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Application of quorum quenching bacteria entrapping sheets to enhance biofouling control in a membrane bioreactor with a hollow fiber module

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ABSTRACT

Quorum quenching (QQ) has been recognized as an innovative approach for biofouling control in membrane bioreactors (MBRs). Recently, QQ bacteria entrapping beads (QQ-beads) have been developed and verified to have excellent anti-biofouling potential in a pilot-scale MBR with flat-sheet membrane modules. In this study, considering the dense structure of hollow fiber (HF) bundles into which QQ-beads can hardly penetrate, QQ bacteria entrapping sheets (QQ-sheets) were developed as a new shape of QQ-media suitable for MBRs with HF modules. In a lab-scale MBR, QQ-sheets with a thickness of 0.5 mm exhibited a greater physical washing effect than did QQ-beads with a diameter of 3.5 mm because the former collided with membrane surfaces at the inner as well as the outer part of HF bundles, whereas the latter only made contact with the outer part. Moreover, QQ-sheets showed 2.5-fold greater biological QQ activity than did QQ-beads due to their greater total surface area at a fixed volume of QQ-media. These results suggest high potential for QQ-sheets to be used in MBRs with HF modules.

1. Introduction

Membrane bioreactors (MBRs) have been widely used as an advanced wastewater treatment technology offering significant advantages, such as a small footprint and high effluent quality. Nevertheless, membrane biofouling (i.e., biofilm formation on the membrane surface) remains a major drawback of MBRs as it significantly reduces membrane permeability, leading to an increase in maintenance and operating costs [1]. Recently, quorum sensing (QS), bacterial cell-to-cell communication via signal molecules, was reported to play a key role in biofilm formation [2,3], and the concept of quorum quenching (QQ), the inhibition of QS, was introduced as a novel molecular biological approach to control biofouling in MBRs for wastewater treatment [4].

First, the enzymatic decomposition of QS signal molecules called *N*-acyl homoserine lactones (AHLs) (i.e., enzymatic QQ) was initiated to inhibit membrane biofouling in an MBR [5–8]. In a follow-up study conducted to overcome the limitations of enzymatic QQ such as high costs and enzyme instability, *Rhodococcus* sp. BH4, which produces AHL-degrading enzyme, was isolated from a wastewater treatment plant and applied as a novel QQ bacterium [9]. Mitigation of biofouling in MBR was achieved by bacterial QQ through microbial vessels encapsulating BH4 bacteria (QQ-vessels) [10–12]. Then, the goal of bacterial QQ shifted toward the development of more efficient QQ-media than the QQ-vessels. Spherical BH4 entrapping beads (QQ-beads) were first reported as moving QQ-media and gave rise to greater biofouling mitigation than the QQ-vessels. It is because they have not only a biological (QQ) effect, but also a physical washing effect through frequent collisions between the media and the biofilm on the membrane surface [13]. In particular, the performance of QQ-beads in biofouling mitigation has been demonstrated not only in lab-scale [13–15] but also pilot-scale MBRs [16,17]. Most recently, cylinder-shaped moving QQ-media (QQ-cylinder [18] and QQ-hollow cylinder [19]) were successively developed for enhanced biological (QQ) and physical washing effects over those with QQ-beads.

However, all previous studies on QQ-media have focused on improving anti-biofouling capabilities without full consideration of membrane module types for water permeation in MBRs. In particular,

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because commercial hollow fiber (HF) modules, deployed in about 75% of total MBR plants [20], are usually composed of HF bundles, the reported media have yet to be ascertained whether their physical washing effect can reach the inner part of a HF module with massed fibers. Moreover, deposition of microbial flocs on the surface of HFs and subsequent clogging problems could attenuate the biological QQ effect of the reported QQ-media due to reduced convection at the inner part of HF modules [21]. Taking into account the structure of a HF module, it is required to design new QQ-media suitable for a HF module.

In this study, we developed QQ bacteria entrapping sheets (QQ-sheets) as new moving QQ-media to enhance the anti-biofouling performance in a QQ-MBR with a HF module. After the application of two types of HF modules, i.e., single-layer hollow fiber (S-HF) and multi-layer hollow fiber (M-HF) modules, both biological (i.e., QQ) and physical washing effects of QQ-sheets were assessed and compared with those of the previously reported QQ-media (QQ-beads). Finally, the anti-biofouling performance of QQ-sheets was evaluated in continuous MBRs.

