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Quorum quenching MBR operations for biofouling control under different operation conditions and using different immobilization media

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ABSTRACT

Biofouling is one of the major problems in reaching the goal of being the only preferable technology for advanced wastewater treatment for membrane bioreactors (MBR). In this study, the quorum quenching mechanism (QQ) was applied to MBR technology with prevention of group behavior of micro-organisms via signaling. This study aims to prepare three different QQ immobilization media, which are (1) cell entrapping bead (CEB), (2) microbial vessel (MV), and (3) rotating microbial carrier frame (RMCF). Two different *Rhodococcus* sp. BH4 (QQ bacteria) amounts and three different fluxes were used to see their effects on QQ mechanism. The flux was more effective than QQ bacteria amount. Transmembrane pressure (TMP) profiles showed that all QQ products were successful in antibiofouling during MBR operation. While CEB was the most effective one, MV had the minimum influence on the TMP reduction. On the other hand, it was found out that RMCF is the most feasible one according to the cost analysis results. This study offers an idea about the potential of QQ applications in pilot- and real-scale MBR plants after examinations on different operation conditions and different QQ products.

Keywords: Membrane bioreactors; Wastewater treatment; Biofouling control; Quorum quenching; High-operation flux

1. Introduction

In today's world, advanced wastewater treatment to get available water has become crucial because of gradual decrease in fresh water resources. As known by the researchers who work on advanced wastewater treatment, membrane bioreactor combining membrane technology, and biological treatment process is one of the most preferred alternatives [1]. Furthermore, it is becoming more popular every day via high-effluent quality and operation conditions which decrease area requirement [2–4]. The achilles heel of this technology with growing popularity is biofouling [5]. The flux of the filtration membrane begins to fall rapidly because of biofilm [6]. Increasing operation pressures in order to ensure the continuity of permeate flux results in an increase in unit cost of wastewater treatment. In the literature, it can be seen that there are a variety of research studies based on chemistry, engineering, etc. to create a solution for biofouling in membrane bioreactors (MBR) [7–12]. Some of these mentioned

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solutions could be inadequate from time to time, since the biofilm blocking feature of some of these could be defeated by time or have high costs. Indeed, the main point of this inadequacy is that biofouling is a totally natural and biological process. Quorum sensing mechanism, which is a quite new concept, can be explained as the communication via signalization and group behavior exhibition by micro-organism [13–15]. The most common one of these group behaviors is biofilm creation [16–18]. Communication mechanism can be interrupted via degradation of N-acyl homoserine lactone (AHL), which is secreted for signalization, by bacteria and this can be called bacterial quorum quenching [19–23].

Bacterial quorum quenching mechanism (QQ) can adapt to MBR operation using various immobilization media in which quorum quenching bacteria are immobilized. There are two main bacterial quorum quenching applications that take place in literature: cell entrapping bead (CEB) and microbial vessel (MV) (polymeric/ceramic) [24–26]. The main aim of bacterial quorum quenching applications is to decrease the transmembrane pressure (TMP). TMP profile related with biofouling could be affected by operation conditions including the flux. The higher the flux causes the higher the TMP values [27]. With this reason; first of all, the effects of operation conditions on bacterial quorum quenching were studied using only one immobilization media type, which is preferred as CEB because of its common usage. Furthermore, it can be said that several successful quorum quenching MBR operation studies were carried with CEB and MVs. However, CEB and MV have disadvantages like low-mechanical durability and low-F:M ratio for immobilized bacteria, respectively. Within this scope, after the effect of MBR operation conditions on bacterial quorum quenching was examined using CEB, potentials on prevention of biofouling and decreasing on unit wastewater treatment cost using different immobilization media such as; CEB, MV, and rotating microbial carrier frame (RMCF) were also studied and tried to be determined under the same operation conditions.

2. Material and method

2.1. Reagents

Commercial C8-HSL (Cayman, USA) was preferred as representative chemical for signal molecules and the stock solution of C8-HSL was stored under −20˚C temperature condition. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was supplied from Sigma-Aldrich (USA) with its powder form and X-Gal solution was stored under −20˚C temperature and dark conditions after prepared with dimethylformamide (DMF) bought from Merck (Germany). Spectinomycin was supplied as a powder from Sigma-Aldrich (USA) and the stock solution of spectinomycin was stored under −4˚C temperature and dark conditions. Tetracycline was also supplied with its powder form from Sigma-Aldrich (USA) and stock solution was kept at −20˚C temperature. Luria Bertani (LB) medium was prepared from its granular form according to the supplier (Merck, Germany) instructions. Lastly, sodium alginate was supplied from Sigma-Aldrich (Germany).

