

# Effect of the Shape and Size of Quorum-Quenching Media on Biofouling Control in Membrane Bioreactors for Wastewater Treatment<sup>S</sup>

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Received: May 10, 2016

Revised: June 14, 2016

Accepted: July 1, 2016

First published online  
July 6, 2016

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**S**upplementary data for this paper are available on-line only at <http://jmb.or.kr>.

pISSN 1017-7825, eISSN 1738-8872

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Recently, spherical beads entrapping quorum quenching (QQ) bacteria have been reported as effective moving QQ-media for biofouling control in MBRs for wastewater treatment owing to their combined effects of biological (*i.e.*, quorum quenching) and physical washing. Taking into account both the mass transfer of signal molecules through the QQ-medium and collision efficiencies of the QQ-medium against the filtration membranes in a bioreactor, a cylindrical medium (QQ-cylinder) was developed as a new shape of moving QQ-medium. The QQ-cylinders were compared with previous QQ-beads in terms of the QQ activity and the physical washing effect under identical loading volumes of each medium in batch tests. It was found that the QQ activity of a QQ-medium was highly dependent on its specific surface area, regardless of the shape of the medium. In contrast, the physical washing effect of a QQ-medium was greatly affected by its geometric structure. The enhanced anti-biofouling property of the QQ-cylinders relative to QQ-beads was confirmed in a continuous laboratory-scale MBR with a flat-sheet membrane module.

**Keywords:** Membrane bioreactor, quorum sensing, quorum quenching, bead, cylinder

## Introduction

Membrane bioreactors (MBRs), in which a biological activated sludge process is combined with membrane filtration technology [8, 16], have been widely used for wastewater treatment and reuse owing to their compactness and high-quality effluent. However, membrane biofouling caused mainly by microbial attachment and growth on the surface of the membrane results in high operating cost, which is one of the critical hurdles in the dissemination of MBRs [3, 6]. Despite various approaches from physical, chemical, and biological viewpoints for overcoming biofouling in MBRs for wastewater treatment [3, 5, 24, 27],

the underlying problem still remains.

Recently, Yeon *et al.* [25, 26] reported that quorum sensing (QS), the bacterial cell-to-cell communication, plays a key role in biofilm formation on the membrane surface in MBRs and that biofouling could be inhibited by enzymatic quorum quenching (QQ), which decomposes signal molecules such as *N*-acyl homoserine lactones (AHLs). To overcome the limitations of enzymatic QQ (*e.g.*, high cost and low stability of enzymes), Oh *et al.* [17] isolated a QQ bacterium, *Rhodococcus* sp. BH4, from a real MBR plant. BH4 produces QQ enzymes, which are capable of decomposing a wide range of AHLs, leading to the mitigation of membrane biofouling in MBRs. After that, many researchers isolated

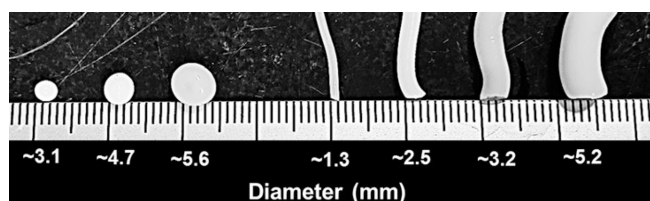
some other QQ bacteria and developed different media for entrapping QQ microorganisms more effectively. Some researchers encapsulated QQ bacteria inside the lumen of microporous membranes, as fixed QQ-media, having only a biological QQ effect [1, 2, 7, 9, 17, 18, 21]. Others entrapped QQ bacteria into spherical beads, as moving QQ-media [11–15].

In particular, spherical QQ-beads have been favored because they have not only a biological (QQ) effect but also a physical washing effect through collisions between the moving beads and the surface of the filtration membrane. Moreover, the spherical QQ-beads were demonstrated to mitigate biofouling under harsh environments in a pilot MBR fed with real wastewater [14]. However, there has been no report on the effect of geometrical changes in the moving QQ-media on their physical washing efficiency/or biological QQ performance. In this study, a cylinder-type medium (QQ-cylinder), as a new shape of moving medium, was prepared to improve the performance of the QQ-MBR. QQ-cylinders with various sizes were prepared and compared with spherical QQ-beads at a batch scale in terms of QQ activity and the physical washing effect under identical loading volumes of each medium. The anti-biofouling capability of the QQ-cylinders was also evaluated in a continuous laboratory-scale MBR with a flat-sheet membrane module. Based on those results, the effects of shape and size on the performance of the QQ-medium were investigated to determine the dominant parameters affecting its performance in QQ-MBRs.

