More Efficient Media Design for Enhanced Biofouling Control in a Membrane Bioreactor: Quorum Quenching Bacteria Entrapping Hollow Cylinder

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S *Supporting Information*

ABSTRACT: Recently, membrane bioreactors (MBRs) with quorum quenching (QQ) bacteria entrapping beads have been reported as a new paradigm in biofouling control because, unlike conventional post-biofilm control methods, bacterial QQ can inhibit biofilm formation through its combined effects of physical scouring of the membrane and inhibition of quorum sensing (QS). In this study, using a special reporter strain (*Escherichia coli* JB525), the interaction between QS signal molecules and quorum quenching bacteria entrapping beads (QQ-beads) was elucidated through visualization of the QS signal molecules within a QQ-bead using a fluorescence microscope. As a result, under the conditions considered in this study, the surface area of QQ-media was likely to be a dominant parameter in enhancing QQ activity over total mass of entrapped QQ bacteria because QQ bacteria located near the core of a QQ-bead were unable to display their QQ activities. On the basis of this information, a more efficient QQ-medium, a QQ hollow cylinder (QQ-HC), was designed and prepared. In batch experiments, QQ-HCs showed greater QQ activity than QQ-beads as a result of their higher surface area and enhanced physical washing effect because of their larger impact area against the membrane surface. Furthermore, it was shown that such advantages of QQ-HCs resulted in more effective mitigation of membrane fouling than from QQ-beads in lab-scale continuous MBRs.
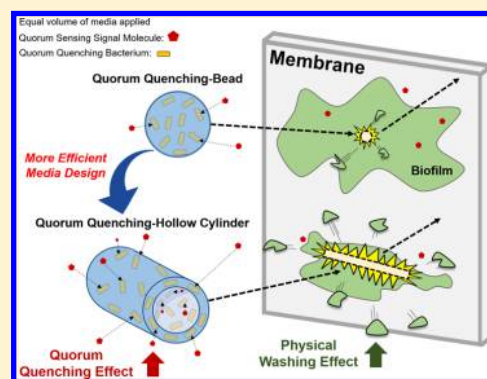
INTRODUCTION

Membrane bioreactors (MBRs) have been in practice for over 2 decades and have evolved into state-of-the-art technology in wastewater treatment and reuse. However, membrane biofouling still remains a major problem that elevates operating and maintenance costs, thereby hampering the spread of MBRs worldwide. Since the introduction of MBR technology, countless studies have been conducted to determine how to reduce biofouling in MBRs, but most of them were limited to physical and chemical strategies, which focus largely on removing already accumulated biomass on membranes.[1] Motivated by the discovery of the involvement of quorum sensing (QS) in bacterial biofilm development,[2] Yeon and his co-workers found that QS also plays a key role in biofilm formation on the membrane surface in a MBR and could be inhibited by enzymes that can decompose signal molecules used for QS [i.e., enzymatic quorum quenching (QQ)].[3,4] This opened a new paradigm in biofouling control in MBRs because, unlike conventional post-biofilm control methods, QQ could inhibit the biofilm formation at its developmental stage.

In a later study, Oh et al.[5] isolated a bacterium, *Rhodococcus* sp. BH4, which produces enzymes that decompose QS signal molecules (i.e., bacterial QQ), from a real MBR plant. They encapsulated *Rhodococcus* sp. BH4 inside the lumen of a microporous hollow fiber membrane to protect the bacteria from attack by other microorganisms co-habiting in the mixed liquor and put this "microbial vessel" into the submerged MBR. As a result, this "microbial vessel" effectively mitigated membrane biofouling and maintained its QQ activity for 80 days. Others have extended the concept of the QQ microbial vessel to the ceramic microbial vessel[6] and the rotating microbial carrier frame,[7] and Weerasekara et al.[8] further
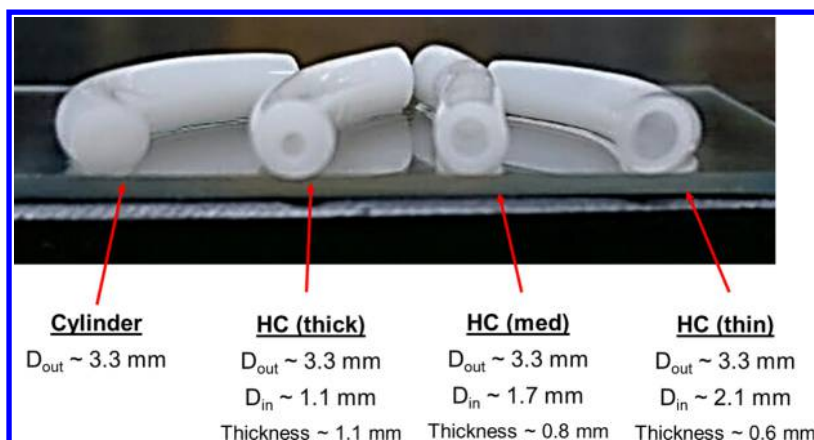
Figure 1. Prepared cylindrical media with different inner diameters and thicknesses but fixed outer diameter.

analyzed the energy savings of the QQ microbial-vessel-applied MBR system.

In 2012, Kim et al.[9] entrapped QQ bacteria in mobile hydrogel porous spherical beads to induce smoother mass transfer across porous beads than the "microbial vessel" provided. Such QQ bacteria entrapping beads (QQ-beads) are imbued with a physical washing effect through frequent contacts between the spherical beads and the membrane surface under the fluidized environment in a MBR. Subsequently, the authors explored opportunities to improve the physical strength of the beads by coating them with a polymeric membrane layer.[10] Rosenberger et al.[11] emphasized the importance of hydrodynamic conditions in the efficacy of mechanical cleaning of plastic and QQ-beads. Recently, Lee et al.[12] expanded the application of QQ-beads from lab-scale MBR fed with synthetic wastewater to a field pilot-plant MBR fed with real wastewater, demonstrating the potential of employing QQ technology in practice. However, all of the above-mentioned studies, which attempted to improve the physical washing and biological QQ effects of static or moving QQ-media, have neglected the interaction between QS signal molecules and QQ-media, i.e., the mass transfer and reactions of QS signal molecules across and within the QQ-media.