2.2. Preparation of QQ products

2.2.1. Bacterial strains and growth conditions

Agrobacterium tumafaciens A136 (Ti-)(pCF218)(pCF372) [25,28,29] was preferred to use for detection of AHL signal molecules using it as a biosensor strain. *A. tumefaciens* A136 was cultured on LB medium containing spectinomycin (50 mg/L) and tetracycline (4.5 mg/L) in order to get two plasmids which can provide the AHL response system. This incubated A136 was used for Quorum Quenching activity bioassay. *Rhodococcus* sp. BH4, which was used as QQ bacteria for this study, cultured on LB broth and incubated in a 30˚C rotary shaker (160 rpm, Nüve, Turkey) for 24 h.

2.2.2. Cell entrapping beads

CEBs were prepared according to the method obtained from [24]. About 100 ml sodium alginate (4% w/v) and 300 ml $CaCl_2$ (3% w/v) solutions were prepared and autoclaved using a steam sterilizer (Nüve OT032, Turkey). After *Rhodococcus* sp. BH4 bacteria solution was centrifuged for 10 min (6,000 rpm, Hettich 320R, Germany), it was added to the sodium alginate solution. This mixture was distilled into $CaCl_2$ solution which is placed on a magnetic stirrer (WiseStir MSH-A, Germany). Created beads were stored at 4˚C for 8 h to increase their strength. At the end of 8 h, beads were stored in physiological saline for the future time.

2.2.3. Microbial vessels

MV was prepared using hydrophilic polyvinylidene fluoride (PVDF) hollow fiber microfiltration membranes (Philos Co. Ltd, Korea) that have 0.2 μm nominal pore size and this method was taken from [25]. A small 10-cm length hollow fiber module, which has a closed and an open end, was manufactured using 0.2 cm^2 membrane. After *Rhodococcus* sp. BH4 bacteria solution was centrifuged for 10 min (6,000 rpm, Hettich 320R, Germany), it was pumped into the fibers using a peristaltic pump (Watson Marlow 323D, USA).

2.2.4. Rotating microbial carrier frame

RMCF is a module composed of a polycarbonate frame and four cubbyholes. The cubbyholes of RMCF were covered with PVDF microfiltration membrane (Microdyn Nadir GmbH, Germany) that has a nominal pore size of 0.20 μm and thickness of 210–250 μm. After *Rhodococcus* sp. BH4 bacteria solution was centrifuged for 10 min (6,000 rpm, Hettich 320R, Germany), it was filled into each cubbyholes using a syringe. The polycarbonate frame of RMCF was sealed with special glue in order to avoid cell escape and each cubbyhole of RMCF has a small channel with 0.75 mm diameter for bacteria solution renewal.

2.3. Quorum quenching activity test

QQ activities of free cells and immobilized cells in beads, MVs and RMCF were measured using a bioassay. In this bioassay, AHL concentrations were determined with indicating agar plate method adopted from Park et al. [30]. Indicating agar plate was prepared by mixing (ratio of 9:1) LB-agar and an overnight culture of A136 that includes spectinomycin (50 mg/L), tetracycline (4.5 mg/L) and X-gal (0.2 g/L). Samples taken regularly from the reaction tube were loaded into the wells of this indicating agar plate. As a result of chain reaction of C8-HSL, A136, and X-gal, the blue color was developed. C8-HSL concentration decreases with time by the degradation of AHL by free or immobilized QQ bacteria could be determined using the relationship equations related with blue color zone areas and standard AHL concentrations.

After QQ activity test, QQ media were added into the MBRs and used during operations. In Fig. 1, schematic drawings of immobilization media and their locations in the MBR can be seen. While CEBs are in a dispersed mode, MV is in a stable mode in the MBR. On the other hand, RMCF is submerged into the MBR using an impeller top and rotated during operation via a rotor.

2.4. MBR system

Within the content of quorum quenching MBR studies, two parallel MBRs were simultaneously operated under the same operation conditions. While one MBR named as control MBR was operated as a conventional MBR, the other one named as QQ MBR was operated with QQ product additions. In this study, MBRs were operated under the constant flux condition. Scheme of the MBR system was given in Fig. 2.

Activated sludge was supplied from Paşaköy Advanced Biological Wastewater Treatment Plant in Istanbul (Turkey) and it was acclimated to synthetic wastewater prior to the MBR operations. The composition of the synthetic wastewater can be listed as follows (mg/L): glucose, 400; yeast extract, 14; bactopeptone, 115; $(NH_4)_2SO_4$, 104.8; KH_2PO_4, 21.75; $MgSO_4$, 15.63; $FeCl_3$, 0.075; $CaCl_2$, 2.45; $MnSO_4$, 1.8; and $NaHCO_3$, 255.5. Control MBR and QQ MBR were connected to a computer and controlled using an automation program that can adjust the values of operating parameters like water levels, pH, temperature, oxidation-reduction potential (ORP), and dissolved oxygen concentration according to the desired levels listed in Table 1. Membrane module was prepared using hollow fiber microfiltration membrane supplied from Philos Co. Ltd (MegaFlux I, Korea).

