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# Effective quorum quenching bacteria dose for anti-fouling strategy in membrane bioreactors utilizing fixed-sheet media

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ABSTRACT

Quorum quenching (QQ) has been recognized as an emerging anti-biofouling strategy for membrane bioreactors (MBRs), but studies on effective QQ bacterial doses are needed. Therefore, in this study, the QQ efficacy for sheet-type media containing different amounts of QQ bacteria (i.e., *Rhodococcus* sp. BH4) with respect to biofouling control during MBR treatment of synthetic and real municipal wastewaters was investigated. The QQ sheets with well-embedded BH4 bacteria were distinctly effective at alleviating biofouling, but their efficacy was highly dependent on the QQ bacterial dose. The activity of the QQ sheet increased with the QQ bacterial density and its pseudo-first order rate constant reached 3.52 $h^{-1}$ with 100 mg BH4 per liter of reactor volume. No QQ activity loss occurred with long-term use of the QQ sheets. The relationship between QQ dose and fouling delay had a strong, linear correlation. Fouling retardation was more pronounced with real wastewater because it had less biofouling propensity owing to its inferior organics and nutrients. In fact, the MBRs fed with real wastewater experienced a dramatic decrease in extracellular polymeric substance (EPS) content responsible for biofouling. QQ suppressed the EPS secretion leading to fouling mitigation, but with no impact on biological treatment performances.

## 1. Introduction

In membrane bioreactors (MBRs), the attachment and growth of microbes on the membrane surface over the long term, i.e., biofouling, is the major hurdle to making MBRs more competitive and economical for field application [1–5]. Various physical/chemical methods have been developed to try to tackle the biofouling problem, such as air scouring/backwashing [6], chemically enhanced backwashing [7], and subcritical flux operation [8,9], but it is difficult to overcome this natural process. It is known that biofouling (caused by biofilm formation on the membrane surface) is closely associated with the microbial cell-to-cell communication through small diffusible signal molecules, i.e., quorum sensing [10–12].

Recently, a novel strategy to interfere with microbial quorum sensing, called quorum quenching (QQ), by injecting an enzyme (e.g., porcine kidney acylase I) that can degrade signal molecules (e.g., N-acylhomoserine lactone), was developed [13–15]. This QQ strategy enabled biofouling to be delayed substantially, but the addition of enzymes to MBRs appeared to be unrealistic for large-scale application. Another approach was developed that employed a QQ bacterium (e.g., *Rhodococcus* sp. BH4), which had been successfully isolated from an MBR plant and enriched in the laboratory, instead of QQ enzymes [16,17]. A QQ vessel comprised of microporous hollow fiber membranes, in the lumen side of which QQ bacteria were confined and sealed, was devised for and applied to MBRs [16,18]. The QQ vessel was able to alleviate membrane fouling substantially in a laboratory MBR. Biofouling was effectively controlled in an MBR with a QQ vessel even though minimal aeration was supplied for membrane scouring, whereas a conventional MBR had severe membrane fouling [18]. This shows the potential for reducing the operational energy consumption for aeration and filtration in MBRs. It was also found that the QQ bacteria in the QQ vessel can control the biofouling that is irremovable via chemically enhanced backwashing with chlorine, and thereby a synergistic effect on biofouling control was observed with the combined use of chlorine injection and QQ [19]. More recently, with the aim of enhanced QQ activity and biofouling control, a new design of a QQ vessel, which contains QQ bacteria in a rotating frame attached to an impeller, was prepared and then tested in a laboratory MBR [20]. Substantial fouling mitigation was achieved with this mobile vessel.

With the goal of higher efficiency biofouling control, several

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different QQ media designs using alginate and hydrogel polymers have been created and evaluated, such as bead [21,22], hollow cylinder [23,24], and sheet [25] media. The dosages of these media were approximately 0.5–1 volumetric percentage of the reactor volume but were not optimized. These moving media had both biological (QQ) and physical effects on biofouling control because they bump into and subsequently detach the biomass from the membrane surface in addition to signaling inhibition. Thin QQ sheets, which can get into the intermembrane fibers and thus effectively scour the membrane surface, showed greater antifouling efficacy than QQ beads did in laboratory MBR tests [25]. However, there is the possibility that sheets can cover or wrap the membrane fibers and hence exacerbate local fouling in hollow-fiber MBRs. There is concern regarding the long-term activity and durability of these alginate/hydrogel-based QQ media. In addition, there has been no comparative study on the efficacy of QQ bacteria doses on the degree of fouling control.

Therefore, the focus of the present study was to investigate and compare the efficiency of QQ sheet media with different QQ bacterial doses in terms of biofouling control and long-term activity. For tests that were more practical, the efficacies of the media were evaluated using real municipal wastewater as well as synthetic feed. During the long-term MBR tests, biological mixed liquor characteristics and treatment performances were continuously monitored and evaluated in terms of biomass concentration, floc size, biopolymer content, and removal of organics/nutrients.

## 2. Materials and methods

### 2.1. Feed wastewater

Two feed wastewater samples were used in this study: synthetic and real municipal wastewater. The synthetic wastewater was prepared in the laboratory daily as described by Weerasekara et al. [18], whereas the real wastewater was collected from the Shincheon Municipal Wastewater Treatment Plant, Daegu, Korea and shipped to the laboratory every two days. During the entire period of MBR operation, the feed wastewater was always stored in the refrigerator at 4 °C. The characteristics of the synthetic and real wastewater samples used are provided in Tables S1 and S2, respectively.