In this study, the route by which QS signal molecules come into contact with QQ bacteria entrapped in a hydrogel medium was monitored using a special reporter strain (*Escherichia coli* JB525), and on the basis of this result, a new geometry of QQ-media, i.e., a QQ hollow cylinder, was designed. A quorum quenching hollow cylinder (QQ-HC) was proposed as a more efficient QQ-medium than a QQ-bead in MBRs because it was expected to have higher QQ activity and a greater physical washing effect (i.e., detachment of accumulated biomass from the membrane surface through scouring upon contact) as a result of its shape. To confirm this, biological and physical aspects of a QQ-HC in biofouling control were assessed in batch scale in comparison to QQ spherical beads and among HCs having the same outer but different inner diameters. Lastly, the anti-biofouling ability of QQ-HCs was evaluated further in continuous MBRs.

■ MATERIALS AND METHODS

Preparation of QQ-Beads and QQ-HCs. For preparation of both QQ-beads and QQ-HCs, *Rhodococcus* sp. BH4 was used as the QQ bacterium because it has been reported to produce *N*-acyl homoserine lactone (AHL) lactonase, which is capable

of decomposing a wide variety of signal molecules of AHLs.[13] Consequently, its QQ ability in a MBR for wastewater treatment has been demonstrated in a number of previous studies.[5,7−10,12−15] Spherical media (QQ-beads) were prepared as described in previous work.[12] Hollow cylindrical media with (i.e., QQ-HC) and without (i.e., vacant-HC) QQ bacteria were prepared from a mixture of poly(vinyl alcohol) (Wako, Japan) and sodium alginate (Junsei, Japan). BH4 grown in Luria−Bertani (LB) broth was centrifuged (6500g for 15 min at 4 °C) and resuspended with deionized (DI) water. The concentration of BH4 was measured by analyzing its dry mass in the resuspended solution.[16] In detail, 0.10 g of BH4 resuspended solution was dropped onto a disk filter (GF/C, Whatman, Pittsburgh, PA), which was then dried in an oven at 105 °C for 1 h. For a QQ-HC, the aliquot amount of BH4 was mixed with the poly(vinyl alcohol)−sodium alginate mixture to yield a final concentration of 7 mg of BH4 (by dry weight)/g of QQ-HC (by dry weight), whereas, for a vacant-HC, an equivalent volume of DI water was added to the polymeric mixture, instead of BH4. Cross-linking of the polymer mixture was performed by extruding it through a nozzle into a $CaCl_2$ (Daejung, Republic of Korea) and boric acid (Samchun, Republic of Korea) solution. Using various sizes of spinneret outlets (2.0−2.5 mm for outer flow and 0.8−2.0 mm for inner flow), cylinders and HCs, with similar outer diameters but different inner diameters, were prepared, as shown in Figure 1. The flow rate of the polymer and cross-linking solutions were adjusted by syringe pumps (KDS Legato 100, KD Scientific, Holliston, MA) to yield linear velocities of fluids of 30−35 mm/s. The densities of all kinds of media were approximately 1.01 (±0.01) g/mL. Also, the unit mass of each medium was approximately 0.035 (±0.001), 0.163 (±0.007), 0.153 (±0.002), 0.123 (±0.003), and 0.109 (±0.001) g for bead, cylinder, HC (thick), HC (med), and HC (thin), respectively.

