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Research Article

DEVELOPMENT OF A NEW APPROACH TO MEMBRANE BIOREACTOR TECHNOLOGY: ENHANCED QUORUM QUENCHING ACTIVITY

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ABSTRACT
Quorum Quenching (QQ) is a mechanism that prevents cell to cell communication and has recently used as an effective control method against biofouling in membrane bioreactors (MBRs). Rotary microbial carrier frame (RMCF) is an innovative application used in QQ-controlled MBRs that provides immobilization medium to QQ bacteria. However, it eventually caused to decrease in QQ activity as a result of a decline in the number of viable QQ cells in the immobilization media over time, especially during long-term MBR operations. In this study, the effect of regeneration of the QQ cells in the immobilization media on biofouling control in MBR was investigated. The growth kinetic of Rhodococcus sp. BH4 as model QQ bacteria was revealed and the bacteria regeneration time was obtained as 28.3 days. In the operation of the regenerated group, an additional QQ activity of 38.2% was achieved compared to control during a 14 day-duration of QQ-controlled MBR operation. It could be indicated that regenerated QQ bacteria reduced SMP and EPS by degrading the signal molecules, thus more efficient control of membrane fouling was achieved.

Keywords: *Wastewater Treatment; Membrane Processes; Biofouling; Quorum Quenching.*

1. INTRODUCTION

Membrane bioreactors (MBRs) have become an attractive process in advanced wastewater treatment. One of the major problems of MBRs is membrane biofouling which causes the flux decline and increases the unit cost of wastewater treatment (Drews, 2010; Lade *et al*., 2014; Wu and Fane, 2012). Various studies have considered the effect of membrane modification with nanoparticles (Koseoglu-Imer *et al*., 2013; Rahimi *et al*., 2015; Ergön-Can *et al*., 2016) and the effect of the varying operating conditions (Ahmed *et al*., 2007; Wu *et al*., 2011; Dvorak *et al*., 2011) in order to prevent and/or mitigate the biofouling. However, biological-based biofouling mitigation techniques have come forward due to the high costs and instability of the physical and chemical-based approaches. Bacteria detect the environment and communicate each-others to form biofilm via signaling molecules (Hammer and Bassler, 2003; Barrios *et al*., 2006; Dong and Zhang, 2005) which is known as quorum sensing (QS). On the other hand, biofilm formation can be inhibited by disrupting the signaling molecules. The inhibition of QS is commonly referred to as quorum quenching (QQ). Recently, the QQ mechanism for the inhibition of biofilm formation was adapted to MBR studies (Yeon *et al*., 2008; Oh *et al*., 2012; Kim *et al*., 2013; Köse-Mutlu *et al*., 2016).

Firstly, Yeon *et al*. (Yeon *et al*., 2009) used the QQ enzyme (acylase) by immobilizing on magnetic particles for effective biofouling control in MBR. Then QQ bacteria having QQ enzyme activity was used by immobilizing in a polymeric microbial vessel due to the instability and purification cost of the enzyme. Up to now, a number of studies have reported that investigate the effect of immobilization media with QQ bacteria such as alginate bead (Kim *et al*., 2013), ceramic microbial vessel (Cheong *et al*., 2014) on the sustainability of the QQ activity. However, QQ applications for biofouling control in the MBR can raise difficulties and carried studies presented that QQ alginate beads as an immobilization media are nondurable and QQ microbial vessels have a low food-to-microorganism ratio to could cause the growth inhibition of QQ bacteria. In our previous studies (Köse-Mutlu *et al*., 2016; Ergön-Can *et al*., 2017) a new approach on the immobilization media to overcome these drawbacks of QQ-controlled MBR has suggested. Rotary microbial carrier frame (RMCF) was manufactured from a polycarbonate multi-frame covered by microfiltration membrane. It was then filled with QQ bacteria and attached to an impeller shaft to allow rotating independently of the main membrane filtration module in the bioreactor. RMCF as an immobilization media provides a durable, renewable and refillable environment for QQ bacteria. However, a decrease in the QQ activity could occur in each cycle of the MBR operation over time. As our knowledge, bacteria can realize the QS and QQ in high levels during their exponential growth phases in which bacterial vital activities are at the highest point (Wagner *et al*., 2003).