2.5. Experimental systematic

With the aim of this study, a two stage experimental systematic was carried. While the first stage of the experimental systematic intended to determine the relationship of QQ effect with the immobilized QQ bacteria amount and flux, the aim of the second stage was the comparison of three different immobilization media about their quorum quenching effects. This first stage can be described as an optimization study. The reason why CEB was selected as the immobilization media for this part of the study was that this immobilization media is one of the main and the most common immobilization media in the literature. Operating strategies for the optimization experiments were given in the Table 2. The experimental systematic of the second stage can be seen in Table 3. During all these studies, mixed liquor suspended solid (MLSS) concentrations in MBRs were maintained within the range of 12,000–13,000 mg/L.

2.6. Analytical methods

During MBR operation some important parameters like MLSS, COD, TKN, and TP were analyzed. While MLSS concentrations of the mixed liquor were determined to check and control the parameters that are directly related with biofouling, COD, TKN, and TP

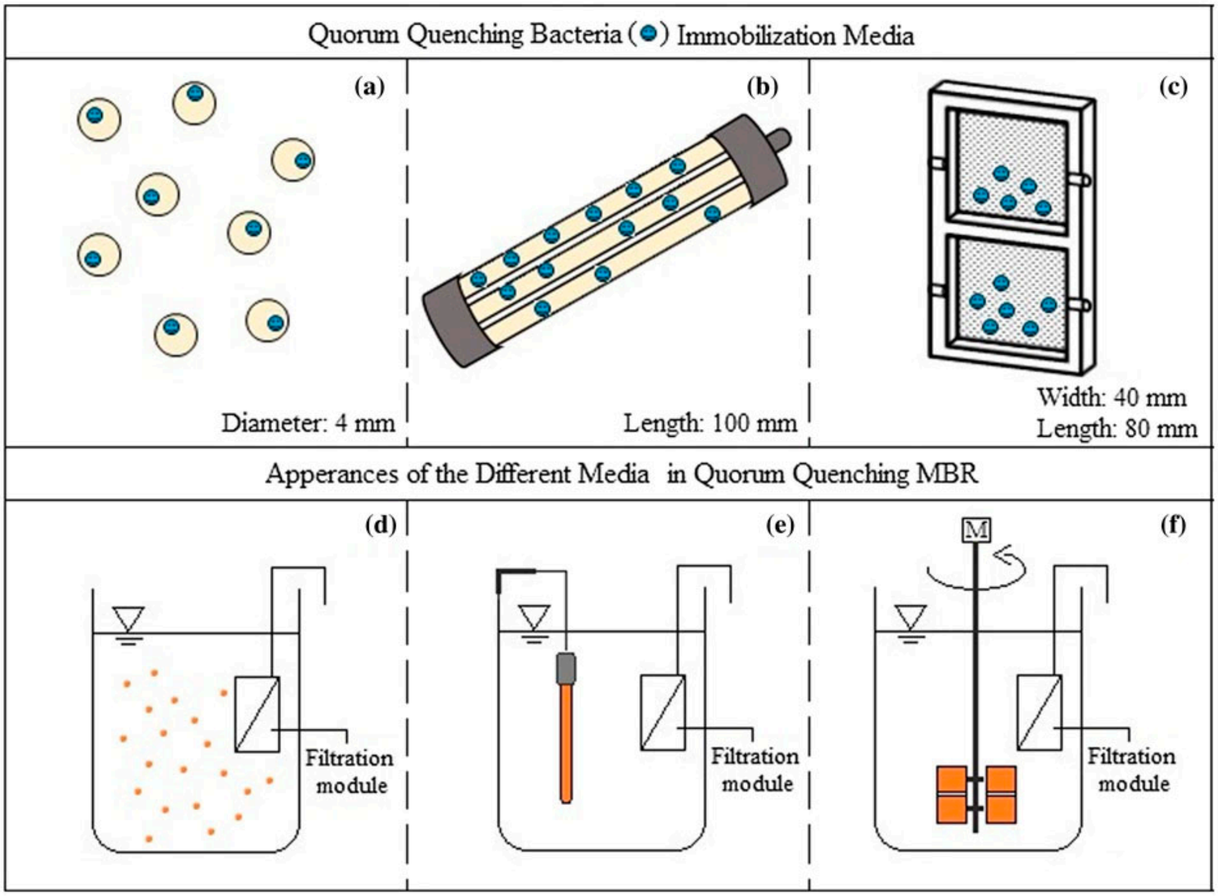

Fig. 1. Different QQ immobilization media: (a) CEB, (b) MV, (c) RMCF, (d) locations of moving CEBs in a MBR, (e) location of a MV in a MBR, and (f) location and rotation of a RMCF in the MBR.

concentrations in feed and permeate flows were analyzed to check whether proper and efficient microfiltration processes in control MBR and QQ MBR were achieved or not. MLSS, COD, TKN, and TP were determined according to Standard Methods [31].