## Materials and Methods

### Preparation of QQ-Beads and QQ-Cylinders

*Rhodococcus* sp. BH4 was used as a QQ bacterium for both QQ-beads and QQ-cylinders because it is capable of degrading a wide variety of signal molecules of AHLs by producing AHL lactonase [18]. Its biofouling control by QQ in MBRs for wastewater treatment has been demonstrated in a number of previous studies [7, 12–15, 17, 18, 21]. Spherical QQ-beads were prepared as described in previous work [14]. QQ-cylinders and cylinders without QQ bacteria (*i.e.*, vacant-cylinders) were prepared from



**Fig. 1.** Image of beads and cylinders of various sizes.

the polymeric mixture of polyvinyl alcohol and sodium alginate. BH4 was mixed with the polymeric mixture for the QQ-cylinders. For the vacant-cylinders, an equivalent volume of DW was added to the polymeric mixture. Cross-linking of the polymer mixture was achieved by extruding it through a nozzle into a  $\text{CaCl}_2$ -boric acid solution. Using various sizes of nozzles (inner diameter of 0.8–3.5 mm), beads and cylinders with various diameters were prepared as shown in Fig. 1. The average densities of the QQ-beads and the QQ-cylinders were approximately 1.06 and 1.05 g/ml, respectively.

### Assessment of Quorum-Quenching Activity

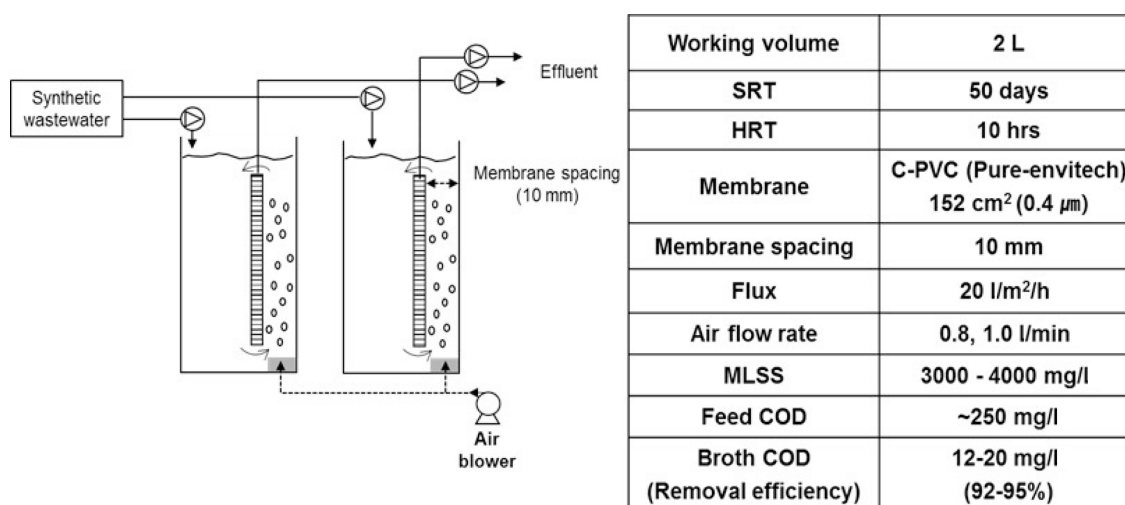
The quorum-quenching activities of various QQ-media were evaluated by measuring the degradation rate of standard C8-HSL (Sigma Aldrich, USA), one of the dominant AHL molecules in MBR [15, 17], by an *A. tumefaciens* A136 bioluminescence assay as described in previous studies [4, 18]. Standard C8-HSL was dissolved in Tris-HCl buffer (50 mM, pH 7) to make a final concentration of 1  $\mu\text{M}$ . The prepared medium with a total volume of 1 ml was inserted in 30 ml of C8-HSL solution in a conical tube, and then the mixture was placed on an orbital shaker at 50 rpm and 25°C. Periodically, 1 ml of solution was taken out of the tube to measure the residual C8-HSL concentration through the A136 bioluminescence assay. The QQ activity was presented as the amount of degraded C8-HSL (nmol) in 60 min in the presence of QQ-media.

### Visualization of the Trace of AHL Molecules in QQ-Cylinders

To visualize signal molecules within the QQ-cylinders, *E. coli* JB525, which produces a green fluorescence protein upon intake of a range of AHLs [23], was used. The JB525 strain was cultured in Luria-Bertani (LB) broth supplemented with tetracycline. QQ-cylinders entrapped with both BH4 and JB525 were prepared. Then, the QQ-cylinders were exposed to 1  $\mu\text{M}$  C8-HSL in Tris-HCl buffer (pH 7) with 1/20 diluted LB broth for 2 h, and their cross-section was visualized through a confocal laser scanning microscope (CLSM, SP8 X; Leica, Germany).