This study aims to maintain a stable and sustainable QQ activity of *Rhodococcus sp.* BH4 as model QQ bacteria in order to prevent biofouling in MBR operation as this specie was preferred in the previous studies and the results can be comparable. It is possible to regenerate QQ bacteria in the RMCF to keep bacteria in the exponential growth phase. RMCF is made of an inert and non-dispersible material/structure and provides an opportunity for the regeneration of immobilized bacteria by using possible discharge/filling lines during the pilot- or full-scale MBR plant operation. Batch and continuous experiments were carried out for the determination of growth kinetics and QQ activity. The net biomass production rate (μ) and the endogeneous decay coefficient (k_d) of *Rhodococcus sp.* BH4 in the MBR conditions was used to calculate bacteria regeneration time. The efficiency of suggested operation conditions was evaluated by comparing the results with the un-regenerated group.

2. MATERIALS AND METHODS

2.1. Reagents

Spectinomycin and Tetracycline were supplied as powders (Sigma-Aldrich, USA), and the stock solutions were stored at 4 °C, in the dark and at -20 °C, respectively. Since it is the most common signal molecule in QS systems, commercial homoserine lactone (C8-HSL) that was supplied from Cayman (USA) was selected and used as the representative chemical for signal molecules. The stock solution stored at -20 °C. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) from Sigma-Aldrich was used with its powder form. After the X-Gal solution was prepared using dimethylformamide (DMF), which was bought from Merck (Germany), it was kept at -20 °C and in the dark. Microfiltration membrane was a polyvinylidene fluoride (PVDF) membrane (Microdyn Nadir GmbH, Germany) and had a nominal pore size of 0.20 μm and thickness of 210–250 μm (Ergön-Can *et al*., 2017). All synthetic wastewater ingredients were also supplied from Sigma-Aldrich (USA).

2.2. Bacterial Strains and Growth Conditions

Agrobacterium tumafaciens A136 (Ti-)(pCF218)(pCF372) was preferred as a biosensor strain for the detection of N-acyl homoserine lactone (AHL) signal molecules (Yeon *et al*., 2008; Oh *et al*., 2012; Kawaguchi *et al*., 2008). A Luria-Bertani (LB) medium containing spectinomycin (50 mg/L) and tetracycline (4.5 mg/L) was used to culture *A. tumefaciens* A136 for the maintaining the two plasmids that provide the AHL response system. *Rhodococcus sp.* BH4, which was isolated from a real MBR plant by Oh *et al*. (2012), was tasked as the QQ bacterium. It was cultured on LB broth and incubated at 30 °C for 24 hours.

2.3. Batch Study on Mass Flux

The mass flux of the microfiltration membrane that is used for covering the RMCF surface was determined. Within this regard, an experimental setup was prepared. This experimental setup included a beaker, which contains synthetic wastewater with 150 mg/L chemical oxygen demand (COD), and a pocket made using an MF membrane, which is filled with distilled water (Fig. 1.A). The composition of the synthetic wastewater was as follows (mg/L): glucose, 133; yeast extract, 4.67;

bactopeptone, 38.3; $(NH_4)_2SO_4$, 34.9; KH_2PO_4, 7.25; $MgSO_4$, 5.21; $FeCl_3$, 0.025; $CaCl_2$, 0.817; $MnSO_4$, 0.6; and $NaHCO_3$, 85.17. Osmotic pressure and total organic matter (TOC) concentration in the synthetic wastewater (outside environment) and in the distilled water (inside environment) were routinely determined. Sampling had been continued until the osmotic pressures on the outside and on the inside were the same. Mass flux was calculated using the equation given below (Eq. 1).

$$J_m=(\Delta TOC)/(\Delta t*A_{mem}) \quad (1)$$

where J_m ($mg.m^{-2}h^{-1}$), ΔTOC (mg/L), and A_{mem} (m^2) are the mass flux through the membrane, the change in the TOC amount in time and the membrane area, respectively.

