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Biofouling control based on bacterial quorum quenching with a new application: Rotary microbial carrier frame

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ABSTRACT

Bacterial quorum quenching (QQ) is a new approach for controlling of membrane fouling in membrane bioreactors (MBRs). In this study, a novel bacterial immobilization medium named rotary microbial carrier frame (RMCF) was applied to inhibit biofilm formation in an MBR. RMCF was prepared by entrapping QQ bacteria (*Rhodococcus* sp. BH4) onto polycarbonate frame covered with microfiltration membrane. RMCF was submerged into the MBR using a rotational axle and rotated independently of the filtration module. QQ effect was examined with short- and long-term MBR operations. In MBRs operated with RMCF, transmembrane pressure (TMP) increasing rate was decreased with 65% efficiency. The prevention of biofouling resulted from both physical (shear forces) and biological (quorum quenching) effects of RMCF, and it was seen that RMCF could sustain its QQ effect over long-term operations. Biofilm formation inhibition by RMCF was confirmed visually using Confocal Laser Scanning Microscopy (CLSM). In addition, microbial population dynamics in MBRs were examined and it was found that RMCF had an effect on microbial diversity. The novelty of RMCF is to immobilize QQ bacteria into a rotating apparatus. With the application of RMCF, the anti-biofouling effect in an MBR had been investigated.

1. Introduction

Membrane bioreactor (MBR) technology has recently received considerable attention due to its high-quality effluent for municipal and industrial wastewater treatment [1,2]. Although it has numerous advantages over conventional wastewater treatment processes, membrane fouling is one of the major drawbacks and process limitations of MBRs [3]. Membrane fouling in MBR, primarily caused by microbial deposition/growth and microbial product accumulation on membranes [4], can be called as biofouling. The control of membrane biofouling in MBR has been recognized as a key factor for reducing the operating and maintenance costs through energy saving biofilm formation on the membrane surface lead to several adverse effects on MBR performance, such as significant increase of transmembrane pressure (TMP), reduction in flux and membrane lifespan [5].

In recent years, a novel approach has been demonstrated that the quorum sensing mechanism is correlated with biofouling in membrane bioreactors [6]. Quorum sensing is a biological communication mechanism between microorganisms which assessing their population densities based on small diffusible signaling molecules called autoinducer like N-acyl homoserine lactones (AHL). When it reaches to an adequate level, QS induces some bacterial behaviors such as bioluminescence, antibiotic production, virulence, biofilm formation and some bacterial activities such as the production of soluble microbial products (SMP) and extracellular polymeric substances (EPS), exocellular enzyme secretion via activating the transcription of specific genes. Thus, quorum quenching, based on the interruption of cell-to-cell communication between microorganisms, has found an application field in MBR as an innovative approach.

Quorum quenching is an efficient mechanism for inhibition of biofilm in the MBR for advanced wastewater treatment. The quorum quenching causes to prevent of biofouling, by which autoinducer-mediated quorum sensing, is interrupted. Three basic QS-based membrane biofouling control strategies have been reported [7]: (i) blockage of AHL production, (ii) interference with the signal receptor, and (iii) inactivation of AHL signal molecules.

Since the concept of quorum quenching was used to control of biofouling in MBRs by Yeon et al. [6], various strategies have been developed. Firstly, they investigated the effects of acylase added in a batch type of MBR on the transmembrane pressure. And then, a magnetic enzyme carrier (MEC) which immobilized acylase onto magnetic particles was produced [8]. Furthermore, acylase and chit-

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osan were deposited and cross-linked on the NF membrane surface [9]. Bacteria, having quorum quenching activity were also immobilized in a hollow fiber membrane [10,11] because the enzymes have disadvantages such as instability and purification cost. Subsequently, quorum quenching bacteria were encapsulated into the alginate beads and biofouling was inhibited successfully [12]. Cheong and his colleagues designed a ceramic microbial vessel to enhance the QQ bacterial viability, as relatively higher F:M ratio inside the vessel rather than inside the hollow fiber membrane [13]. It is a fact that all of the studies mentioned above were successful as progressive applications. Besides, QQ applications for biofouling control in the MBR can have some limitations and previous studies showed that QQ beads are non-durable and QQ vessels have small food-to-microorganism ratio.

In this study, an immobilization medium has been designed as an alternative to the present bacterial QQ studies such as QQ beads and QQ vessels in MBR. On the other hand, different membrane concepts have widely been used for the process enhancements in various biotechnological applications like microorganism-based product obtainments during fermentation [14–16] and rapid biomass increase during an adhesive cell cultivation in the bioreactor [17–20]. It is possible to evaluate these studies within these frameworks: i) more effective filtration with rotational membrane filtration modules and ii) effective cell cultivation with rotational membrane-based apparatus. From this point of view, a new immobilization medium can be manufactured. This immobilization medium named as “rotary microbial carrier frame” (RMCF) was designed to be able both to carry the microorganisms and to prevent the microorganism leakage into the environment, which is the activated sludge in the MBR, while helps to create the bacterial effects at the same time. Within this regard, the RMCF was manufactured with small multi-frames and QQ bacteria was immobilized under the flat sheet microfiltration membrane in the cubbyholes. This immobilization medium was submerged at the bottom of the bioreactor and rotated by the means of the impellers independently of the main membrane filtration module and was tasked for the prevention of biofilm formation on the main membrane filtration module during an MBR operation. Finally, besides the anti-biofouling effect of RMCF via quorum quenching, RMCF is a new immobilization medium for bacteria which is renewable, refillable, and durable.

