average floc size and distribution, sludge volume index, MLSS, MLVSS were carried out through standard operation procedures.

Results and Discussions

Evaluation of TMP trends in QQ-MBR versus C-MBR with and without backwashing

The combined effect of backwashing and quorum quenching of polymer coated QQ beads and vacant beads on TMP trends were evaluated from two parallel MBRs, fed with real wastewater. QQ-MBR having *Rhodococcus* sp. BH4 entrapped CEBs and C-MBR with vacant CEBs were operated without backwashing (Phase I) and with backwashing (Phase II) mechanisms. In the present study, after 55 days of operation in phase I and 68 days in phase II, polymer coated alginate CEBs were present almost at the same percentage as they were at the start of each exhibiting stronger physical stability in comparison with our previous study [12] using alginate beads where the beads completely disappeared in 45 days of operation. TMP profiles comparison of QQ-MBR and C-MBR in both modes of operations (phases) has been shown in Fig. 2. It took 13-15 days (Fig. 2-a) for the TMP to reach 30kPa in C-MBR without backwashing while 21-23 days (Fig. 2-b) with backwashing. On the other hand, TMP of QQ-MBR took 55 days (Fig. 2-a) to reach 30kPa without backwashing whereas it took 68 days (Fig. 2-b) with backwashing. These results deduce that QQ-MBR has four times longer filtration cycle than C-MBR without backwashing while three times longer filtration cycle with backwashing. Similar impact of QQ was observed in previous MBR studies treating synthetic wastewater [7, 12, 28]. Moreover, the dominant effect of backwashing on both the MBRs filtration capability was clearly evident with C-MBR exhibiting 8 d longer and QQ-MBR 13 d longer filtration cycle, respectively exhibiting greater impact of backwashing on QQ-MBR in comparison with C-MBR.

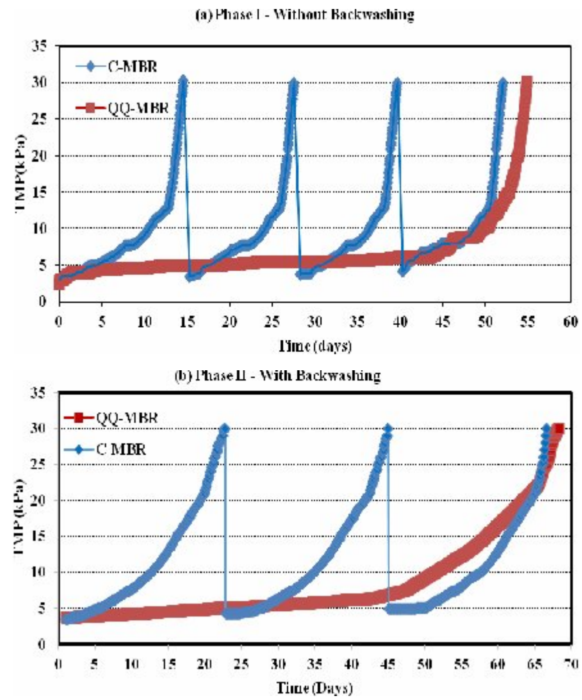


Fig. 2: TMP profiles of C-MBR and QQ-MBR; (a) Without Backwashing; (b) With Backwashing.

Relating the TMP profiles of QQ-MBR with C-MBR under the two operational modes, the overall delay in TMP rise can be attributed mainly to quorum quenching (biological mechanism) under the second stage of long-term TMP rise while the backwashing (physical mechanism) mainly influences the third stage of rapid TMP rise. The C-MBR and QQ-MBR were under similar scouring effect due to moving beads either vacant or imbedded with QQ bacteria. Furthermore, backwashing retards the TMP jump resulting in longer filtration cycle by inhabiting pore narrowing/pore blocking by colloidal matter and solute substances under the mature filtration stage. Under this stage of filtration, the proportion of membrane pores, open to filtration, is much less as compared to chemically-cleaned membrane/virgin membrane *i.e.*, the local TMP within the membranes pores, open to filtration, is significantly greater. In presence of cyclic backwashing, proportion of membranes pores open to filtration can be prolonged till TMP reaches terminal value of 30kPa. In comparison with previous conventional and QQ-MBR studies [2, 11, 12, 18, 29-31], the main contribution of our findings is evident from the delayed TMP jump in proportion to steady-state TMP due to filtration/backwashing cycle as illustrated in Table-3.