2.4. Batch Study on Growth Kinetic

After the mass flux was determined, the growth kinetics of *Rhodococcus sp.* BH4 in the reactor conditions was tried to be revealed by using a batch study. With this aim, a batch experimental setup was prepared. Inoculated BH4 bacteria were centrifuged and BH4 with the same amount used in the RMCF studies (Köse-Mutlu *et al*., 2019; Ergön-Can *et al*., 2017), which is approximately 25 mg dry weight of biomass per ml, added into the synthetic wastewater. The composition of the synthetic wastewater was the same as the recipe given in the section of the batch study. The determination of the elemental concentrations in synthetic wastewater was based on the calculations related to the dilution of the feed in the reactor caused by existing water in it. The measurements carried with the samples taken from the supernatant of the activated sludge (data not shown). During the 6 days-duration incubation of BH4 in this synthetic wastewater, the optical density (OD) of the bacterial solution was routinely measured by using an ultraviolet (UV) spectrophotometer (Hach Lange, DR 500, USA). Moreover, TOC concentrations were also measured in order to see the rate of organic matter removal by the microorganisms. According to the results of these measurements, μ and k_d coefficients were found out using Eq. 2 and Eq. 3 and minimum mean cell residence time (MCRT) (Equation 4) was calculated. μ and k_d coefficients were found out by using the slope of the exponential growth and exponential decay phases, respectively (Metcalf and Eddy, 1980). According to these values, the minimum MCRT was calculated and used as the regeneration time. All studies had carried out duplicated.

$$\mu_m=(\ln(OD_{t1})-\ln(OD_o))/\Delta t \quad (2)$$
$$k_d=-(\ln(OD_{t2})-\ln(OD_{t3}))/\Delta t \quad (3)$$
$$\mu = \mu_m - k_d \quad (4)$$
$$1/\Theta_{min} = \mu$$

where μ_m is the maximum specific bacterial growth rate (d^{-1}), ODt is optical density at time t, OD_o is optical density at the beginning, k_d is endogenous decay coefficient (d^{-1}), μ is the net biomass production rate (d^{-1}), and Θ_{min} is the minimum mean cell residence time (d).

2.5. Continuous Study on Quorum Quenching Activities

In the light of idea about the positive effect of bacteria regeneration on the quorum quenching activity, an experimental study was realized with a continuous feed. Sterilized synthetic wastewater, which is the same as the used one in the growth kinetic study, was used as inoculation media, and COD concentration was kept constant and at 150 mg/L according to the previously determined organic matter removal rates. 10 mg BH4 (dry weight) were inoculated on the first day. This study was carried with two groups: 1) control group and 2) regenerated group. While the control group was inoculated without an interception, a determined volume of the regenerated group was removed from the flask according to the sludge retention time calculations. The aim was this removal was to provide a constant QQ bacteria number and log phase for the regenerated group. Besides, a control group can carry on their routines like growing, divisions, aging and cannibalism in an interference-free environment. All studies had carried out duplicated.

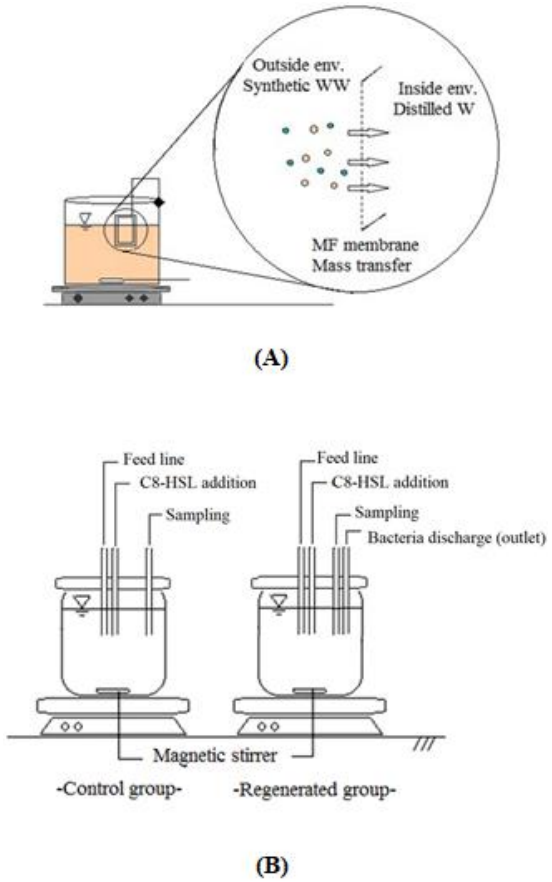

Fig. 1. (A) Experimental setup for mass flux determination (WW: wastewater, W: water, env.: environment) and (B) experimental setup of continuous study on the regeneration