2. Materials and methods

2.1. Preparation of QQ-media

Two different shapes of QQ-media (i.e., QQ-sheets and QQ-beads) were prepared using *Rhodococcus* sp. BH4 as a QQ bacterium, as it has been reported to degrade AHL signal molecules and control biofouling in MBRs [9]. BH4 grown in Luria-Bertani (LB) broth was collected by centrifugation at 6000 rpm for 10 min. The pellet was re-suspended in deionized (DI) water. At the same time, we prepared a mixed solution of polyvinyl alcohol (Wako, Japan) and sodium alginate (Junsei, Japan) with the mass ratio of 10 to 1, and then the polymer solution was mixed with the re-suspension of BH4. For the preparation of QQ-sheets, the BH4-polymer mixture was cast on a glass board using a micrometer film applicator (Sheen, UK). The cast BH4-polymer mixture was cross-linked via submersion in a boric acid and $CaCl_2$ solution with the mass ratio of 7 to 4, and subsequently in 0.5 M sulfate solution. The cross-linked sheet was cut into small pieces using 10 mm X 10 mm sized cutting press for finalizing the preparation of QQ-sheets. Using this cutting press, four pieces of QQ-sheets were produced at one time of cutting. On the other hand, QQ-beads were prepared using the dripping method, as described in a previous study [17]. The concentration of entrapped BH4 in both QQ-sheets and QQ-beads was 5 mg of BH4 (in dry weight) per g of QQ-media (in dry weight). The non-cell entrapping media (i.e., Vacant-beads and Vacant-sheets) were prepared as controls using the same methods as described above, except with an equivalent volume of DI water instead of the BH4 re-suspension. The density of all media prepared in this study was approximately 1.0g/mL. The procedure for measuring thickness of QQ-sheets is as follows: after measuring the mass of each piece of QQ-sheet, the mass was converted to the volume considering the density of media (approximately 1.0g/mL). The resulting value of volume was divided by width (10 mm) and height (10 mm), and thus the thickness of each QQ-sheet was yielded. The thickness of thin QQ-sheets, medium QQ-sheets, and thick QQ-sheets was approximately 0.48 (± 0.02), 0.73 (± 0.02), and 1.06 (± 0.07) mm.

2.2. Fabrication of HF modules

To fabricate two different HF modules (i.e., S-HF and M-HF) for MBR operation, a polyvinylidenefluoride (PVDF) HF with outer diameter of 1.9 mm (Zeeweed 500, GE-Zenon, USA) was used. Parts for module assembly were prepared using a 3-D printer (Stratasys Object 30, USA). The S-HF (Fig. 1a) had 13 hollow fibers with a total effective filtration area of 0.0152 m^2 and spacing of 2 mm, while the M-HF (Fig. 1b) consisted of 25 (5 by 5) hollow fibers with a total effective filtration area of 0.0208 m^2 and spacing of 1 mm. The packing density of the M-HF was around 39%, which is close to that of commercial HF modules from other companies (usually above 45%) [22].

2.3. Fabrication of polyacrylic stick modules

For the assessment of the physical washing effect of the prepared media for HF modules, single-layer (Fig. 1c) and multi-layer (Fig. 1d) polyacrylic stick modules were prepared from polyacrylic sticks with the same physical dimensions as the HFs for the actual HF module used in MBR operation. They were fabricated using the same methods as those described in Section 2.2 for the HF modules.

2.4. Assessment of physical washing effect

The physical washing effects of Vacant-sheets and Vacant-beads were examined in three batch reactors (working volume of each: 2.5 L) with 10-fold concentrated synthetic wastewater and 3 mL of activated sludge inoculum. The single-layer (Fig. 1c) or multi-layer (Fig. 1d) polyacrylic modules were inserted into each of three batch reactors: control reactor without any medium, reactor with Vacant-beads, and reactor with Vacant-sheets. The loading volume of each medium was fixed at 0.5% (volume of media per volume of reactor). All three batch reactors were operated in parallel at an aeration rate of 1.5 L/min, which is the same as the operating condition of continuous MBRs. After operation for 20 h, the bio-fouled modules were taken out of each reactor and were stained with 0.2% crystal violet (CV) for 10 min, followed by a gentle wash with DI water. The CV-stained sticks in single-layer module (200 mm) were individually dissolved in 25 mL of 95% ethanol for 1 h. In case of the CV-stained stick in multi-layer module, individual CV-stained stick (140 mm) was dissolved 10 mL of 95% ethanol for 1 h. Finally, the absorbance at 570 nm (OD_{570}) of the CV-dissolved solution was measured using a spectrophotometer (Epoch, Biotek, USA). The optical densities of the polyacrylic sticks from the module were averaged, representing the amount of biofilm formed. Since CV was slightly adsorbed on fresh polyacrylic stick, the value of CV concentration of the fresh stick, which was immersed in CV solution for 10 min, was used as a blank for the physical washing test. The physical washing effects of Vacant-sheets and Vacant-beads were represented as the percentage of biofilm reduced relative to the control reactor without media.

2.5. Assessment of QQ activity

The QQ activities of the prepared QQ-media were evaluated in terms of the degradation rate of standard *N*-octanoyl-$_{DL}$-homoserine lactone or C8-HSL (Sigma-Aldrich, USA), one of the major QS signal molecules of AHLs in MBRs [4]. The concentration of this signal molecule was measured via a bioluminescence assay using the reporter strain *A. tumefaciens* A136. In detail, the prepared QQ-media with a fixed volume of 1.0 mL were individually added to 20 mL of 1 μM C8-HSL dissolved in 20 mM phosphate buffer, and the mixture was incubated at 30 °C with shaking at 200 rpm. After 30 min, the solution was sampled for measurement of the concentration of residual C8-HSL through an A136 bioassay. The reporter strain A136 and the C8-HSL samples were mixed and loaded onto a microwell plate. The microwell plate was placed in an incubator at 30 °C for 90 min, after which the Beta-Glo Assay System (Promega, USA) was added to the solution for a luminescent reaction with β-galactosidase produced by the reporter strain. After 40 min of reaction, luminescence was measured by a luminometer (Synergy 2, Biotek, USA). The amounts of C8-HSL were calculated using relationship equations based on the calibration curve derived from standard samples of C8-HSL. The QQ activity was presented as the number of nanomoles of degraded C8-HSL per min, which was measured after 30 min in the presence of QQ media.