### Assessment of the Physical Washing Effect

The physical washing effect of the media was assessed in a batch reactor with a working volume of 1.1 L. A polycarbonate (PC) plate was vertically inserted into the reactor, in which the interval between the PC plate and the nearest wall of the reactor was 10 mm. Then, each batch reactor was filled with concentrated synthetic wastewater, and 5 ml of activated sludge was inoculated. The batch reactors were then operated with or without vacant-media (0.5% (v/v)) at an aeration rate of 0.5 l/min. After 24 h, the cell density in the broth of each reactor was measured by a spectrophotometer at 600 nm. Simultaneously, the biofouled PC plate was taken out of each reactor, and the upstream side of the PC plate was stained with crystal violet. Crystal violet on the PC plate was rinsed with 100 ml ethanol solution, and the concentration of crystal violet was measured by a spectrophotometer at 570 nm.



**Fig. 2.** Scheme and operating conditions of the continuous laboratory-scale MBRs.

SRT, solid retention time; HRT, hydraulic retention time; MLSS, mixed liquor suspended solids; COD, chemical oxygen demand.

The physical washing effect was defined as the ratio of the amount of normalized detached biofilm ( $OD_{570}/OD_{600}$ ) in a reactor with vacant-media to that in the control reactor without media.

#### Trajectory of Circulating Media in a Reactor

Vacant-media with different shapes were stained with Congo red (Sigma-Aldrich) to clearly observe the motion and to monitor the trajectory of each medium when passing through the space between the PC plate and the reactor wall. They were stained to more clearly distinguish the media from air bubbles. The snapshot images, consecutively taken every 0.1 sec, were combined into a time-lapse composite image by a free image processing program (Startrails).

#### MBR Configurations and Operation Conditions

Two continuous MBRs with a working volume of 2 L were operated in parallel. A flat-sheet membrane (C-PVC; Pure-Envitech, Korea) with a pore size of 0.4 μm and an effective surface area of 152 cm<sup>2</sup> was used for filtration. The distance between the membrane and the reactor wall was 10 mm (Fig. 2). Activated sludge was taken from a wastewater treatment plant (Tancheon, Korea) and was acclimated to the synthetic wastewater before starting the experiments. The composition of the synthetic wastewater in this study was as follows (mg/l) [17]: glucose, 200; yeast extract, 7; bactopeptone, 57.5; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 52.4; KH<sub>2</sub>PO<sub>4</sub>, 10.9; MgSO<sub>4</sub>·7H<sub>2</sub>O, 16; FeCl<sub>3</sub>·3H<sub>2</sub>O, 0.06; CaCl<sub>2</sub>·2H<sub>2</sub>O, 1.6; MnSO<sub>4</sub>·5H<sub>2</sub>O, 1.4; and NaHCO<sub>3</sub>, 127.8. The filtration flux was fixed at 20 l/m<sup>2</sup>/h without relaxation. The aeration rate for both air supply and membrane scouring was 0.8 or 1.0 l/min. The concentration of medium applied to each reactor was 0.5% (v/v) (volume of media/volume of reactor). The transmembrane pressure (TMP) was continuously monitored to evaluate the extent of biofouling during the operation of each MBR.

#### Analytical Methods

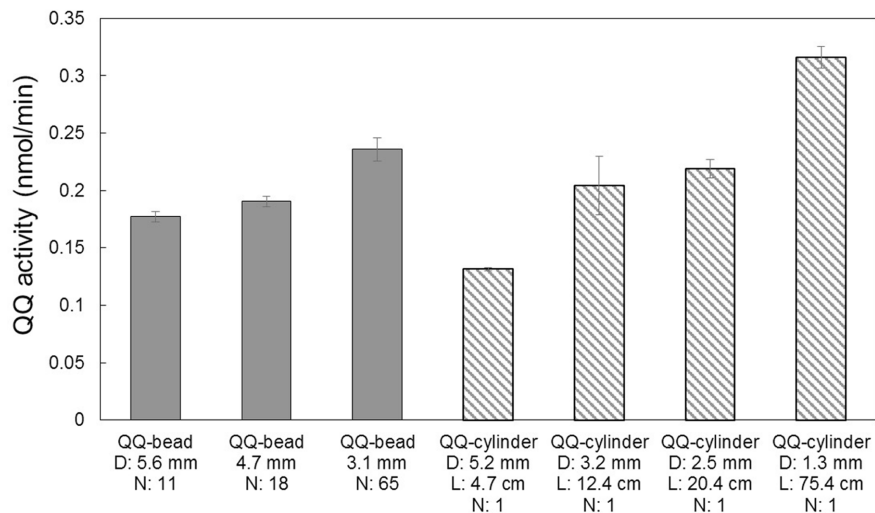
Mixed liquor suspended solids (MLSS) and chemical oxygen demand (COD) were measured according to standard methods [10].