This experiment was carried out under the aseptic conditions. The experimental setup can be seen in Fig. 1.B. QQ activities of these two groups were daily

determined by adding 200 nM C8-HSL and taking samples at 0^{th}, 10^{th} and 30^{th} minutes. AHL concentrations were determined with the indicating agar plate method from Park *et al*. (2003). Indicating agar plate was prepared as follows: 1) mixing (ratio of 9:1) LB-agar, 2) an overnight culture of *A. tumefaciens* A136 including spectinomycin (50 mg/L), tetracycline (4.5 mg/L), and X-gal (0.2 g/L), 3) AHL degradation by *Rhodococcus sp*. BH4 occurred in the reaction tube (the initial concentration of C8-HSL was 200 nM), and 4) reaction mixtures were added into the well of plates. The residual amounts of AHL were determined by the equations that were based on the color zone sizes.

2.6. MBR Operation

Three parallel MBRs were operated under the same operating conditions as MBR-A, MBR-B, and MBR-C. MBR-A was a conventional MBR. While MBR-B was operated with RMCF, MBR-C was a parallel operated MBR with RMCF having QQ bacteria regeneration lines. The scheme of the MBR system was depicted in Fig. 2. Activated sludge was supplied from an advanced biological wastewater treatment plant in Istanbul. The activated sludge was subjected to an acclimation before the operation. The composition of the synthetic wastewater was the same used in the batch study on the mass flux. The elemental concentrations in the MBR were the same ass the concentrations in the batch and the continuous studies by using this recipe taking the dilution into the consideration. MBRs were connected to a computer to control all operating parameters like water levels, pH, temperature, oxidation-reduction potential (ORP) values and dissolved oxygen concentrations (Table 1). All membrane bioreactors were operated under the steady-state conditions following the previous studies carried out during several months (Ergön-Can *et al*., 2017).

Table 1. MBR operation details

Parameter	Unit	Value or information
pH		6.8~7.2
Dissolved oxygen	mg/L	4.2-6.6
ORP	mV	170-210
Temperature	^oC	24±2
Membrane type		Hollow fiber (Philos MegaFluxI)
Membrane area	cm^2	100
Flux	$L/m^2/h$	60
COD removal efficiency	%	~ 96
MLSS	mg/L	12,000
Feed COD	mg/L	440
Seeding sludge		Paşaköy Advanced Biological WWTP (Istanbul/Turkey)
Reactor volume	L	4.75

MBR-A, MBR-B, and MBR-C were operated under the constant flux condition by using a microfiltration system consisting of the hollow fiber membrane module. Membrane module properties and dimensions were the same as in the previous study (Ergön-Can *et al*., 2017). While the effective area of the hollow fiber membranes in the module was around 100 cm^2, the hydraulic retention time (HRT) and sludge retention time (SRT) were kept as 13 h and 30 d, respectively. The mixed liquor suspended solids (MLSS) concentrations in MBRs were maintained within the range of 11000-12000 mg/L.

2.7. Analytical Methods

MLSS and COD were determined according to Standard Methods. TOC concentrations were measured using the TOC instrument (Shimadzu, Japan). Osmotic pressures were determined with a 3250 model instrument of Advanced Instruments Inc. (USA).

3. RESULTS AND DISCUSSION

3.1. Mass Flux

Firstly, the mass flux of the microfiltration membrane that is used for coverage of the RMCF and in which *Rhodococcus sp*. BH4 is immobilized was determined. The change of the osmotic pressures of the inside and of the outside environments in time was monitored. In addition to this, organic matter concentrations in these environments were determined and mass flux was calculated. Osmotic pressures in two environments were equal after a time and this result can mean that nutrients and minor elements could pass through the microfiltration membrane as expected. At the end of the 8^{th} hour, the microfiltration membrane totally soaked, and the osmotic pressures had started to change. The osmotic pressures in the two environments were nearly equal at the end of the 12^{th} hour. After 24 hours, the immobilized bacteria can achieve to get the total nutrient mass because of the total equalization. Moreover, the mass flux of total organic carbons throughout the microfiltration membrane was calculated as 2.26 $mg.m^{-2}h^{-1}$ using the data obtained from this side-study. Because there is no filtration process in which driving force is an applied pressure, the mass transfer was realized as a result of the osmotic pressure differences.