Visualization of QS Signal Molecules in Beads. To visualize the signal molecule within the media, *E. coli* JB525, which produces green fluorescence protein (GFP) upon intake of a range of AHLs,[17] was used. Two types of beads were prepared, one with only JB525 entrapped and another with both BH4 and JB525 entrapped, following the procedure described in the previous section. The beads were then exposed to 1 μM *N*-octanoyl-DL-homoserine lactone (C8-HSL) in 20 mM phosphate buffer (PB) at pH 7 with $^1/_{10}$ diluted LB broth for 2 h, and their cross-sections were observed with a fluorescence microscope (Eclipse E600, Nikon, Japan). Similarly, the JB525 only and JB525 and BH4 together
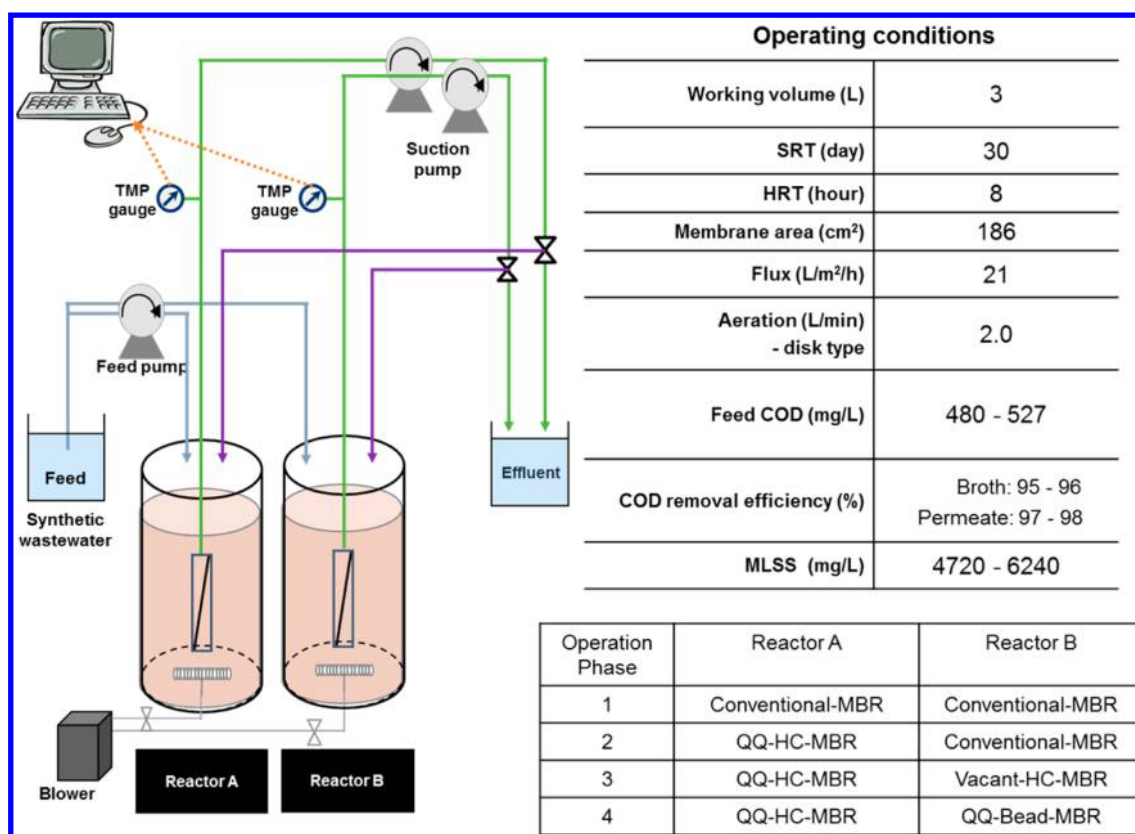
Figure 2. Schematic, operating conditions, and operation phases of the two MBRs.

entrapping HCs were reacted with 1 μM C8-HSL, and then their cross-sections were observed with a confocal laser scanning microscope (CLSM, SP8 X, Leica, Germany). The control experiment with whole cells of BH4 and JB525 was conducted as follows. An overnight culture of JB525 or BH4 was centrifuged (6500g for 15 min at 4 °C) and resuspended to $1/10$ its original volume with $1/5$ diluted LB broth. To yield a final concentration of C8-HSL of 0 or 1 μM, 0.5 mL of BH4 or JB525 resuspended solution or the mixture of 0.25 mL of BH4 and 0.25 mL of JB525 resuspension solutions was mixed with 0.5 mL of 0 or 2 μM C8-HSL dissolved in 20 mM PB at pH 7. The mixture was left to react on an orbital shaker at 70 rpm and 25 °C for 2 h and observed with a fluorescence microscope.

Assessment of QQ Activity. QQ activity of the media was analyzed in terms of the degradation rate of commercially available C8-HSL (Sigma-Aldrich, St. Louis, MO), a QS signal molecule. The concentration of this molecule was measured using the reporter strain *Agrobacterium tumefaciens* A136 as described in previous studies.[9,12] C8-HSL was used as the model QS signal molecule in this experiment because it was previously reported to be one of the major QS signal molecules.[3] In detail, QQ-media, prepared by fixing either their volume at 1.0 mL or their number at 10 pieces, were added to 20 mL of 1 μM C8-HSL dissolved in 20 mM PB (pH 7) and left to react on an orbital shaker at 70 rpm and 25 °C. After 60 min, 0.5 mL of the solution was sampled. The sample was filtered using a 0.45 μm syringe filter and then immediately immersed in a 100 °C water bath for 15 min. The C8-HSL concentration was measured through an A136 bioassay as follows. The reporter strain *A. tumefaciens* A136 and samples were mixed and loaded into a 96-well plate, which then was incubated at 30 °C for 90 min. The Beta-Glo assay system

(Promega, Madison, WI) was added to the wells, and the plate was stored at 25 °C for 30 min. The luminescence intensity was measured by a luminometer (Synergy 2, BioTek, Winooski, VT), and the AHL concentration was determined by plotting the calibration curve of standard C8-HSL. The QQ activity was presented as the number of nanomoles of C8-HSL degraded per minute, which was measured over 60 min.

Assessment of the Physical Washing Effect. The physical washing effect of a prepared medium was assessed in a batch reactor (working volume of 3 L) with concentrated synthetic wastewater and 5 mL of activated sludge inoculum. Instead of using an actual membrane module, a polycarbonate (PC) plate module of identical physical dimensions was used. Four PC coupons were placed on the PC plate. The batch reactors were operated with or without vacant media at an aeration rate of 1.5 L/min. After 24 h, the biofouled PC plate was taken out of the batch reactor and stained with 0.1 wt %/vol crystal violet and dried for 24 h. Crystal violet on the coupons was dissolved in ethanol by separately immersing the coupons in 10 mL of ethanol solution for 30 min. The crystal violet concentration in the ethanol solutions was measured using a spectrophotometer set at 570 nm. The optical density values of the four coupons from one PC plate were averaged, and their standard deviation was calculated and plotted as an error bar.