3. Results and discussion

3.1. *QQ MBR operation under different operation conditions*

3.1.1. *Quorum quenching activities of CEBs in a standard AHL solution*

Optimization studies on effect of operation conditions were carried as four QQ MBR operations using CEBs according to the experimental systematic given in Table 2. The aim of this study was to see the effects of immobilized QQ bacteria amount and flux on the QQ mechanism efficiency. It is important to determine the QQ effect (AHL degradation rate) of manufactured CEBs in a standard AHL solution using bioassay before adding to the reactor for healthy evaluations of different QQ mechanism efficiencies resulted from the operation conditions. This bioassay is based on AHL, signal molecule, and degradation rate with time in a standard 200 nM C8-HSL solution. The rate of degradation in the standard AHL solution is directly related with the immobilized QQ bacteria amount. AHL degradation rates of four different CEBs which have two different immobilized QQ bacteria amounts as 1.5 and 7.5 mg BH4/cm^3 are given in Fig. 3.

As seen in Fig. 3, while CEB that includes 1.5 mg BH4/cm^3 could degrade 49% of 200 nM C8-HSL in 120 min, CEBs that include 7.5 mg BH4/cm^3 could show QQ mechanism efficiencies, which can be defined as the degradation efficiencies of AHL in a certain time, as the average of 85.8% ± 2.3 in the same duration. It can be said that QQ mechanism efficiency could increase with the increase in the immobilized QQ bacteria amount and three CEBs including 7.5 mg BH4/cm^3 had quite close QQ mechanism efficiencies.

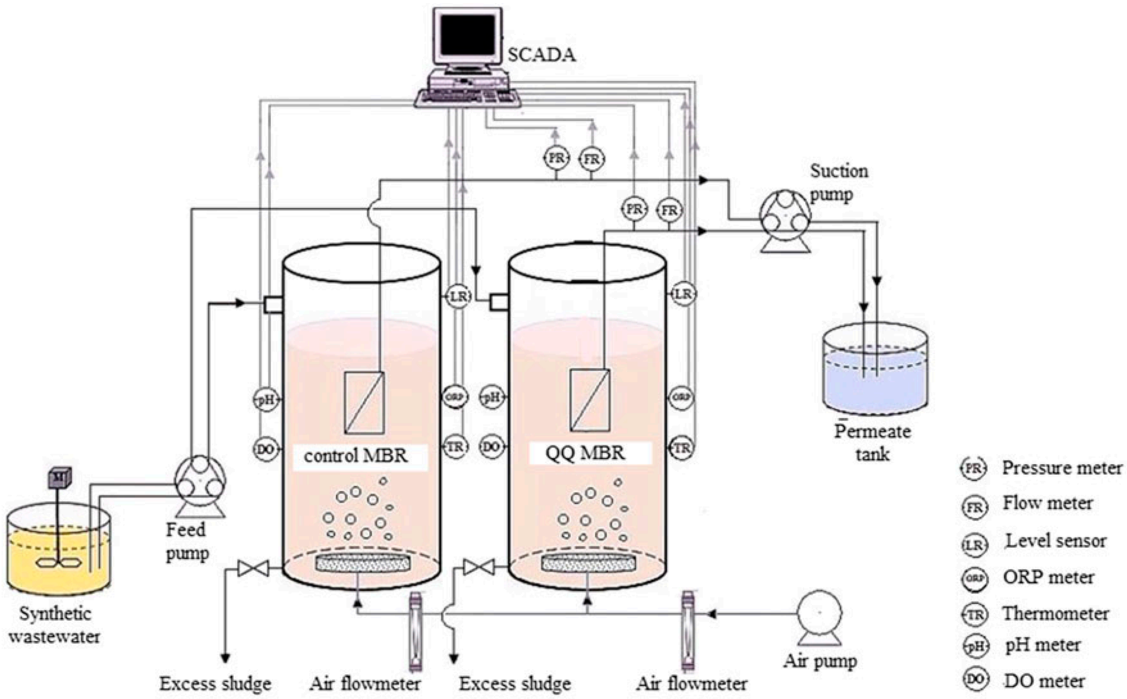

Fig. 2. MBR system.

Table 1
Operating parameters and conditions of the MBR

Parameter	Unit	Value
Working volume	L	5
pH	–	6.8–7
Dissolved oxygen	mg/L	4.2–6.5
ORP	mV	170–200
Temperature	℃	20 ± 2
SRT	d	30
HRT	h	13
Membrane area	m^2	0.01
COD removal efficiency	%	~95
Feed COD	mg/L	440

Vacant CEB that was used as a control sample for this study did not include bacteria, and it was seen that this CEB showed also a small amount of decrease in AHL concentration in the environment (~9.5%). This decrease could be evaluated as the surface adsorption of the CEB and this is negligible.