3.2. Growth Kinetics

After the mass flux was determined, a growth kinetic study was carried. The growth kinetic study was realized under the conditions which are seen in the membrane bioreactors operated in this study, and the result of the study was given in Fig. 3. As shown in the figure, bacteria can degrade the synthetic wastewater with 150 mg COD/L with 60% efficiency in their first 24 hours. At the end of the 48 hours, the rate of substrate degradation started to decrease. The reason can be explained as the beginning and the end of the log phase of the culture. Bacteria could easily reach the substrate and use for their rapid metabolism in the first 48 hours. According to the slope changes in the biomass graph, growth phases could be determined. As expected, the bacteria stayed in a stable situation in their first 23

hours. At the end of the 23rd hour, divisions immediately started and this was the siren of the start of the exponential growth phase. The exponential growth phase had continued until the end of the 50th hour. Because there was feed for once at the beginning, bacteria had been faced with the substrate limiting condition between 48th and 74th hours. At the end of the stationary phase, the culture started to show a decay situation which can get by looking at the high negative slope of the OD graph

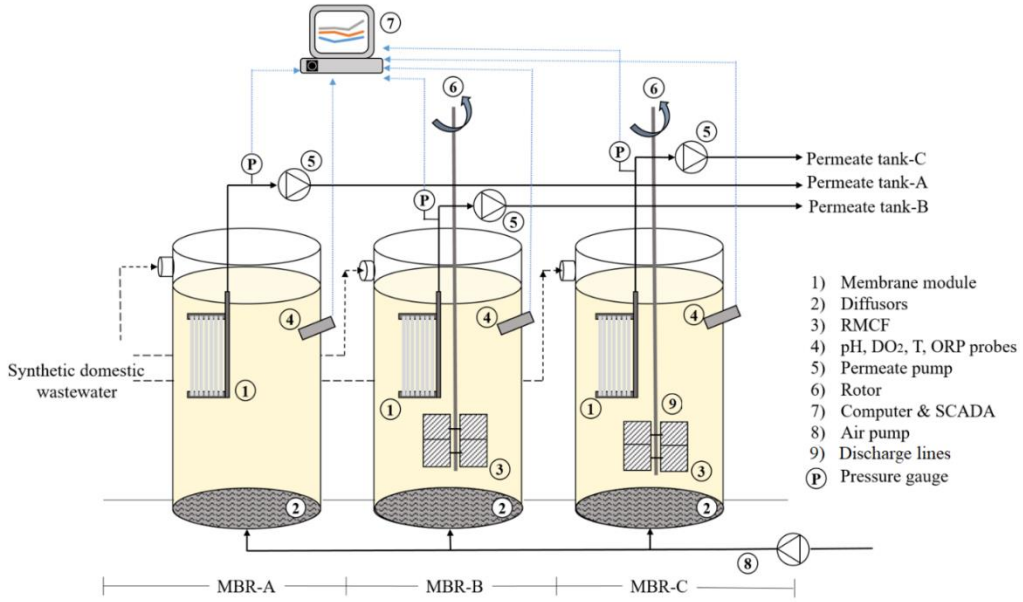

Fig. 2. Schematic description of the MBR set-up

At the end of the inoculation, the OD values were lower than the OD values at the beginning. This final term was the decay phase of the inoculated species. By using the slope values, the growth, and the decay rate constants can be determined. In this study, Eq. 2, Eq. 3 and Eq. 4, and data from Fig. 3 was used, and bacteria retention time of *Rhodococcus sp.* BH4 was determined as 28.3 days. This means that 3.5% of the total volume can be daily removed, all volume can be renewed at the end of this 28.3 days-duration.

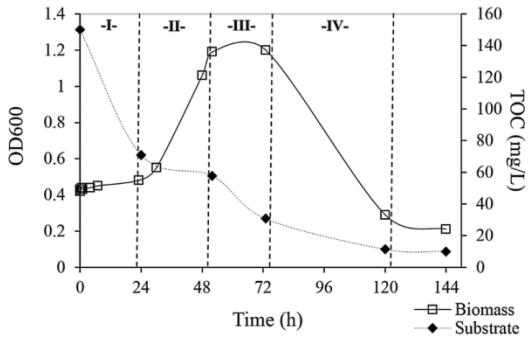

Fig. 3. Growth phases of BH4

This means that daily removal of 3.5% of the total immobilized bacteria amount from the medium may help the bacteria regeneration, constant bacteria OD, prevention of the bacterial aging, and increase of the QQ activity, which are closely related together. In this study, the application of the daily bacteria removal from the immobilization medium was tried to be explained by the means of the "regeneration of the immobilized culture" statement.