MBR Operation. Two MBRs, reactors A and B, were operated continuously in parallel, as shown in Figure 2. Activated sludge was taken from a wastewater treatment plant (Tancheon, Korea) and acclimatized until the MBR operating parameters stabilized. The concentration of media applied to each reactor was 1% (v/v, volume of media/volume of reactor), unless stated otherwise. Operation of the two MBRs was
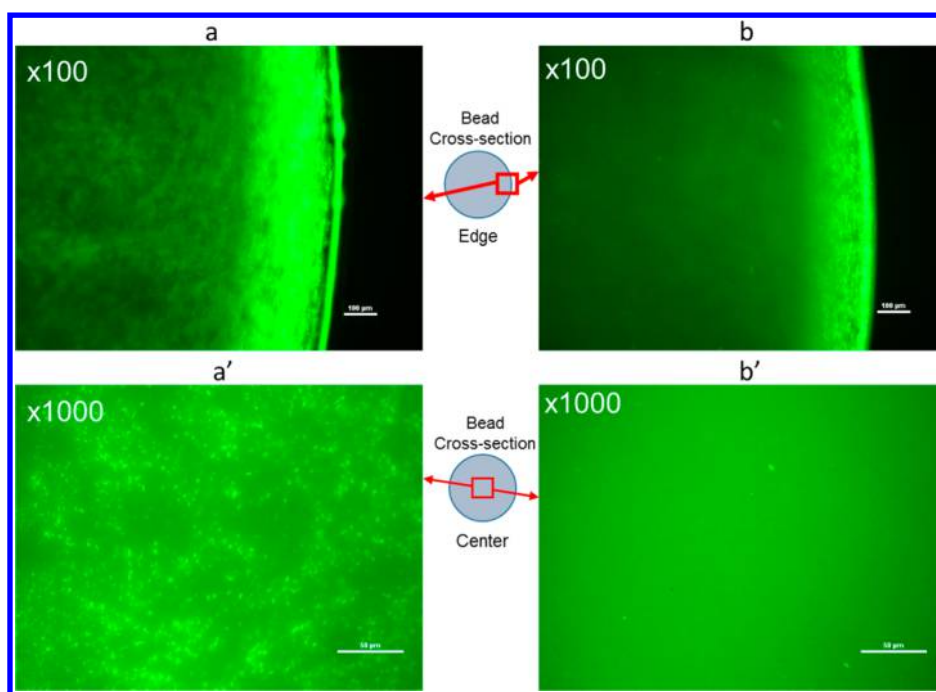
Figure 3. Fluorescence images of bead cross-sections entrapped with JB525 only (a, near surface; a′, center) and with both JB525 and BH4 (b, near surface; b′, center).

performed in four different phases, as listed in the lower table in Figure 2. The composition of the synthetic wastewater in this experiment was as follows (all in mg/L):[5] 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. A hollow fiber membrane (ZeeWeed 500, GE-Zenon, Trevose, PA) with an effective filtration area of 186 cm^2 was used for filtration. The MBRs were operated at constant flux of 20 L m^{-2} h^{-1} (LMH) with an aeration rate of 2.0 L/min. Membranes that became fouled during operation were taken out of the reactor and cleaned by soaking them in 1000 ppm of NaOCl for 3 h. Prior to phase 1 (Figure 2), the mixed liquors of the two reactors (A and B) were mixed and then divided equally back into reactors A and B. The transmembrane pressure (TMP) in each reactor was monitored to estimate and compare the extent of biofouling in the MBRs. For comparison of anti-biofouling performance of QQ-HCs in a MBR at each operation phase, the average number of days for one TMP jump to occur (T_{TMP}) was calculated by dividing the operation period in days by the number of TMP jumps during the operation period.

Analytical Methods. Mixed liquor suspended solids (MLSS) and chemical oxygen demand (COD) were measured according to standard methods.[18]

Statistical Analysis. The QQ activity and physical washing effect of different media were compared by a one-way analysis of variance (ANOVA) test or a one-tailed t test assuming unequal variance.

■ RESULTS AND DISCUSSION

Interaction between QQ-Beads and QS Signal Molecules. To design a more efficient QQ-medium, the interaction mechanism between QS signal molecules and QQ-media when they came into contact with each other needed to be studied. In other words, it was necessary to look deeply at how QS signal molecules cross the medium, diffuse toward the

core of the medium, and react with QQ bacteria entrapped in such a hydrogel medium. For this analysis, two types of bacteria entrapping beads were prepared: (1) beads entrapped with only the AHL reporter strain *E. coli* JB525 and (2) beads entrapped with both JB525 and the AHL decomposing QQ bacteria BH4. Because the bacteria and polymer solution were mixed thoroughly before being shaped into beads, BH4s were likely to be well-distributed throughout the entire area of a bead. This was confirmed by observing a cross-section of the media using a CLSM (data not shown). When JB525 reacts with AHL, it produces GFP, which can be visualized using a fluorescence microscope. On the other hand, GFP expression was not observed when BH4 was exposed to C8-HSL (see Figure S1 of the Supporting Information). Thus, by observation of the cross-section of a JB525 entrapping bead exposed to AHLs, diffusion of QS signal molecules through pure bead material without the QQ bacteria, BH4, can be monitored. In other words, whether AHLs can diffuse freely within the bead can be verified visually. Furthermore, by observation of the cross-section of JB525 and BH4 entrapping beads exposed to AHLs, diffusion of the AHLs to be decomposed by BH4 can be monitored.