3.1.2. Quorum quenching activities of CEBs in the MBR

After QQ mechanism efficiency determination, CEBs were used in MBRs. In these four studies, while control MBR was operated with vacant CEBs, QQ MBR was operated with QQ CEBs having the same amount. Control MBR and QQ MBR were operated parallel and under the same and constant flux conditions. At the beginning of each study, activated sludge of the control MBR and QQ MBR was totally mixed in a tank and added to the control MBR and QQ MBR after it was divided into two. New membrane modules were used for each operation. The rate of TMP increase in control and QQ MBR were determined using the TMP profiles obtained from both reactors. It is a known fact that TMP values increase with the biofouling formation in time. Areas under the TMP graphs drawn after operations were calculated via taken the integrals of the formulas of TMP profiles, and fouling reductions were parameterized via percentage statement of the differences between these areas. TMP profiles for four studies can be seen in Fig. 4.

As seen in Fig. 4, while TMP values increased via fouling in the control MBRs, TMP values in QQ MBR were kept lower than TMP values of control MBRs. This means that in all studies, quorum quenching was achieved. The first CEB study was carried out under low flux and low-immobilized QQ bacteria amount conditions, and biofouling could be prevented with 78.7% efficiency. In the second study, while flux value was kept the same with the first study, the amount of the immobilized QQ bacteria was increased four times

Table 2
Experimental systematic used for the comparison of QQ effects obtained under the different operation conditions

Study	QQ bacteria amount in immobilization media	Flux (LMH)	MLSS (g/L)	Aim of the operation
CEB I	1.5 mg BH4/cm^3	20	12–13	QQ effect determination
CEB II	7.5 mg BH4/cm^3	20	12–13	Effect of immobilized QQ bacteria amount on QQ effect
CEB III	7.5 mg BH4/cm^3	30	12–13	Effect of an increase of 50% in flux on QQ effect
CEB IV	7.5 mg BH4/cm^3	50	12–13	Effect of an increase of 50% more in flux on QQ effect

Table 3
Experimental systematic used for the comparison of QQ effects obtained via different quorum quenching media

Study	QQ bacteria amount in immobilization media	Flux (LMH)	MLSS (g/L)	Aim of the operation
CEB IV	7.5 mg BH4/cm^3	50	12–13	QQ effect of CEB in high-flux MBR operation
MV	7.5 mg BH4/cm^3	50	12–13	QQ effect of MV in high-flux MBR operation
RMCF	7.5 mg BH4/cm^3	50	12–13	QQ effect of RMCF in high-flux MBR operation

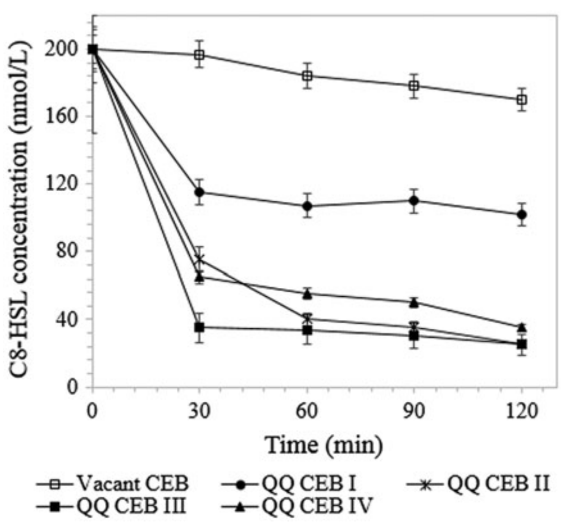

Fig. 3. QQ activities of CEBs (vacant beads did not include QQ bacteria).

and 99.3% biofouling prevention efficiency was achieved with a 21% efficiency increase. After the second study, the flux was increased to see the efficiency of the same amount immobilized QQ bacteria under the harder conditions. In this study, study CEB III, the efficiency about biofouling prevention was found as 96.1%. It can be said that efficiency decreased with flux increase; however, high-quorum quenching efficiency could be achieved for this flux. It is a fact that very high flux values could result in rapid and irreversible fouling in MBR treatment. In the fourth study, control MBR and QQ MBR were operated as high-flux MBRs and it was desired to see the potential quorum quenching effect of immobilized QQ bacteria on rapid fouling. Finally, quorum quenching efficiency was 77.7% for this study. This efficiency result is close to the result of the first study. In the light of these results, it can be mentioned that four times increase in the immobilized QQ bacteria amount could hardly meet the one and a half times increase in flux. As a result, the effect of flux on TMP increase was higher than the effect of the immobilized QQ bacteria amount on TMP decrease.