2. Materials and methods

2.1. Reagents

Spectinomycin was supplied as a powder (Sigma-Aldrich, USA), and the stock solution was stored at 4 °C, in the dark. Tetracycline was also supplied as powder form (Sigma-Aldrich, USA) and stock solution was stored at −20 °C. Commercial C8-HSL from Cayman (USA) was preferred as a representative chemical for signal molecules and the stock solution was stored at −20 °C. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was supplied from Sigma-Aldrich with its powder form and the X-Gal solution was stored at −20 °C and in dark after prepared by using dimethylformamide (DMF) which is bought from Merck (Germany).

2.2. Bacterial strains and growth conditions

Agrobacterium tumafaciens A136 (Ti^-)(pCF218)(pCF372) [6,21,22] was used for detection of *N*-acyl homoserine lactone (AHL) signal molecules as a biosensor strain. *A. tumefaciens* A136 was cultured on Luria-Bertani (LB) medium containing spectinomycin (50 mg/L) and tetracycline (4.5 mg/L) to maintain the two plasmids that provide the AHL response system. *Rhodococcus* sp. BH4, isolated from a real MBR plant by Oh et al. [10], was used as a QQ bacterium and cultured on LB broth and incubated in a 30 °C rotary shaker (160 rpm) for 24 h.

2.3. Quorum quenching activity test

QQ activities of both free and immobilized cells in RMCF were measured respectively. AHLs were detected using the indicating agar plate method adopted from Park et al. [23]. Indicating agar plate was prepared by mixing (ratio of 9:1) LB-agar and an overnight culture of *A. tumefaciens* A136 including spectinomycin (50 mg/L), tetracycline (4.5 mg/L), and X-gal (0.2 g/L). AHL degradation by *Rhodococcus* sp. BH4 occurred in the reaction tube with the initial concentration of C8-HSL with 200 nM. The reaction mixtures were loaded into the well of plates overlaid with *A. tumefaciens* A136. The residual amounts of AHL were calculated using the relationship equations based on the color zone size and known amounts of AHLs.

2.4. Manufacturing of RMCF

The mobile immobilization medium composed of a polycarbonate frame including four cubbyholes was covered with a polyvinylidene fluoride (PVDF) membrane (Microdyn Nadir GmbH, Germany). PVDF membrane has a nominal pore size of 0.20 μm and thickness of 210–250 μm. Because this membrane tasked as a semi-permeable barrier that prevents the QQ bacteria leakage in the bioreactor and allows the transfer of needed nutrient and oxygen by bacteria in the cubbyhole without any vacuum force, the properties like high hydrophilicity, efficient nutrient and gas transportation, and smaller pore size were reasons for the preferring of this PVDF membrane. *Rhodococcus* sp. BH4 culture was filled into each cubbyhole with an amount of 1 ml (~25 mg dry weight of biomass per ml) using a sterilized syringe. Polycarbonate frame was completely sealed with glue avoiding cell leaking. Cubbyholes have inlet and outlet channels to refill with the cell suspension. In order to reach the desired total QQ bacteria amount, the number of RMCF can be adjusted. In this study, three of the mobile modules (the total PVDF membrane area: 0.0108 m^2) were mounted to impeller before the submerging to MBR. A photography and illustrations of RMCF were given in Fig. 1. Quorum quenching mechanism of RMCF in MBR was shown in Fig. 2.

2.5. Membrane bioreactors

Short term and long term quorum quenching activities of RMCF were studied in MBRs. Two parallel MBRs were operated under the same operating conditions as the MBR-A and MBR-B. While MBR-A was a conventional MBR, MBR-B was a parallel operated MBR with Quorum Quenching product. Scheme of the MBR system was depicted in Fig. 3.

Activated sludge was taken from an advanced biological wastewater treatment plant in Istanbul, Turkey, and prior to the MBR operations, it was acclimated to synthetic wastewater during one month. The composition of the synthetic wastewater was 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. MBRs were connected to a computer and controlled by an automation program, according to the target ranges of operating parameters like water levels, pH, temperature, oxidation-reduction potential (ORP) values and dissolved oxygen concentrations (Table 1).

MBR-A and MBR-B were operated under the constant flux condition using the hollow fiber membrane modules. The effective area of the hollow fiber membrane module was around 100 cm^2. The hydraulic retention time (HRT) and sludge retention time (SRT) were 13 h and 30 d, respectively. The mixed liquor suspended solid (MLSS) concentrations in MBRs were determined within the range of 11000–12000 mg/L. Five different experimental studies were carried out to see the effects of QQ bacteria as well as RMCF on membrane fouling in short- and long-term operations. RMCFs were fastened to the impellers of an axial axle and submerged into the QQ MBR at the beginning of