## Results and Discussion

#### Effect of the Shape and Size of QQ-Media on QQ Activity

Three sizes of QQ-beads (diameter: 3.1–5.6 mm) and four sizes of QQ-cylinders (diameter: 1.3–5.2 mm; length: 4.7–75.4 cm) were prepared to investigate the effect of the shape and size of the media on QQ activity. Having a total volume of QQ-media fixed at 1 ml (*i.e.*, the total mass of QQ-media including polymeric substances and BH<sub>4</sub> was fixed), the degradation rates (nmol/min) of standard C8-HSL by those media were measured in a conical test tube containing 30 ml of C8-HSL solution and compared with one another (Fig. 3). Under an identical loading volume of QQ-media (1 ml), the QQ activities of the beads increased as the number of beads increased, but their diameters decreased. In contrast, the QQ activities of the cylinders increased as their diameters decreased, but their lengths increased even though the number of cylinders was the same (*i.e.*,  $N = 1$  for all types of cylinders).

Based on the results of Fig. 3, the correlation between the QQ activity and total surface area of the media was analyzed. Regardless of the shape of the QQ-media, the QQ activity of the media was proportional to their total surface area ( $R^2 = 0.95$ ), as shown in Fig. S1. This indicates that the net QQ activity of the QQ-media, excluding the physical washing effect, was not dependent on the shape of the



**Fig. 3.** QQ activities of three QQ-beads and four QQ-cylinders having the same total volume of 1.0 ml. D, L, and N represent the average diameter, length, and number of the media, respectively. Error bar: standard deviation ( $n = 3$ ).

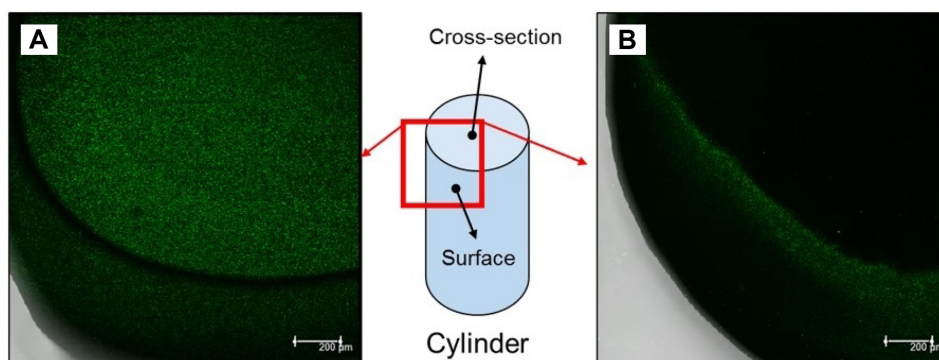
media (bead or cylinder) under the identical loading volume of QQ-media. However, the total surface area was found to be important for the biological QQ activity under identical loading volume. In other words, QQ activity would be proportional to the surface area to volume ratio of the QQ-media. It is because the mass transfer rate of signal molecules through the QQ-media would be significantly dependent on their effective surface area [22]. To confirm and elucidate this finding more clearly, the interaction between the QQ-media and the QS signal molecules was investigated using a reporter strain, JB525, which produces green fluorescence protein upon intake of a range of AHL signal molecules.

#### Effective Region in a QQ-Medium for Quorum Quenching

To elucidate the interaction mechanism between QS signal molecules and QQ media when they come into

contact with each other, two types of cylinders were prepared: (i) cylinders entrapped with only JB525 (an AHL reporter strain), and (ii) cylinders entrapped with both JB525 and BH4 (QQ bacteria). The two types of cylinders were exposed to 1  $\mu$ M C8-HSL in 30 ml of Tris-HCl buffer (pH 7) with 1/20 diluted LB broth for 2 h, and their cross-sections were visualized by fluorescence microscopy.