Table-3: Three stages of membrane fouling in terms of TMP rise in Conventional and QQ MBRs

	Stage I Conditioning fouling (initial rise)	Stage II Steady-state fouling	Stage III TMP Jump	Reference
Conventional MBRs with relaxation only	27 %	64%	9%	[28]
	24%	62%	14%	[29]
	19%	54%	27%	[30]
QQ - MBR	16%	75%	9%	[17]
	5%	84%	11%	[11]
	7%	83%	10%	[2]
QQ – MBR with relaxation and Backwashing	1%	65%	33%	Present Study

Table 4: Fouling resistances of C- MBR and QQ- MBR membranes

Resistance	Without Backwashing (Phase-I)		With Backwashing (Phase-II)	
	C-MBR(x^{12} 1/m)	QQ-MBR(x^{12} 1/m)	C-MBR(x^{12} 1/m)	QQ-MBR(x^{12} 1/m)
Total Hydraulic Resistance - (R_t)	3.06	3.54	4.11	3.76
Cake layer resistance - (R_c)	1.14	1.06	1.58	1.02
Pore blocking resistance - (R_p)	1.08	1.82	1.18	1.71
Intrinsic membrane resistance - (R_m)	0.90	0.66	0.94	0.17
R_c/R_t (%)	37	30	43.3	27.1
R_p/R_t (%)	35.3	51.4	28.7	39.5
R_m/R_t (%)	29.5	18.7	22.8	4.6

Effect of QQ-beads and backwashing on membrane fouling resistance

The membrane resistances are reported in Table-4 including total hydraulic resistance (R_t), cake layer resistance (R_c), pore block resistance (R_p) and intrinsic membrane resistance (R_m). The total hydraulic resistance (R_t) of the membrane in QQ-MBR was slightly higher than the C-MBR after 55 and 68 days QQ-MBR operations as compared to 13-15 and 21-23 days of C-MBR operations under phase I and II, respectively. Resistance of cake layer (R_c) supported the major portion of R_t in the C-MBR while pore block resistance (R_p) posed the major share of R_t in the QQ-MBR as shown in Table-4. Physical cleaning can help to remove cake layer but pore clogging is irreversible in nature and requires physical as well as chemical cleaning [31]. Membrane module of C-MBR was chemically cleaned three to four times within the QQ-MBR operational period based upon TMP rise to 30kPa. Due to successive chemical cleaning, the internal resistance (R_m) of C-MBR membrane was found to be continuously increasing between filtration cycles, indicating permanent clogging *i.e.* irremovable fouling of the membrane. Under phase I, the R_p to R_t ratio was 35 and 51% for QQ-MBR and C-MBR respectively, while under phase II, the R_p to R_t ratio was 29 and 40% for QQ-MBR and C-MBR, separately. These results indicate that due to prolonged exposure of QQ membrane in real wastewater, soluble organic and complex bio-polymeric compounds were adsorbed directly onto the surface of membrane and inside pores of the membrane in the absence of cake layer or biofilm resulting in higher R_p in QQ-MBR versus C-MBR. It was further revealed that QQ membrane exhibited lower R_c due to significant reduction in cake layer formation through quorum quenching activity. The values of all the resistances are higher in C-MBR under Phase II (backwashing) as compared to Phase I (without backwashing) because of the historical irremovable fouling experienced by the C-MBR membrane.