3.3. Effect of the Regeneration on the Quorum Quenching Activity

An experimental study with a control group was carried in order to check the possible effect of the bacterial regeneration on the quorum quenching activity. QQ activities of control and regenerated groups during the 4 days were daily determined. Daily removal of 3.5% of the total immobilized bacteria amount from the group, which is named as the regenerated group, was realized during this continuous study. AHL degradations were given in Fig. 4.A. The detailed results can be seen in the Appendix. Correlation coefficients were higher than 0.953 for each quorum quenching activity experiment, and the results can be used if the correlation coefficient should be higher than 0.95 for this type of bio-assay. The initial AHL concentration was 200 nmol/L for both groups at the beginning of each AHL degradation rate study carried daily. Because both groups include a high amount of *Rhodococcus sp*. BH4 in their environments, AHL degradation was realized dramatically. In 30 minutes *Rhodococcus sp*. BH4 could degrade the AHL up to 50 mg/L and lower concentrations. It is a known fact that *Rhodococcus sp*. BH4 is a highly effective QQ specie used before for biofouling control by the researchers (Dong and Zhang, 2005; Jahangir *et al*., 2012). It can be said that while the regenerated group could save its quorum quenching

activity, control group started to lose its quorum quenching activity after 24 hours. On the first day, the two groups showed the same quorum quenching activity (87% for the control group and 88% for the regenerated group), and they could rapidly degrade the AHL in their environment. 24 hours after the first bacteria removal application; the QQ activities were determined as 80% and 88% for the control group and regenerated group, respectively. It may be said that the control group had started to lose its activity because this group had lived in the stationary phase for that 24 hours-duration. After 48 hours, the time was the 72nd hour and the quorum quenching activities were 72% and 82% for the control group and regenerated group, respectively. The decrease in the QQ activity of the control group as 8% may result from the late stationary phase and the start of the decay phase. In the last day of the experiments; it was seen that the regenerated group could sustain its quorum quenching activity, the control group showed a quorum quenching activity with 65% efficiency. This means that quorum quenching bacteria can lose its AHL degradation efficiency with time in the environment because of the limiting factors and living population ratio in the suspension. In light of this study, it may be mentioned that bacteria removal from the cubbyholes of the RMCF can result in sustained quorum quenching activities.

3.4. Effect of Regeneration on Quorum Quenching Activites During the MBR Operation

In order to examine the effect of bacteria regeneration in QQ MBR operation, two parallel MBRs were operated for two weeks. Transmembrane Pressure (TMP) profiles obtained from operations were given in Fig. 4.B. In order to determine the TMP decreasing effect caused by the daily bacteria removal, the areas under the TMP graphs were calculated by taking integrals of the line equations having correlation coefficients higher than 0.997.

$$\text{Decreasing percentage (\%)} = \frac{(\int_0^t f\,control\,(t)\,dt - \int_0^t f\,regenerated\,(t)\,dt)}{\int_0^t f\,control\,(t)\,dt} * 100 \quad (5)$$

where $f_{control}$ is the equation of the TMP profile of MBR-A and $f_{regenerated}$ is the equation of the TMP profile of MBR-B. According to these TMP profiles, it can be said that the regeneration of bacteria resulted in an additive effect on biofouling prevention via quorum quenching. There is a slight difference between the TMP profile of MBR-A and the TMP profile of MBR-B. While TMP values reached 200 mbar in MBR-A, it was around 100 mbar in MBR-B. Because these both MBRs were operated as QQ MBRs, TMP values could not reach high values during these 14 days of operation. According to the decreasing percentage calculations, it can be mentioned that there was an additional 38.2% TMP decrease via daily bacteria removal from the cubbyholes of RMCF. The TMP decrease with RMCF usage against conventional MBR usage resulted in a 60% TMP decrease (Köse-Mutlu *et al*., 2016). Bacterial regeneration may create an increase in the quorum quenching effect of 15%, which means an additional decrease of 3.4% on the total MBR operation costs.