In a cylinder entrapped with only JB525, green fluorescence indicating the presence of QS signal molecules (C8-HSL) was observed across the cylinder (Fig. 4A). This means that free diffusion of C8-HSL occurred within the cylinder. In contrast, in a cylinder entrapped with both JB525 and BH4, green fluorescence was observed only along the surface of the cylinder, and it faded out toward the center (Fig. 4B). Note that the entrapped BH4 produced the endo-enzyme [18], which is capable of decomposing AHL signal molecules only if they diffuse into the cell.



**Fig. 4.** CLSM images of cylinders entrapped with (A) only JB525 and (B) both JB525 and BH4.

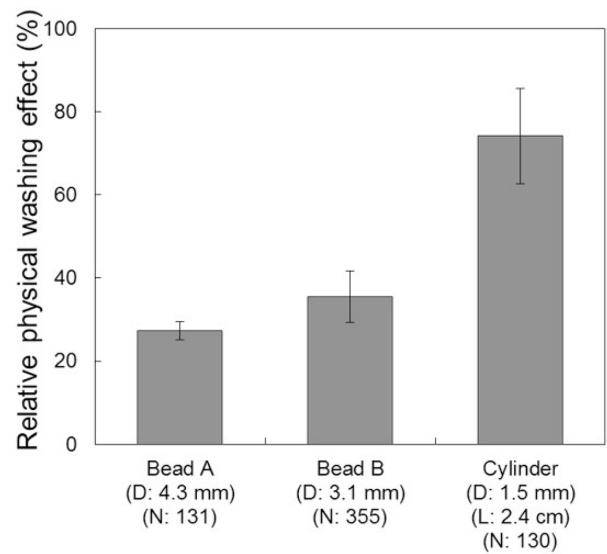
Based on the contradictory results from observing the two different cylinders, it had been concluded that the presence of BH4 (QQ bacteria) limits the diffusion of signal molecules toward the center of the medium because most AHLs were degraded by the BH4 located near the surface of the medium. Consequently, QQ bacteria at the inner part of a medium may be deprived of opportunities to encounter and decompose signal molecules.

It is also worth noting that, although QQ activity can be increased by reducing the diameter of a cylinder and increasing its length, a QQ-cylinder with a small diameter but longer length may be disadvantageous for its practical application in real MBRs for wastewater treatment. This is because a longer cylinder type of the QQ-medium could more easily become wound on or caught in other equipment in a bioreactor, such as a membrane module or aeration device.

#### Comparison of the Physical Washing Effect of the QQ-Media (Bead vs. Cylinder)

To compare the physical washing effect of various media shapes, different shapes of vacant-media (without QQ bacteria) were prepared, and their physical washing capabilities were tested in a batch reactor (1.1 L). The interval between the PC plate and wall of the batch reactors (10 mm) was designed to be identical to the membrane spacing (10 mm) of laboratory-scale MBRs. As the loading volume of each medium was fixed at 0.5% (v/v) (total media volume/reactor volume), the number of media tested varied depending on the size of the medium. In Fig. 5, the physical washing effect of bead B, with a smaller diameter (3.1 mm) but higher number ( $N = 355$ ), was approximately 30% greater than that of bead A, with a larger diameter (4.3 mm) but lower number ( $N = 131$ ). However, when the shape was changed from bead to cylinder (diameter: 1.5 mm; length: 2.4 cm), with nearly the same number ( $N = 130$ ), the effect of the cylinder was approximately 170% greater than that of bead A. It turned out that the physical washing was greatly affected by media shape, and the cylinder shape is more desirable than the bead shape.

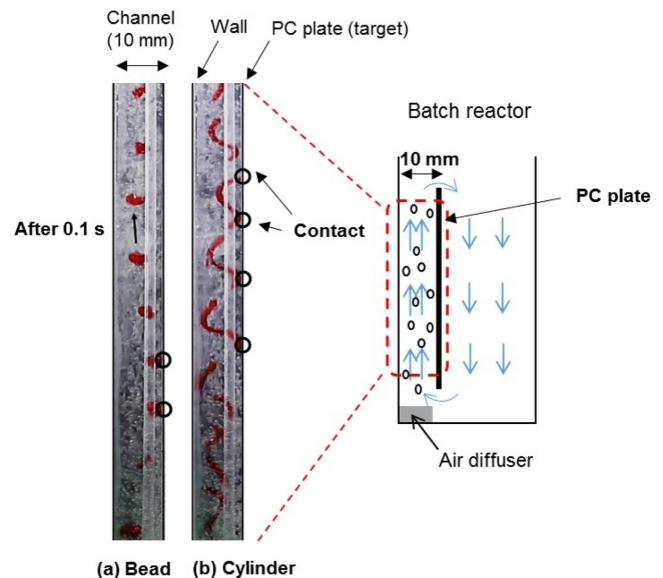
To deeply investigate the effect of media shape on physical washing, the circulation of bead A and the cylinder (Fig. 5) was monitored when passing through a confined space between the reactor wall and PC plate in a batch reactor, between which the interval was 10 mm, as shown in Fig. 6. Each medium was stained with Congo red for clear observation. During their movement, snapshots were taken at every 0.1 sec for each medium passing through the