Effect of QQ-beads on EPS production along with backwashing

Extracellular polymeric substances (EPS) are a complex mixture of humic acids, proteins (PN), polysaccharides (PS) and other compounds. EPS is an important parameter for the evaluation of quorum quenching activity in activated sludge. Due to its significant role in biofouling, EPS is divided into two categories *i.e.* soluble and bound EPS. Initially concentrations of PS and PN in MBR sludge of both systems were the same because the reactors were inoculated with the same activated sludge. Under Phase I (without backwashing), the total soluble and the total bound EPS in the mixed liquor of QQ-MBR were reduced by approximately 69% (Fig. 3-a) and 10% (Fig. 3-b), respectively as compared to C-MBR while under Phase II (with backwashing) 61% (Fig. 3-c) and 15% (Fig. 3-d) reduction in soluble and bound EPS, respectively, was witnessed in the C-MBR as compared to QQ-MBR.

Based on the previous studies [2, 11, 12, 32-34] and these results, it is likely that the reduction in EPS was only due to quorum quenching activity whereas backwashing has insignificant effect in this regard. It is also inferred that due to the EPS reduction in QQ-MBR, the filtration period prolonged. Similarly, Jiang *et al.* [35] reported less soluble and bound EPS in QQ-MBR in comparison with C-MBR, however, Weerasekara *et al.* [32] detected that there was no considerable difference in soluble and bound EPS of conventional and QQ MBRs which may be resulted from different immobilization method or QQ agents used.