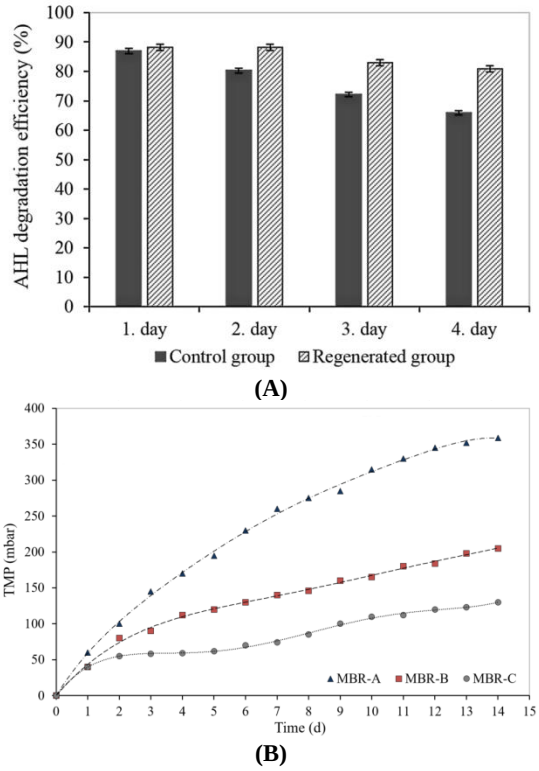

Fig. 4. (A) The effect of the regeneration on the QQ activity and (B) TMP profiles obtained from the parallel MBR operations

4. CONCLUSION

Within the scope of this study, the possible positive effect of bacterial regeneration on the quorum quenching activity was examined. The younger bacteria in their log phases results in the higher efficiencies of many vital activities. Because of this reason, it was thought that a definite percentage, which was decided according to the net biomass production rate which indicates the difference between growth rate and decay rate, of the *Rhodococcus sp.* BH4 solution in the RMCF can be removed, and this can be adapted to the MBR operation. The concluding remarks of this study can be listed as:

- The nutrient mass transfer throughout the microfiltration membrane used for the immobilization of *Rhodococcus sp.* BH4 in RMCF was totally achieved in 12 hours, and the mass flux was determined as 2.26 $mg.m^{-2}h^{-1}$.
- Over the first 23 hours, *Rhodococcus sp.* BH4 was in lag-phase and reached in the exponential growth-phase between 23rd and 50th hours. After a 23 hour-duration stationary-phase, specie had started to live its decay-phase. In light of the net growth rate calculations, it was found out that the regeneration of the immobilized culture may be realized by the means of a volumetric removal of 3.5%.
- According to the QQ activity tests, it was seen that while the QQ activity of the control group had decreased from 87% to 65%, the regenerated group could sustain its QQ activity.

- The sustainability of the QQ activity via bacteria regeneration was also tried to prove with QQ-MBR operations, and an additional QQ activity of 38.2% could be obtained during a 14 day-duration MBR operation.

This concept and the mechanism mentioned in this paper should be studied to enlighten all the effects of various parameters. Since it is an innovative idea, this study is the first for the researchers focused on the QQ MBR. Finally, it can be said that the application of this idea was successful during MBR operation, and it will be possible, especially for the pilot- and full-scale MBR plants with bacteria discharge lines.

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REFERENCES

Ahmed Z, Cho J, Lim BR, Song KG, Ahn KH. (2007) "Effects of sludge retention time on membrane fouling and microbial community structure in a membrane bioreactor". *Journal of membrane science.* Jan 15; 287(2):211-8.

Barrios AF, Zuo R, Hashimoto Y, Yang L, Bentley WE, Wood TK. (2006) "Autoinducer 2 controls biofilm formation in Escherichia coli through a novel motility quorum-sensing regulator (MqsR, B3022)". *Journal of bacteriology.* Jan 1;188(1):305-16.

Cheong WS, Kim SR, Oh HS, Lee SH, Yeon KM, Lee CH, Lee JK. Design of quorum quenching microbial vessel to enhance cell viability for biofouling control in membrane bioreactor. J. Microbiol. Biotechnol. 2014 Jan 1;24(1):97-105.

Dong YH, Zhang LH. (2005) "Quorum sensing and quorum-quenching enzymes". *The Journal of Microbiology*. Feb;43(1):101-9.

Drews A. (2010) "Membrane fouling in membrane bioreactors—characterisation, contradictions, cause and cures". *Journal of membrane science*. Nov 1;363(1-2):1-28.

Dvořák L, Gómez M, Dvořáková M, Růžičková I, Wanner J. (2011) "The impact of different operating conditions on membrane fouling and EPS production". *Bioresource technology*. Jul 1;102(13):6870-5.