### 2.2. Preparation of QQ sheets

QQ sheets containing different amounts of *Rhodococcus* sp. BH4 (50 mg, 75 mg, or 100 mg per sheet) were prepared using sodium alginate and polyvinyl alcohol solutions as described elsewhere by Nahm et al. [25]. Briefly, BH4 cultivated in Luria-Bertani broth was collected and resuspended in deionized water. A mixture of polyvinyl alcohol and sodium alginate (the mass ratio 10:1) was prepared separately. The polymer solution and BH4 suspension were then mixed, cast on a glass board, submerged in a cross-linking solution (the mass ratio of boric acid to $CaCl_2$ 7:4), stabilized in 0.2 M $Na_2SO_4$ solution, and finally cut into sheets. For the control run, vacant sheets were also prepared using sodium alginate and polyvinyl alcohol solutions, but without QQ bacteria. Detailed information on the QQ and vacant sheets is provided in Table S3. A sheet-type QQ media module consisted of two sheets, each of which was first placed between plastic screen meshes with sieve dimensions of 1.5 × 1.5 mm and then in a plastic frame.

### 2.3. Operation of membrane bioreactor systems

Two identical laboratory-scale submerged MBRs were constructed (Fig. 1) and operated under various operating conditions. MBR operation was divided into six phases according to their corresponding operating conditions, as described in Table 1. From phase 1 through phase 3, two reactors were operated using synthetic wastewater as the feed. After each phase, the mixed liquor of both reactors was collected, homogenized, and divvied between the two reactors. One reactor (reactor 1) was the control, i.e., an MBR with vacant sheets. The other one (reactor 2) was the quorum quenching MBR (QQMBR), with fixed sheet-type QQ media. From phase 4 to phase 6, only one reactor (reactor 2) was operated being fed with real municipal wastewater. The QQ media were installed in phase 5 only. No QQ media were used during phases 4 and 6, i.e., the reactor was operated in the conventional MBR mode.

During the experimental runs, the MBRs received a new membrane module at the start of each phase. The laboratory-scale small membrane modules were prepared using Kolon polyvinyl difluoride hollow fiber membranes with a pore size of 0.1 μm and an effective surface area of 94.2 $cm^2$. Both MBRs (control MBR and QQMBR), with working volumes of 2 L, were operated at a constant flux of 30 L/$m^2$-h with a hydraulic retention time of 7 h and a solids retention time of 100 d. The reactor volume was maintained at a constant level using an electric level switch. The reactor temperature was maintained at 25 °C throughout the experimental period using a temperature-controlled water bath. Continuous air sparging (1.0 L/min corresponding to 72 $s^{-1}$) was applied to MBRs via an air diffuser beneath the membrane module. The aeration intensity was within the recommended velocity gradient range (40–80 $s^{-1}$) for biological treatment systems [18,26]. Membrane operation was cyclical, with 19-min filtration followed by 1-min relaxation, which was controlled by a programmable logical controller (K7M-DR30S, LG, Korea). The transmembrane pressure (TMP) of each reactor was recorded continuously with a data logging system comprising a pressure transducer (ZSE40F, SMC, Japan), multimeter (M-3850D, Metex, Korea), and laptop computer.

### 2.4. Assessment of quorum quenching activity

The activity of the QQ media was evaluated in terms of the degradation of a signal molecule, e.g., N-octanoyl-L-homoserine lactone (C8-HSL). Each QQ medium was placed in 200 mL of 175 nM C8-HSL solution, and then the mixture was agitated at 200 rpm. An aliquot (100 μL) of each solution was collected at 0, 15, 30, 60, 120, and 240 min. The concentration of C8-HSL in each sample was determined via a bioassay test using agar indicator plates. A reporter strain, *Agrobacterium tumefaciens* A136, was cultured in Luria-Bertani broth appended with the two antibiotics tetracycline and spectinomycin. Each agar indicator plate was prepared by adding 10 mL of Luria-Bertani agar as a supporting layer followed by the functional layer containing Luria-Bertani agar, the antibiotics, *Agrobacterium tumefaciens* A136, and X-gal, as described by Yeon et al. [14]. Two distinct, shallow holes were made in the solidified agar with the help of sterilized Pasteur pipettes. Each hole was then loaded with 20 μL of sample, and finally the agar plate was incubated for 24 h at 30 °C. The unknown C8-HSL concentrations were calculated based on the size of the blue-colored circle after calibration using known C8-HSL concentrations. The pseudo-first order reaction rate constant of C8-HSL degradation was defined as the QQ sheet activity in this study.

### 2.5. Analytical methods

The amounts of soluble microbial products (SMPs) and extracellular polymeric substances (EPSs) were determined as described elsewhere [18,27,28]. A mixed liquor sample was centrifuged for 10 min at 4000 rpm (2951 g) followed by filtration of the supernatant with a 0.45 μm membrane filter (Millipore). The filtrate was defined as the SMPs, and the residual pellet was further suspended in 30 mL of a 0.9% NaCl solution for EPS extraction. Subsequently, the suspension was heated for 30 min in a water bath at 80 °C and then allowed to cool down at room temperature. Finally, it was centrifuged at 4000 rpm for 20 min, and the EPSs were collected by filtrating the supernatant using a 0.45 μm membrane filter. For both the SMP and EPS fractions, the concentration of proteins present was determined using a modified