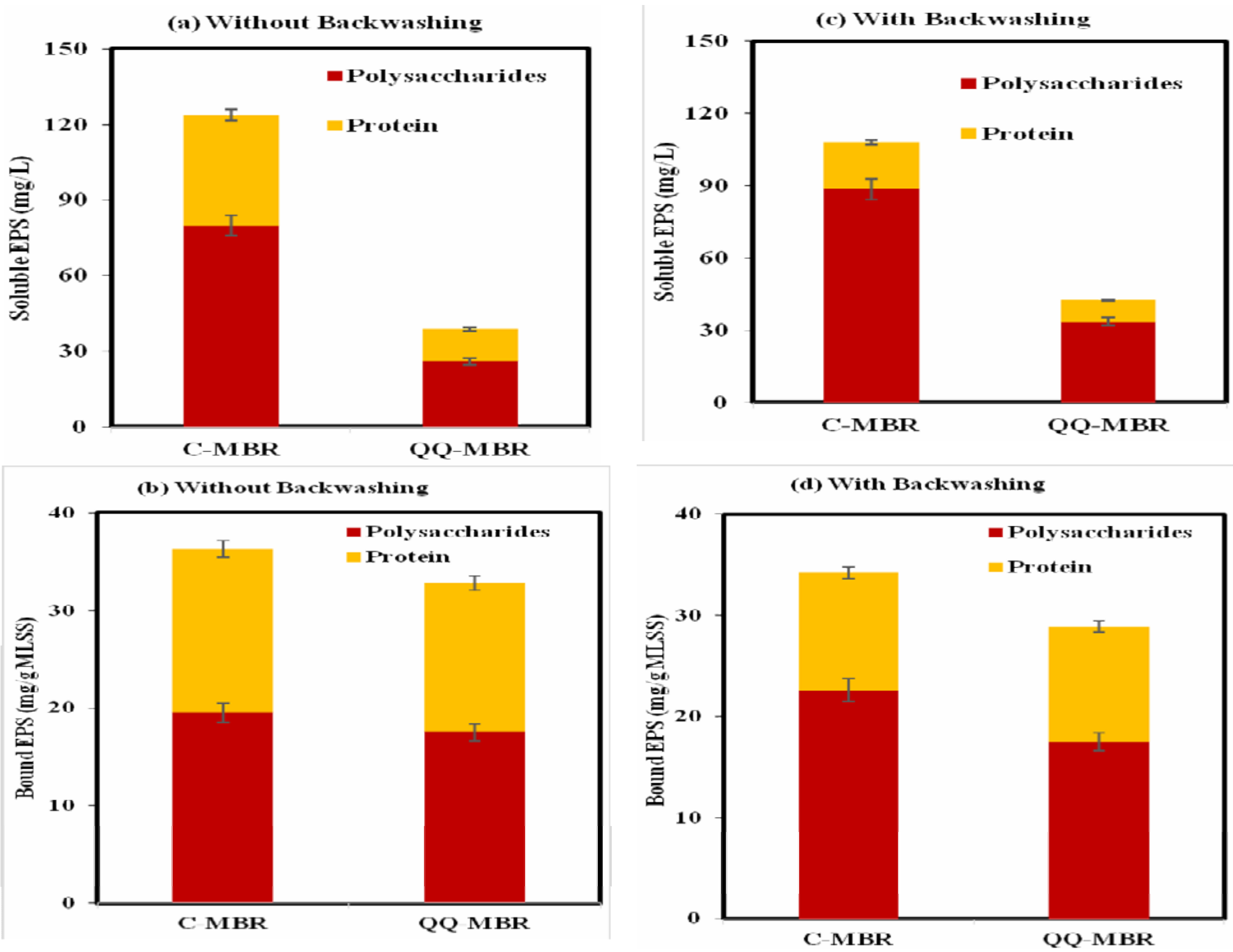


Fig. 3: (a) Soluble EPS in C-MBR and QQ-MBR without backwashing; (b) Bound EPS in C-MBR and QQ-MBR without backwashing; (c) Soluble EPS in C-MBR and QQ-MBR with backwashing; (d) Bound EPS in C-MBR and QQ-MBR with backwashing.

Comparison of sludge characteristics and permeate quality in QQ-MBR versus C-MBR

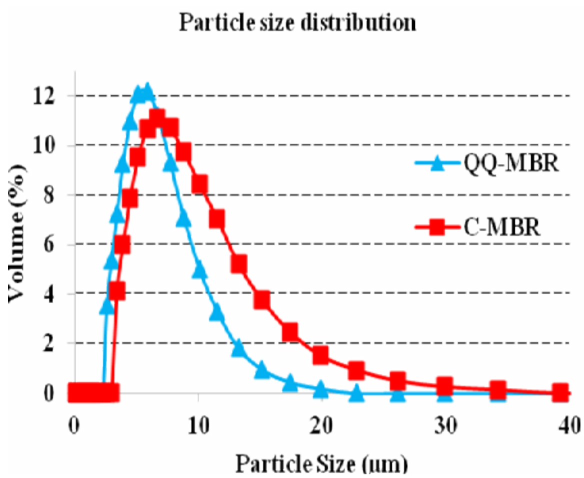
To further investigate the effects of QQ on mixed liquor characteristics, capillary suction time (CST), SVI and pollutant removal aptitudes of both the MBRs were monitored regularly under both modes (phases) of operation. Sludge dewaterability is one of the most important sludge properties that can have a direct impact on membrane filtration performance. CST measurement is used to characterize the sludge dewaterability through capillary suction. Higher CST in C-MBR could be the result of increased concentration of soluble matter including soluble EPS because of which filterability deteriorated [36] and vice versa for QQ-MBR.

Organic removal capabilities of both the MBRs were found to be excellent in terms of COD, ammonium-nitrogen and phosphate-phosphorous concentrations as reported in Table-5 for both modes (phases) of operation. Both MBRs achieved more than 90% removal of the COD and ammonium-N under both phases.