Ergön-Can T, Köse-Mutlu B, Koyuncu İ, Lee CH. (2017) "Biofouling control based on bacterial quorum quenching with a new application: rotary microbial carrier frame". *Journal of membrane science.* Mar 1;525:116-24.

Ergön-Can T, Köseoğlu-İmer DY, Algur ÖF, Koyuncu İ. (2016) "Effect of different nanomaterials on the metabolic activity and bacterial flora of activated sludge medium". *CLEAN–Soil, Air, Water*. Nov;44(11):1508-15.

Hammer BK, Bassler BL. (2003) "Quorum sensing controls biofilm formation in Vibrio cholerae". *Molecular microbiology*. Oct;50(1):101-4.

Jahangir D, Oh HS, Kim SR, Park PK, Lee CH, Lee JK. (2012) "Specific location of enca.psulated quorum quenching bacteria for biofouling control in an external submerged membrane bioreactor". *Journal of membrane science*. Sep 1;411:130-6.

Kawaguchi T, Chen YP, Norman RS, Decho AW. (2008) "Rapid screening of quorum-sensing signal N-acyl homoserine lactones by an in vitro cell-free assay". *Applied Environmental Microbiology.* Jun 15;74(12):3667-71.

Kim SR, Oh HS, Jo SJ, Yeon KM, Lee CH, Lim DJ, Lee CH, Lee JK. (2013) "Biofouling control with bead-entrapped quorum quenching bacteria in membrane bioreactors: physical and biological effects". *Environmental science & technology.* Jan 4;47(2):836-42.

Koseoglu-Imer DY, Kose B, Altinbas M, Koyuncu I. (2013) "The production of polysulfone (PS) membrane with silver nanoparticles (AgNP): physical properties, filtration performances, and biofouling resistances of membranes". *Journal of membrane science.* Feb 1;428:620-8.

Köse-Mutlu B, Ergön-Can T, Koyuncu İ, Lee CH. (2016) "Quorum quenching MBR operations for biofouling control under different operation conditions and using different immobilization media". *Desalination and Water Treatment.* Aug 14;57(38):17696-706.

Lade H, Paul D, Kweon JH. (2014) "Quorum quenching mediated approaches for control of membrane biofouling". *International Journal of Biological Sciences.* 10(5):550.

Metcalf & Eddy B, (1980). "Wastewater engineering: Treatment disposal reuse." Central Book Company. New york, USA.

Oh HS, Yeon KM, Yang CS, Kim SR, Lee CH, Park SY, Han JY, Lee JK. (2012) "Control of membrane biofouling in MBR for wastewater treatment by quorum quenching bacteria encapsulated in microporous membrane". *Environmental science & technology*. Apr 11;46(9):4877-84.

Park SY, Lee SJ, Oh TK, Oh JW, Koo BT, Yum DY, Lee JK. (2003) "AhlD, an N-acylhomoserine lactonase in Arthrobacter sp., and predicted homologues in other bacteria". *Microbiology.* Jun 1;149(6):1541-50.

Rahimi Z, Zinatizadeh AA, Zinadini S. (2015) "Preparation of high antibiofouling amino functionalized MWCNTs/PES nanocomposite ultrafiltration membrane

for application in membrane bioreactor". *Journal of Industrial and Engineering Chemistry*. Sep 25;29:366-74.

Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH. (2003) "Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment". *Journal of bacteriology*. Apr 1;185(7):2080-95.

Wu B, Fane AG. (2012) "Microbial relevant fouling in membrane bioreactors: influencing factors, characterization, and fouling control". *Membranes.* Aug 15;2(3):565-84.

Wu B, Yi S, Fane AG. (2011) "Microbial community developments and biomass characteristics in membrane bioreactors under different organic loadings". *Bioresource technology.* Jul 1;102(13):6808-14.

Yeon KM, Cheong WS, Oh HS, Lee WN, Hwang BK, Lee CH, Beyenal H, Lewandowski Z. (2008) "Quorum sensing: a new biofouling control paradigm in a membrane bioreactor for advanced wastewater treatment". *Environmental science & technology.* Dec 18;43(2):380-5.

Yeon KM, Lee CH, Kim J. (2009) "Magnetic enzyme carrier for effective biofouling control in the membrane bioreactor based on enzymatic quorum quenching". *Environmental science & technology.* Sep 2;43(19):7403-9.