The two types of beads were exposed to 1 μM C8-HSL in 20 mM PB (pH 7) with $^1/_{10}$ diluted LB for 2 h, and then cross-sections of the beads were observed with a fluorescence microscope. Figure 3 shows the cross-sectional images of the near surface (Figure 3a) and center (Figure 3a′) regions of JB525 only entrapping beads. In panels a and a′ of Figure 3, the bright green spots represent expression of GFP by JB525, indicating the presence of QS signal molecules (C8-HSL). The near surface (Figure 3a) and center (Figure 3a′) regions are expressing GFP, indicating free diffusion of signal molecules within the bead. However, in beads entrapped with both JB525 and BH4, a thin bright band of fluorescence is observed along the surface only (Figure. 3b), which rapidly fades toward the center, leaving only a few bacteria expressing GFP (Figure. 3b′). For quantitative comparison, we quantified the area of
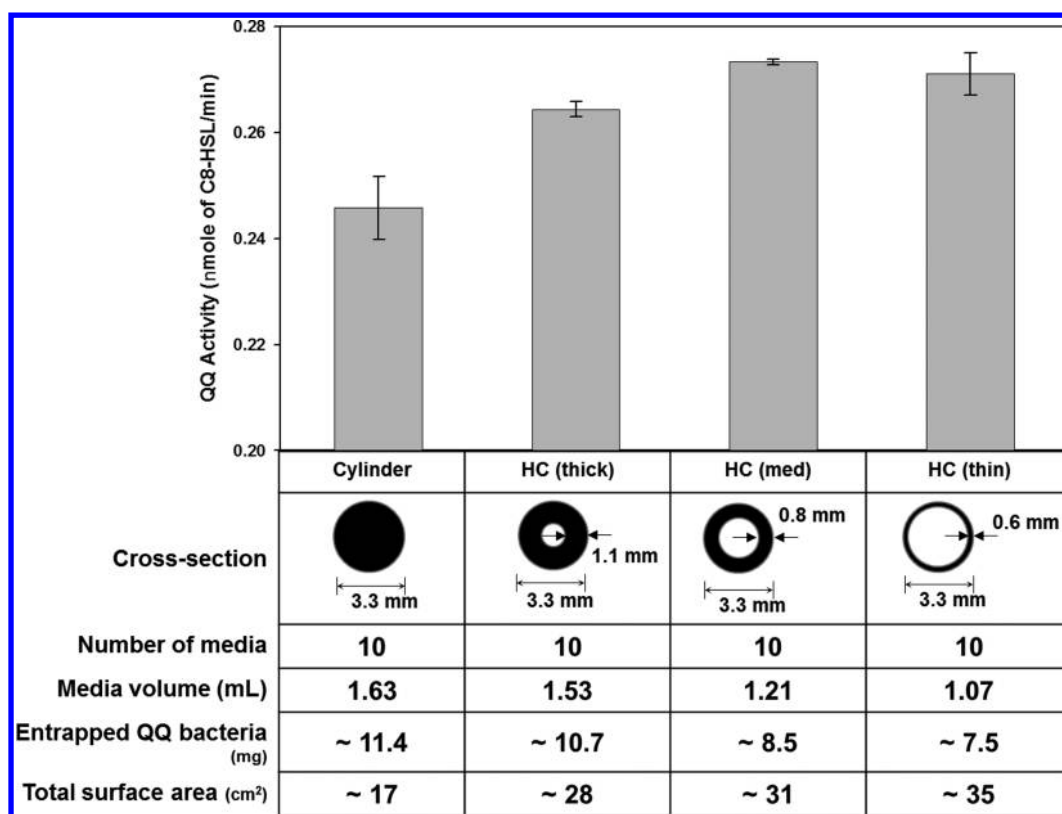
Figure 4. Comparison of QQ activity between four different QQ-cylinders that have the same outer diameter but different inner diameters. Error bar = standard deviation ($n = 3$).

GFP expression from images in Figure 3 using ImageJ software. All color images in Figure 3 were first converted to an 8-bit image (black and white), and then threshold values were set to 50 and 255 for all images. Percent area occupied by GFP, expressed by JB525 in each image, was calculated using the Analyze Particles tool. The results were 16 and 3.8% for panels a and b of Figure 3, respectively, and 2.2 and 0.18% for panels a′ and b′ of Figure 3, respectively. This indicates that GFP was expressed much more in the JB525 only entrapping bead (panels a and a′ of Figure 3) than in the JB525 and BH4 together entrapping bead (panels b and b′ of Figure 3). Consequently, we could conclude that the C8-HSL concentration was higher in the JB525 only entrapping bead than in the JB525 and BH4 together entrapping bead.

From the analysis of the results from observing the two types of beads, the presence of BH4 in a bead seems to limit the diffusion of QS signal molecules into the bead, not by exerting resistance to diffusion but by rapidly decomposing the signal molecules at the outer surface of the bead. In other words, even though AHLs can diffuse freely to the center of a bead, BH4 located near the surface decomposes most of the QS signal molecules, leaving only a few AHLs available for degradation at the center and, thereby, depriving the QQ bacteria located deep in the medium of opportunities to encounter and degrade QS signal molecules. Therefore, QQ bacteria located in the inner part of a medium may appear to have no QQ activity. This result also implies that the surface area of a QQ-medium is a more dominant parameter in QQ activity over the total mass of entrapped QQ bacteria. On the basis of this, a QQ-HC was designed and prepared as a new QQ-medium. Preparation of two types of HCs, one entrapping JB525 only and the other entrapping JB525 and BH4 together, and exposure of them to 1

μM C8-HSL HC yielded the same result: most AHLs were degraded near the surface (see Figure S2 in the Supporting Information). As such, a high surface area per unit medium volume of QQ-HC was expected not only to enhance QQ activity but also to enable the efficient use of QQ bacteria in the medium. Furthermore, a QQ-HC could be separated more easily from activated sludge microbial flocs than a QQ-bead as a result of its relatively longer length. A QQ-HC was also expected to have a greater physical washing effect because the contact site between a QQ-HC and the membrane surface would be a line, whereas that between a QQ-bead and the membrane surface is a point.