**Fig. 5.** Comparison of the physical washing effect between bead A, bead B, and the cylinder.

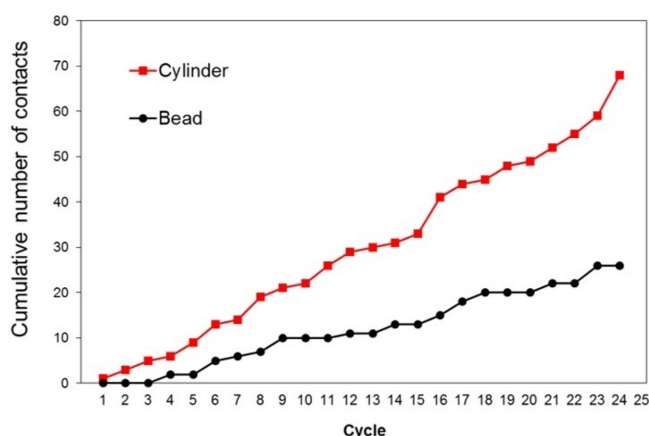
D, L, and N represent the average diameter, length, and number of the media, respectively. Error bar: standard deviation ( $n = 3$ ).

confined space and then converged to make time-lapse images. As each medium passed once through the channel, bead A contacted the PC plate two times (Fig. 6A), whereas the cylinder contacted the plate four times (Fig. 6B). To



**Fig. 6.** Time lapse (snapshot time = 0.1 sec) images of media passing through the channel.

(A) Bead with a diameter of 4.3 mm. (B) Cylinder with a diameter of 1.5 mm and a length of 2.4 cm.



**Fig. 7.** Cumulative number of contacts for a bead with a diameter of 4.3 mm, and a cylinder with a diameter of 1.5 mm and length of 2.4 cm.

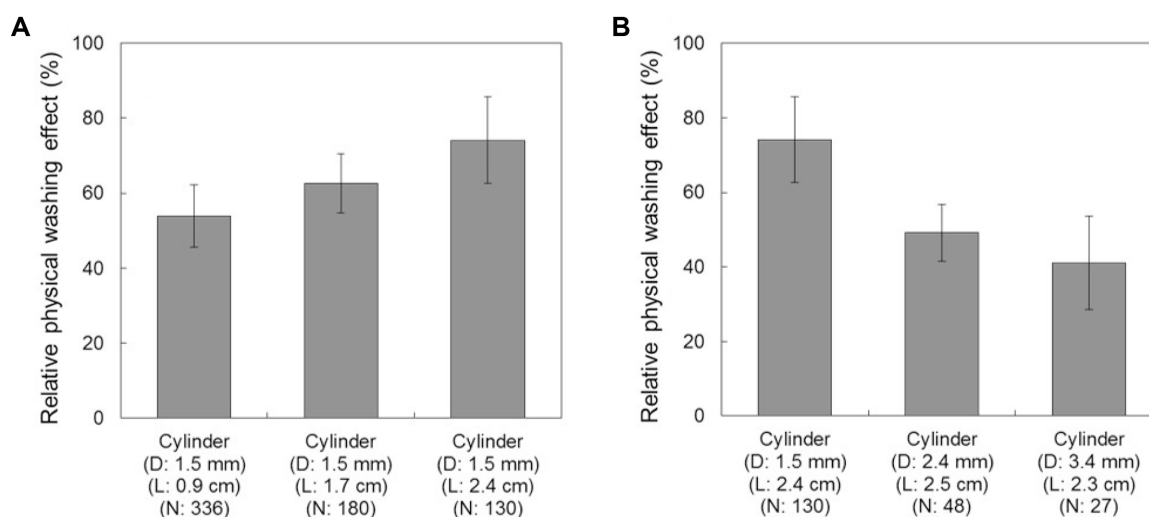
quantify the contact frequency for a longer period, all of the contacts were counted and cumulated during 24 repeated passes of each single medium through the channel (Fig. 7). The single cylinder contacted the PC plate a total of 68 times, and the average contact number per cycle was  $2.8 (\pm 2.1)$ , whereas bead A contacted a total of 26 times, and the average per cycle was  $1.1 (\pm 1.3)$ . The higher contact frequency of the cylinder compared with that of the spherical bead may be attributed to its geometric structure, as the length of the cylinder was longer than the channel spacing. In summary, substances attached to the PC plate could be expected to be detached, at least partly, by collisions

between the PC plate and the media. Consequently, the QQ-cylinders are expected to have a greater physical washing effect than the QQ-beads owing to their higher contact frequency.