3.1.3. Removal of organics

It is necessary to check whether microfiltration process had properly been carried during the MBR operation or not to be able to mention about the QQ as a result of TMP decrease. In this regard, COD and TKN removal efficiencies of control MBR and QQ MBR were determined. It can be said that efficiencies were similar for control MBR and QQ MBR and quorum quenching did not affect the removal of organics during operation (Fig. 5).

3.2. QQ MBR operations with different immobilization media

3.2.1. Quorum quenching activities of immobilization media in a standard AHL solution

Because it was found out that flux has a higher effect on the quorum quenching efficiency than immobilized QQ bacteria amount, three different immobilization media were aimed to be compared via

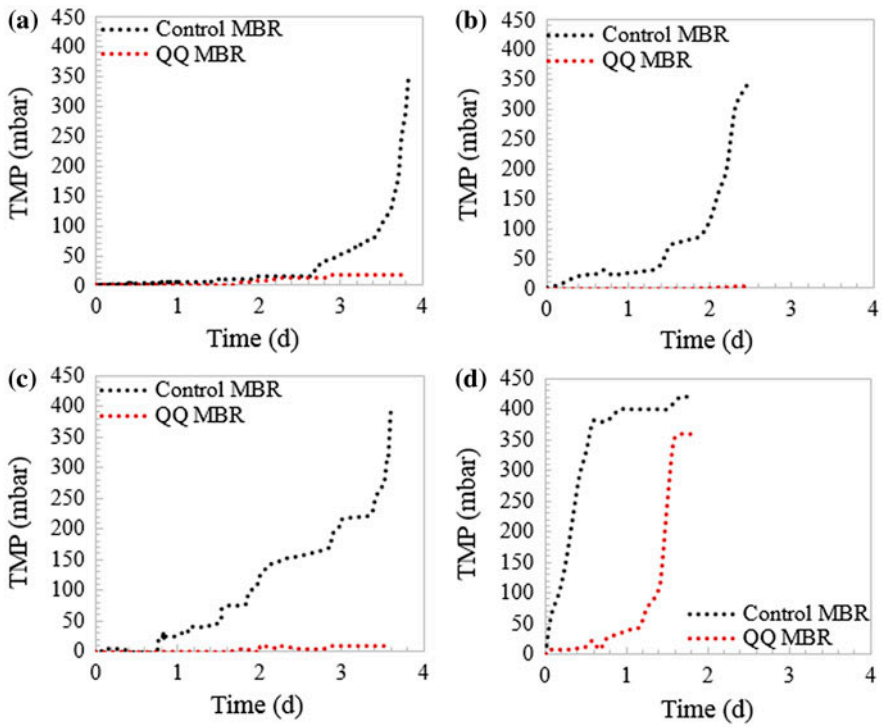

Fig. 4. TMP profiles obtained from CEB studies for different operation conditions (a) study CEB I, (b) study CEB II, (c) study CEB III, and (d) study CEB IV.

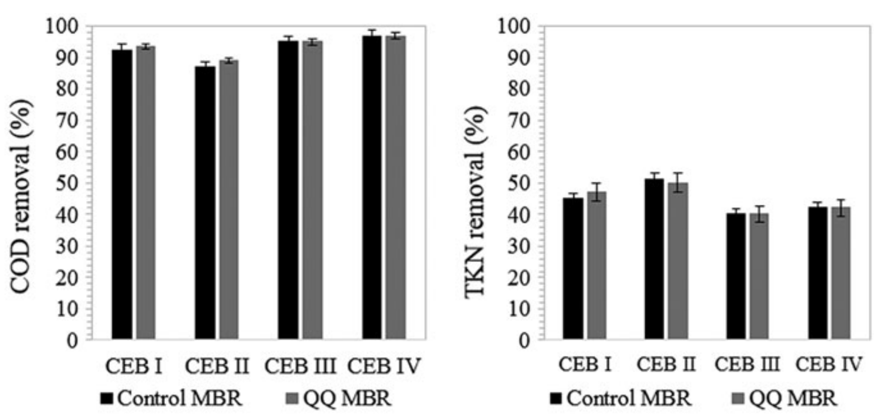

Fig. 5. COD and TKN removal efficiencies obtained from CEB studies for different operation conditions.

their efficiencies under the high-flux conditions. With this aim, two different immobilization media (MV and RMCF) were also manufactured with the same amount of QQ bacteria in the study CEB IV for MBR operations. AHL degradation rates of the vacant and QQ bacteria immobilized vessel and RMCF in a standard AHL solution were determined before operations and given in Fig. 6 with study CEB IV results.

It can be seen in the figure that the immobilization media material or design has no effect on QQ activity potential. The only parameter was the amount of immobilized QQ bacteria for a batch study in a standard solution. While decreasing of the AHL amount in the solution by vacant immobilization media was 13.6% ± 3.6, it was averagely 76.5% ± 4.5 for CEB, MV, and RMCF.