QQ Activity of the Inner Part of QQ-Media. To confirm how those expectations based on the fluorescence image analysis may turn out, four different QQ cylindrical media with various inner diameters but fixed outer diameters were prepared as follows: (1) cylinder, outer diameter (D_{out}) of ~3.3 mm and inner diameter (D_{in}) of 0 mm; (2) HC (thick), D_{out} of ~3.3 mm and D_{in} of ~1.1 mm; (3) HC (med), D_{out} of ~3.3 mm and D_{in} of ~1.7 mm; and (4) HC (thin): D_{out} of ~3.3 mm and D_{in} of ~2.1 mm. The QQ activities of each medium were compared to one another (Figure 4). Because the media were prepared with the homogeneous mixture of BH4 and polymer solution, BH4 was considered to be distributed well throughout the medium. As the inner diameter increases, the proportion of inner material (i.e., BH4 plus polymer) decreases, because the volume of material used for media preparation decreases as the surface area increases. Thus, as the inner diameter increases, the amount of entrapped BH4 decreases. Nevertheless, the QQ activity shows an increasing trend as the inner diameter of the cylinder increases, as shown in Figure 4. More specifically, as the inner diameter increases from that of a QQ-HC (thick) to

that of a QQ-HC (med), the QQ activity was enhanced ($p <$ 0.05, one-tailed t test with unequal variance), whereas the QQ activity of a QQ-HC (thin) was not greater than that of a QQ-HC (med) ($p > 0.05$, one-tailed t test with unequal variance). It can be hypothesized that an increase in the total surface area as a result of an increase in the inner diameter up to that of a QQ-HC (med) enhanced QQ activity of the medium to a greater degree than the loss of QQ activity, resulting from the reduction in the total amount of entrapped QQ bacteria. In other words, in the range of an inner diameter increase from that of a QQ-cylinder to that of a QQ-HC (med), the surface area of the QQ-medium might be a more dominant parameter for QQ activity over the total mass of entrapped QQ bacteria, which coincides with the result from Figure 3, predicting a negligible contribution toward QQ activity from the QQ bacteria located deep within the medium. However, the QQ activity of a QQ-HC (thin) had a similar value to that of a QQ-HC (med). It seems that, at this point, the contribution toward QQ activity from the amount of entrapped QQ bacteria became significant, exceeding that from the surface area. In other words, there is a balancing point between the amount of entrapped QQ bacteria and surface area that maximizes the QQ effect of a QQ-medium.

Comparison of QQ Activity between QQ-HCs and QQ-Beads. The QQ activity of a QQ-HC was compared to that of a QQ-bead because the latter has been often reported in studies on QQ MBRs.[9−11,15] The volume of the media was fixed at 1 mL, which corresponds to approximately 7 mg of the entrapped BH4. Then, the degradation rate (mol/min) of QS signal molecules (C8-HSLs) by the media was measured over a 60 min period. The result indicates higher QQ activity for HCs than for beads (Figure 5) ($p < 0.05$, one-tailed t test with
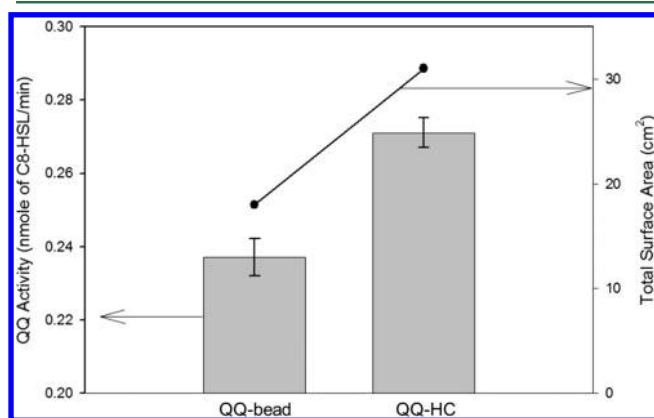

Figure 5. Comparison of QQ activity (columns) and surface area (line) between QQ-beads (diameter of ∼3.3 mm) and QQ-HCs (inner diameter of ∼2.1 mm, outer diameter of ∼3.3 mm, and length of ∼20 mm). Error bar = standard deviation ($n = 3$).

unequal variance). Because the media volume was the same for both media, the HC resulted in a higher total surface area as a result of its greater "surface area to volume ratio" at the given dimension. Because a higher surface area can facilitate a higher mass transfer of signal molecules through the media, the higher surface area of HC is thought to enhance its QQ activity over that of a bead.

Comparison of the Physical Washing Effect between Vacant-HC and Vacant-Bead. Because QQ-media circulate all of the time together with activated sludge under the aeration environment in a MBR for wastewater treatment, they may

reduce biofouling through not only biological QQ but also collisions with and removal of attached biocakes on a membrane surface (i.e., physical washing).[9−11,15] Figure 6
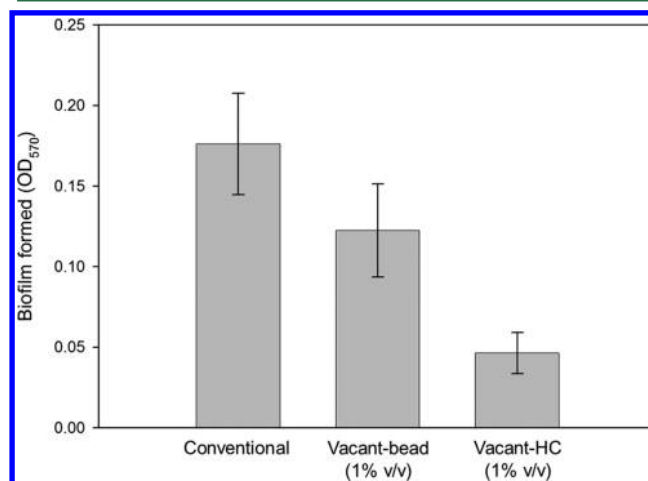

Figure 6. Comparison of the physical washing effect between vacant-beads (diameter of ∼3.3 mm) and vacant-HCs (inner diameter of ∼2.1 mm, outer diameter of ∼3.3 mm, and length of ∼20 mm) at the loading volume of 1% of the bioreactor. Error bar = standard deviation ($n = 4$).

shows the comparison of the physical washing effect between vacant-beads (diameter of ∼3.3 mm) and vacant-HCs (inner diameter of ∼2.1 mm, outer diameter of ∼3.3 mm, and length of ∼20 mm) at the same loading volume of 1% of the bioreactor volume. Three batch reactors were operated in parallel with 10-fold concentrated synthetic wastewater and 5 mL of activated sludge inoculum. The same medium loading volume of 1% (v/v) resulted in 780 pieces of vacant-beads and 280 pieces of vacant-HCs. The reactors were run with aeration for 24 h, after which the PC module was taken out of each reactor and stained with crystal violet.