### Correlation of Cylinder Size with the Physical Washing Effect

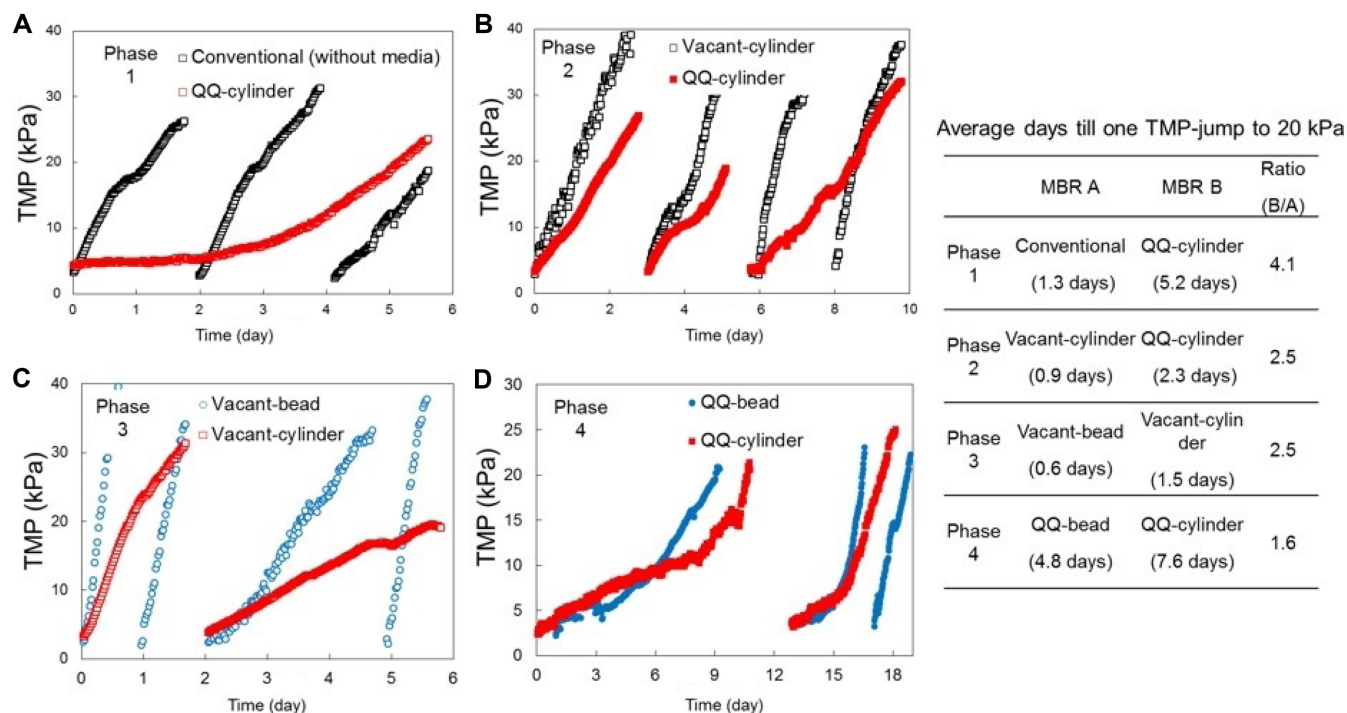
It has been reported that the motion of flexible fiber in shear flows can be affected by its geometry, such as the length and aspect ratio (length/diameter) [19, 20]. First, under the same loading volume (0.5% (v/v)), cylinders with identical diameters but different lengths and numbers were compared with each other in terms of their physical washing effect. The physical washing effect of the cylinders increased with the increase in their length, despite a decrease in number (Fig. 8A), indicating that the length of the cylinder is more important than the number in terms of the physical washing effect. Next, under the same loading volume (0.5% (v/v)), cylinders with similar lengths but different diameters and numbers were compared with each other. The cylinders with greater numbers and smaller diameters showed better physical washing (Fig. 8B). The two parameters (length and diameter) were combined into one parameter (*i.e.*, the aspect ratio; ratio of length to diameter), and then the physical washing effect was plotted vs. the aspect ratio. It was found that the physical washing effect of the cylinders tended to show a positive relationship with the aspect ratio (Fig. S2).