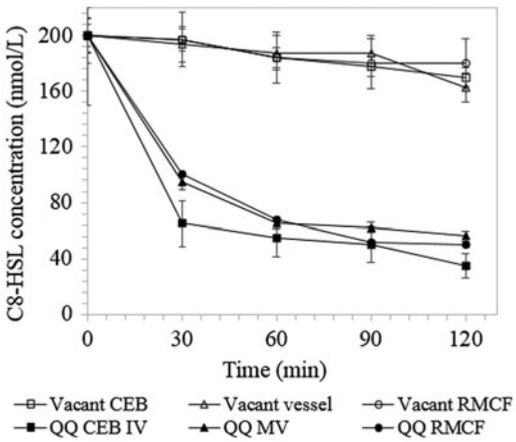

Fig. 6. QQ activities of CEB, MV, and RMCF.

3.2.2. *Quorum quenching activities of immobilization media in the MBR*

After the bioassays, QQ mechanism efficiency studies of MV and RMCF were carried with MBR operations. Firstly, MV study was realized with a parallel MBR operation using a control MBR operated with a vacant vessel and a QQ MBR operated with the MV in which BH4 is immobilized. After MV study, RMCF study was realized with the same conditions. For MV and RMCF studies, control MBR and QQ MBR were operated under the same and constant flux conditions. At the beginning of these each two studies, activated sludge of the control MBR and QQ MBR was totally mixed and added to the control MBR and QQ MBR after it was divided into two again. New membrane modules were used for these operations like CEB studies. The TMP profiles obtained from MV and RMCF studies are given in Fig. 7.

In these two studies, while TMP values of control MBR increased in time rapidly, TMP values of QQ MBR increased more slowly. This prevention of the rapid TMP increase in QQ MBR is a result of QQ mechanism. MV and RMCF could result in 44 and 59.7% TMP decrease during operation, respectively. If the results of the CEB IV (Fig. 6), MV (Fig. 7(a)) and RMCF (Fig. 7(b)) were evaluated together, it can be said that CEB is the most successful one for biofouling control under the high-flux condition. As seen, RMCF had also an important achievement for biofouling control with prevention around 60%. The minimum efficiency was obtained from the MV study and these results are similar to previous studies in the literature [24,25]. First of all, it can be mentioned that the reason why the highest efficiency obtained from CEB study is the high-F:M ratio for immobilized QQ bacteria in the CEB and physical cleaning of the membrane surface via the mobility of CEB in the reactor. In addition to this, RMCF has motion property and provide higher F:M ratio when it is compared to MV. Furthermore, the rotation motion of the RMCF under the membrane module also helps to create shear forces and physical cleaning even if it is not a totally spread media through the reactor like CEB. The biggest disadvantage of MV is immobility as an immobilization medium.

3.2.3. *Removal of organics*

It was also checked whether microfiltration process had properly been carried during the MV and RMCF studies or not to be able to mention about the QQ as a result of TMP decrease. COD and TKN removal efficiencies of control MBR and QQ MBR were also determined. The COD and TKN removal efficiencies for study CEB IV, study MV, and study RMCF were given in Fig. 8 together. It may be said that efficiencies were similar for control MBR and QQ MBR and quorum quenching did not affect the removal of organics during operations.

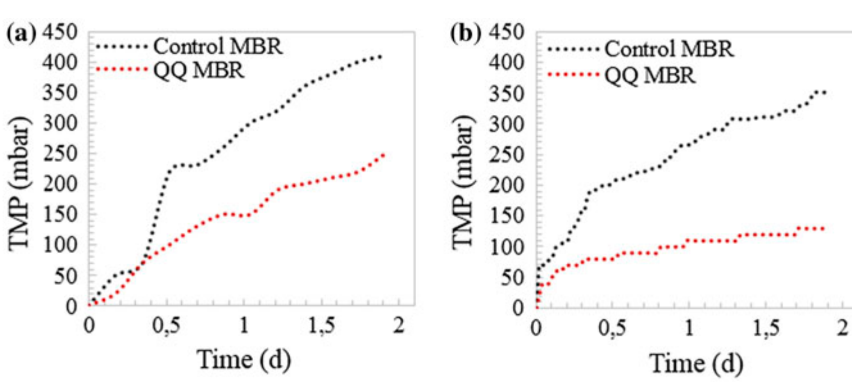

Fig. 7. TMP profiles obtained from MV and RMCF studies (a) study MV and (b) study RMCF.