The y axis represents the amount of biofilm formed, derived from measuring the concentration of crystal violet, which is proportional to the amount of biofilm attached to a PC plate. The statistical analysis showed that the physical washing effects of the three cases were different at medium loading of 1% (v/v) ($p < 0.05$, one-way ANOVA test). In detail, the conventional reactor, having no physical washing as a result of a lack of mobile media, had the highest amount of biofilm on the PC plate, whereas the reactor with vacant-beads had the second highest amount of biofilm and the reactor with vacant-HCs had the lowest amount of biofilm, despite the fact that the number of HCs was approximately more than 3 times fewer than the number of beads. This result is indicative of the greater cleaning efficiency of HCs, possibly as a result of the larger contact area of HCs with the membrane surface because of their cylindrical geometry. In addition, the mass per unit medium of a HC (0.109 g/HC) was greater than that of a bead (0.035 g/bead). Hence, HCs may have had higher impact momentum than beads, which facilitated the detachment of biofilms that were more strongly adhered to the PC coupon. Recollecting that the inner part of QQ-media had trivial contribution toward QQ activity, we became interested in testing whether the inner part of a medium would influence its physical washing efficiency.

Assessment of the Physical Washing Effect of the Inner Part of QQ-Media. To examine closely whether the inner part of a medium influences its physical washing effect,

vacant-cylinders (D_{in} = 0), vacant-HCs (thick) (D_{in} = 2.1 mm), and vacant-HCs (thin) (D_{in} = 1.1 mm) were prepared and their physical washing effects were assessed (Figure 7). For this
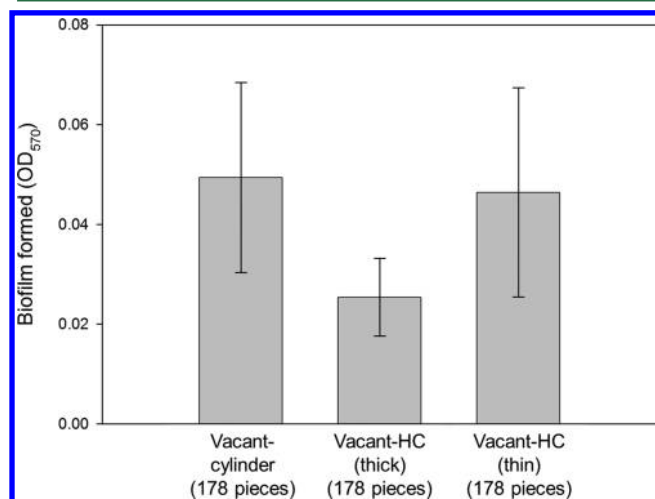

Figure 7. Physical washing effect comparison between vacant-cylinders with the same outer diameter (~3.3 mm) but different inner diameter [vacant-cylinder, 0 mm; vacant-HC (thick), 1.1 mm; and vacant-HC (thin), 2.1 mm]. Medium loading was 178 pieces each. Error bar = standard deviation (n = 4).

comparison, instead of loading every medium at 1% (v/v), the same number of pieces (178), which translates to a net volume of 30 mL [1% (v/v)] of vacant-cylinder, 26 mL [0.9% (v/v)] of vacant-HC (thick), and ~17 mL [0.6% (v/v)] of vacant-HC (thin), for each medium loaded. The result shows there was no significant difference in the physical washing effect among the cylinders, regardless of their inner diameter size ($p > 0.05$, one-way ANOVA test). It is interesting to note that the inner material of a mobile medium does not seem to contribute significantly toward physical washing of a membrane. The hydrogel used to prepare QQ-media and the MLSS have wet densities that are very close to that of water (approximately 1.0 g/mL), and the cylindrical media have almost the same outer

dimension (D_{out} of ~3.3 mm and length of ~20 mm). Consequently, the filling of the lumen of HC with MLSS may make the effective mass of the HC similar to the mass of a cylinder, thereby rendering the impact momenta of a HC and a cylinder indifferent. In addition, their similar outer diameter (~3.3 mm) and length (~20 mm) may result in similar membrane contact areas. For such reasons, the physical washing effects of HCs and a cylinder may not have seemed different. However, further analysis would be required to confirm the exact mechanism and key parameters in physical scouring of a membrane surface by mobile media in a submerged MBR.

Comparison of Biofouling Mitigation among Various Media in Continuous MBRs. TMP rise-up was monitored in MBRs with different media (Figure 2), and the profiles of such are shown in Figure 8. In addition, for quantitative comparison, T_{TMP} was calculated and its value for each MBR was compared (Table 1). Fouled membrane modules were cleaned and reused in the next cycle. The possible change in intrinsic membrane resistance resulting from the different number of chemical washing in reactors A and B was assumed to be negligible.