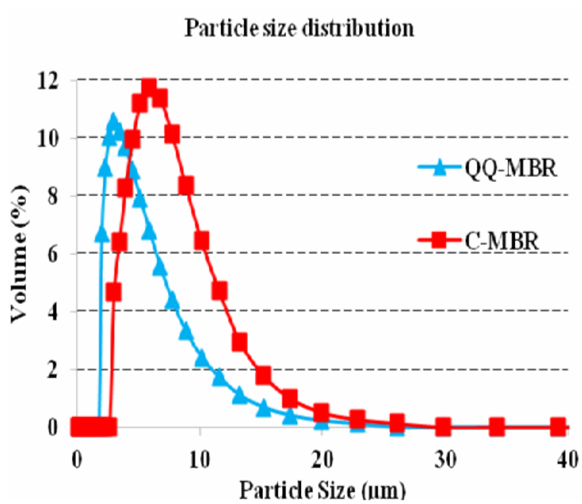
In addition, the floc size distribution (PSD) and average particle size in mixed liquor of both MBRs were measured using particle size analyzer as shown in Fig. 4 and the average floc size in QQ-MBR was 5.76µm and 4.39µm in phase I&II, respectively which was slightly smaller than C-MBR *i.e.*, 7.84µm and 6.45µm in phase I&II, respectively. The C-MBR sludge exhibited larger flocs and wide range of PSD as compared to QQ-MBR in both the phases. Floc breakage is due to the shear force exerted by beads kept in suspension in both reactors. The slightly decreased floc size and relatively skewed PSD detected in QQ-MBR as compared with C-MBR may be due to the collision of relatively heavier CEBs as compared to vacant beads with microbial flocs. Similar reduction in floc size was observed in hybrid MBRs with the presence of moving bio-media (K1, kaldnes®) [35, 37].

Table-5: Comparison of treatment routine of C-MBR and QQ-MBR.

Pollutants	QQ-MBR Effluent (mg/L)	QQ-MBR (Present Removal)	C-MBR Effluent (mg/L)	C-MBR (Present Removal)
Phase I - Without Backwashing				
COD	25.07 (60) ± 17.85	91%	19.94 (60) ± 9.55	93%
PO_4^{-3}-P	5.81 (60) ± 0.88	62%	6.53 (60) ± 0.27	55%
NH_4^{+1}-N	3.40 (60) ± 2.72	90%	2.04 (60) ± 1.95	94%
Phase II - With Backwashing				
COD	17.82 (60) ± 2.82	90 %	13.06 (60) ± 2.43	93%
PO_4^{-3}-P	6.19 (60) ± 0.47	64 %	7.47 (60) ± 0.96	57 %
NH_4^{+1}-N	1.98 (60) ± 1.03	90 %	1.56 (60) ± 0.88	92 %



(a) Phase I - Without Backwashing



(b)Phase II - With Backwashing

Fig. 4: Particle size distributions of C-MBR and QQ-MBR; (a) Phase I-Without Backwashing; (b) Phase II-With Backwashing.

Conclusions

The study elaborates the presence of vacant beads versus *Rhodococcus* sp. embedded beads in C-MBR and QQ-MBR along with backwashing respectively treating real wastewater. The presence of CEBs extended the filtration cycle of membrane in QQ-MBR by mitigating the biofouling, due to reduction in soluble EPS concentration. Whereas, backwashing helped to retard the TMP jump by inhabiting pore narrowing and pore blocking. Under this stage of filtration, the proportion of membrane pores, open to filtration, are much less as compared to chemically-cleaned membrane/virgin membrane *i.e.*, the local TMP within the membranes pores, open to filtration, is significantly greater. In presence of cyclic backwashing, proportion of membranes pores open to filtration can be prolonged till TMP reaches terminal value of 30 kPa. At the same time, relatively high values of R_p in case of QQ-MBR indicate a disadvantage of permanent pore blocking of membrane or irreversible fouling. This lead to shorten the membrane life. Hence, QQ is not sustainable in the long run for full scale MBRs. The CEBs improved the sludge dewaterability resulting in enhanced sludge filtration capability. The polymer coated alginate beads wererelatively stable in the MBR system over prolong filtration duration in treating real domestic wastewater. However very small particle size distribution was observed mainly due to low sludge retention time and physical collision of beads with the flocs.

Acknowledgements

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