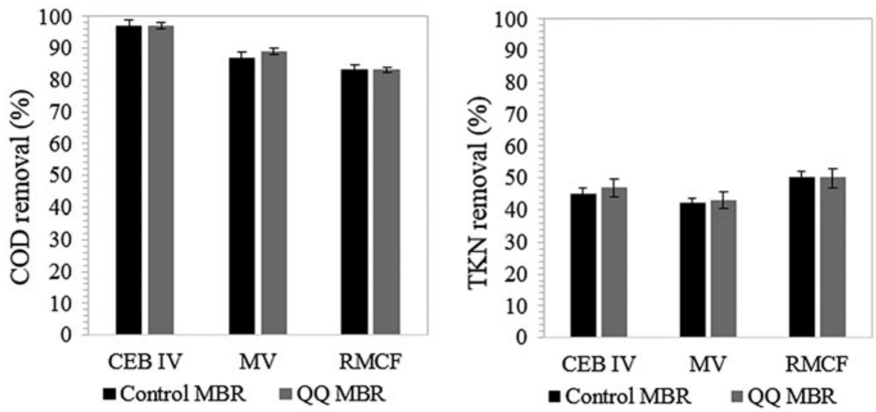

Fig. 8. COD and TKN removal efficiencies obtained from CEB IV, MV, and RMCF studies.

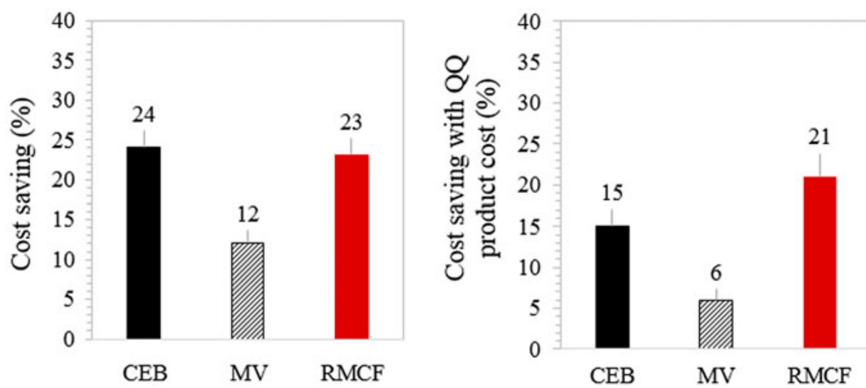

Fig. 9. Cost savings via QQ MBR operations (left: total cost savings and right: cost savings with QQ product costs).

3.3. Energy saving and cost analysis

It is a well-known fact that the successful prevention of biofouling results in reduction of TMP values and this reduction minimizes the spending item for suction pump power, which is one of the most important spending items. Although immobilization media have remarkable high success on TMP decrease, the main point that should be taken into consideration is their manufacturing costs and lifetimes. For instance, the material of CEB shows generally low durability and CEBs should be renewed periodically. Because MV and RMCF are manufactured from inert and durable material, they do not need to be renewed during long-term operations. In this regard, cost analysis with the manufacturing costs and without the manufacturing costs were done to see the rational reduction on unit wastewater treatment cost via quorum quenching. The unit energy cost of domestic wastewater treatment with MBR technology is approximately 0.8 kW h/m^3 [32]. MBR operation cost items with their contributions to the total operation cost can be listed as: feed pumps (10%), activated sludge aeration (20%), membrane aeration (36%), suction pumps (21%), online control (7%), and chemical cleaning (6%) [33,34]. The using of CEB, MV, and RMCF can reduce the costs of two items like suction pump and chemical cleaning via their biofouling prevention in direct proportion to their own QQ mechanism efficiencies in MBR. In addition to this, CEB and RMCF have a reduction on the membrane aeration cost of 10 and 20%, respectively. Total operation costs for MBR operation with/out QQ product were determined and the gained rational reductions on the total operation cost via QQ product usage are given in Fig. 9(left). The results if manufacturing costs were also involved in total operation costs are given in Fig. 9(right).

By looking at Fig. 9(left), it can be said that while CEB and RMCF can create cost savings of 24 and 23% via their higher biofouling prevention efficiencies, respectively, the usage of MV could only reduce the cost 12%. Because of its immobile property MV fell behind about cost saving. Besides, high-manufacturing cost and renewal need of CEB may cause to reduce the saving from 24 to 15%. There is no need for material renewal for MV and RMCF. However, the manufacturing cost of MV is higher than the manufacturing cost of RMCF. Because of this reason while RMCF may provide a cost saving of 23%, MV can only save 6%. To summarize, it can be said that the most feasible immobilization media is RMCF and the unit cost of domestic wastewater treatment with MBR technology would be 0.61 kW h/m^3 with RMCF preference.

In this study, the effects of QQ bacteria amount and flux on application of QQ mechanism to MBR technology were examined. With the results of the studies, it can be said that the flux increase can affect dramatically the QQ mechanism in the MBR. After that, the biofouling prevention efficiencies of three different QQ immobilization media under the same conditions, in which QQ mechanism had difficulty in working properly, were studied. It was seen that CEB is very successful in biofouling prevention; however, RMCF is more feasible for pilot- and real-scale MBR plants when the manufacturing costs of QQ products are taken into consideration.

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