In phase 1, reactors A and B were operated in conventional mode, meaning no medium was inserted. Almost identical TMP profiles yielded a ratio of T_{TMP} values between the two reactors of 1.0, which indicates that the two reactors have similar microbial broths and, thus, similar fouling tendencies. In phase 2, QQ-HCs were inserted into reactor A (QQ-HC-MBR), which had been operated as a conventional-MBR. Although there were four TMP jumps in the conventional-MBR (T_{TMP} = 4.8 days), only one TMP jump occurred in the QQ-HC-MBR (T_{TMP} = 21.6 days) over 22 days of operation. This translates into a ratio of T_{TMP} values of 4.5, meaning that the occurrence of TMP jump was approximately 4.5 times more frequent in the conventional-MBR than the QQ-HC-MBR. In phase 3, vacant-HCs were added to reactor B (vacant-HC MBR), which had been operated as the conventional-MBR. The T_{TMP} values for the QQ-HC-MBR and vacant-HC-MBR were 25.9 and 12.8 days, respectively, and the ratio of their T_{TMP} values was 2.0. Because the number of hollow cylinders
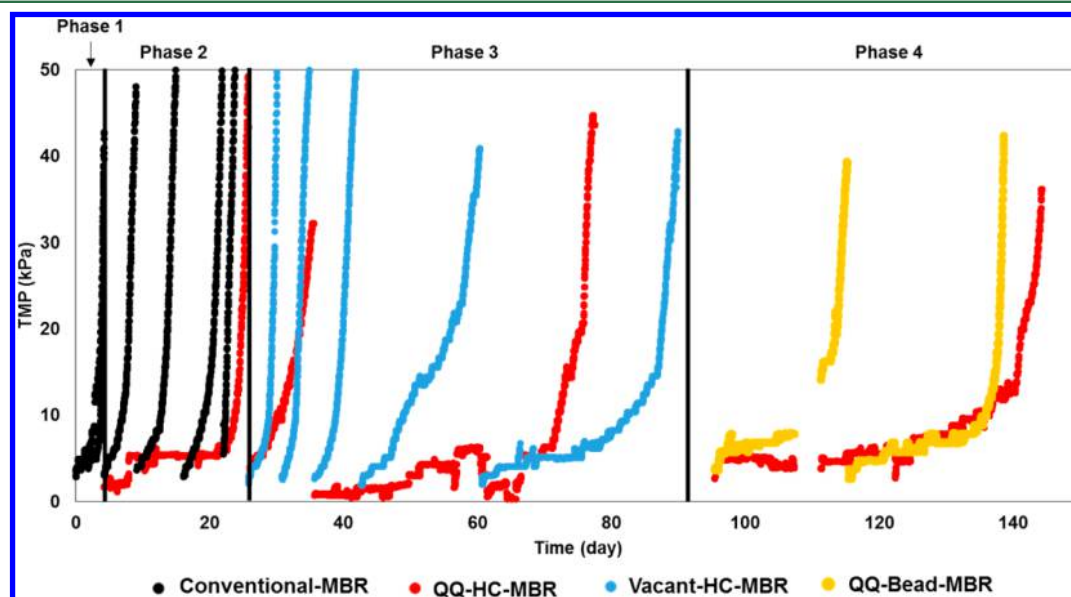

Figure 8. TMP profiles of MBRs with different media.

Table 1. Average Number of Days for One TMP Jump, T_{TMP}, To Occur in Reactors A and B in Each of the Four Phases

phase	reactor A	reactor B	ratio (A/B)
1	4.2 days (conventional-MBR)	4.1 days (conventional-MBR)	1.0
2	21.6 days (QQ-HC-MBR)	4.8 days (conventional-MBR)	4.5
3	25.9 days (QQ-HC-MBR)	12.8 days (vacant-HC-MBR)	2.0
4	48.8 days (QQ-HC-MBR)	21.6 days (QQ-bead-MBR)	2.3

inserted in both reactors was the same and, hence, it could be assumed that the physical washing effect in the two reactors was the same, the difference in the T_{TMP} values (i.e., greater membrane biofouling retardation in QQ-HC-MBR) is likely attributable to the greater QQ activity of QQ-HCs than of vacant-HCs. In phase 4, vacant-HCs were removed and QQ-beads were inserted, in reactor B. Additionally, the QQ-HCs in reactor A were replaced by freshly prepared QQ-HCs, so that the comparison between the two reactors was performed with equally aged QQ bacteria. The ratio of T_{TMP} values of the QQ-HC-MBR to the QQ-bead-MBR was calculated as 2.3, which indicates that the QQ-HC was more than twice as effective as the QQ-bead in mitigating biofouling in a MBR under the operating conditions in this study. As demonstrated in batch studies, the enhancement of mitigation of membrane fouling in a QQ-HC-MBR may be attributed to the higher QQ activity and greater physical washing effect of QQ-HCs than those of QQ-beads.

In summary, the MBR with QQ-HCs was approximately 4.5 times less prone to membrane fouling compared to the conventional-MBR that lacked media. When only the QQ activity of the QQ-HCs was taken into account (phase 3), the biological QQ effect alone reduced membrane fouling by half. In comparison to QQ-beads, QQ-HCs were about 2.3 times more effective in mitigating biofouling. This is possibly caused by greater QQ activity of a QQ-HC, resulting from its higher surface area and enhanced physical washing effect because of its larger impact area against a membrane surface. Through various tests in batch and continuous lab-scale MBRs with different mobile media, it has been concluded that the hollow cylindrical geometry of a QQ-HC is a more efficient QQ-media design that delivers more enhanced biofouling control in MBRs than a QQ-bead.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01221.

Test of fluorescence expression by BH4 and JB525 (Figure S1) and CLSM images of the cross-section of (a) JB525 entrapping HC and (b) JB525 and BH4 entrapping HC obtained from 2 h after exposure to 1 μM C8-HSL (Figure S2) (PDF)

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Notes
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