In this study, the effects of the shape and size of QQ-medium on QQ activity and physical washing effect were



**Fig. 8.** Comparison of the physical washing effect of various vacant-media.

(A) Cylinders with the same diameter but different lengths. (B) Cylinders with different diameters but the same length. D, L, and N represent the average diameter, length, and number of the media, respectively. Error bar: standard deviation ( $n = 3$ ).



**Fig. 9.** Comparison of transmembrane pressure profiles in MBRs at four different phases.

(A) Control and QQ-cylinder (D: 1.6 mm; L: 3 cm; N: 168). (B) Vacant-cylinder (D: 1.7 mm; L: 3.1 cm; N: 140) and QQ-cylinder (D: 1.7 mm; L: 3 cm; N: 146). (C) Vacant-bead (D: 3.4 mm; N: 517) and vacant-cylinder (D: 1.6 mm; L: 3 cm; N: 168). (D) QQ-bead (D: 3.4 mm; N: 490) and QQ-cylinder (D: 1.7 mm; L: 3 cm; N: 147). D, L, and N represent the average diameter, length, and number of the media, respectively.

separately investigated in different batch tests. However, there might be a synergistic effect between the QQ activity and the physical washing, in that the efficiency of QQ activity would be improved owing to detachment of the biofilm by physical washing.

#### Assessment of Biofouling Mitigation with Various QQ-Media in Continuous MBRs

Four different phases of MBR sets were operated in a continuous mode to compare biofouling mitigation between various vacant- or QQ-media configurations. TMP rise-up was monitored, and their profiles are shown in Fig. 9. In addition, for quantitative comparison,  $T_{\text{TMP}}$ , the average number of days taken to reach a TMP of 20 kPa, was calculated in each phase.

In phase 1, conventional- (without media) and QQ-cylinder MBRs were run in parallel at an airflow rate of 1.0 l/min (Fig. 9A).  $T_{\text{TMP}}$  was approximately 1.3 days in the conventional-MBR, whereas it was 5.2 days in the QQ-cylinder MBR. In other words, the rate of TMP rise-up, which reflects the extent of membrane biofouling, was delayed by approximately 300% with the insertion of the

QQ-cylinder. In Fig. 9B, the  $T_{\text{TMP}}$  in the vacant-cylinder and QQ-cylinder MBRs was 0.9 days and 2.3 days, respectively, resulting in a ratio of  $T_{\text{TMP}}$  value of 2.5. Because the physical washing effect was the same with vacant-cylinders and QQ-cylinders, the delay of TMP rise-up in the QQ-cylinder MBR could be attributed to the biological QQ activity of the QQ-cylinders. In phase 3, the vacant-cylinder MBR was compared with the vacant-bead MBR (Fig. 9C). The ratio of  $T_{\text{TMP}}$  was 2.5, indicating that the rate of TMP rise-up in the vacant-cylinder MBR was delayed approximately 150% longer than that in the vacant-bead MBR. Consequently, the better physical washing effect of the cylinders compared with the beads was confirmed in continuous MBR.

Finally, the QQ-cylinder MBR and the QQ-bead MBR were operated in parallel at an airflow rate of 0.8 l/min. The ratio of  $T_{\text{TMP}}$  value of the QQ-cylinder MBR to that of the QQ-bead MBR was calculated to be 1.6, which indicates that the TMP rise-up of the QQ-cylinder MBR was delayed more by about 60% compared with that of the QQ-bead MBR (Fig. 9D). As demonstrated in batch studies, the improved inhibition of biofouling in the QQ-cylinder MBR may be attributed to the higher QQ activity and physical

washing effect of the QQ-cylinders compared with QQ-beads.

In summary, a new shape of moving media, a cylinder-type medium (QQ-cylinder), was developed. The QQ activities and physical washing effect of beads and cylinders of different sizes were compared in batch tests. It was found that the QQ activity of the QQ-medium was highly dependent on its total surface area regardless of the medium shape. In contrast, the physical washing effect of the media was greatly affected by the medium shape. It was confirmed that the physical washing effect of the cylinders was higher than that of the beads owing to their high contact frequency with the membrane surface. Moreover, the physical washing effect of the cylinders was in close association with the aspect ratio. The enhanced performance of the QQ-cylinder was confirmed in a laboratory-scale continuous MBR with a flat-sheet membrane module.

## Acknowledgments

This research was supported by the Convergence Technology Program (2015001640001) funded by the Korea Ministry of Environment (MOE). The authors wish to thank Prof. Michael Givskov at the University of Copenhagen for kindly providing *E. coli* JB525.

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