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Design of Quorum Quenching Microbial Vessel to Enhance Cell Viability for Biofouling Control in Membrane Bioreactor

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Quorum quenching (QQ) with a microbial vessel has recently been reported as an economically feasible biofouling control platform in a membrane bioreactor (MBR) for wastewater treatment. In this study, a quorum quenching MBR with a ceramic microbial vessel (CMV) was designed to overcome the extremely low F/M ratio inside a microbial vessel. The CMV was prepared with a monolithic ceramic microporous membrane and AHL-degrading QQ bacteria, *Pseudomonas* sp. 1A1. The "inner flow feeding mode" was introduced, under which fresh feed was supplied to the MBR only through the center lumen in the CMV. The inner flow feeding mode facilitated nutrient transport to QQ bacteria in the CMV and thus enabled relatively long-term maintenance of cell viability. The quorum quenching effect of the CMV on controlling membrane biofouling in the MBR was more pronounced with the inner flow feeding mode, which was identified by the slower increase in the transmembrane pressure as well as by the visual observation of a biocake that formed on the used membrane surface. In the QQ MBR with the CMV, the concentrations of extracellular polymeric substances were substantially decreased in the biocake on the membrane surface compared with those in the conventional MBR. The CMV also showed its potential with effective biofouling control over long-term operation of the QQ MBR.

Keywords: Membrane bioreactor (MBR), quorum sensing, quorum quenching, ceramic microbial vessel, inner flow feeding, biofouling

Introduction

Commercial use of membrane bioreactors (MBRs) has increased dramatically over the last two decades owing to the high quality of its effluent and compactness. However, biofouling, filterability loss caused by natural biocake formation on the membrane surface, still remains unsolved, significantly reducing its efficiency [1, 3].

Recently, the concept of quorum sensing has been introduced as a novel biofouling control strategy, ever since Yeon *et al*. [22] revealed that quorum sensing is closely related to biofouling in MBRs for wastewater treatment. Quorum sensing (QS) is the density-dependent regulation of versatile microbial functions such as biofilm formation using signaling molecules called autoinducers [6, 7, 19, 20]. Based on QS mechanisms, decomposition of signal molecules, (*i.e*., quorum quenching (QQ)) has been reported as an effective membrane biofouling control technique [15, 16, 18, 21, 23]. For example, acylase, which can hydrolyze *N*-acyl homoserine lactone (AHL)-type autoinducers from gram-negative bacteria, was successfully applied to mitigate biofouling in MBRs as well as in nanofiltration with various enzyme immobilization techniques [10, 14, 23].

To achieve economic feasibility of quorum quenching for anti-biofouling in MBRs, however, "enzymatic quorum quenching" has been replaced by "bacterial QQ" using a "microbial vessel" or "cell entrapping beads," respectively. Oh *et al*. [18] isolated QQ bacteria, *Rhodococcus* sp. BH4, from a real MBR plant and prepared a microbial vessel encapsulating QQ bacteria (BH_4) to insert the "microbial

vessel" into an MBR. Cheong *et al*. [2] also isolated the natural AHL-degrading *Pseudomonas* sp. 1A1 strain from a real municipal MBR plant to prepare a "microbial vessel." Furthermore, Kim *et al*. [16] prepared "free moving beads" by entrapping the same QQ bacteria. All of them, "microbial vessels" and "moving beads," were successful in substantially mitigating biofouling in MBRs.

However, in QQ MBRs with a polymeric microbial vessel [2, 9, 18], the food-to-microorganism (F/M) ratio inside the microbial vessel was extremely small compared with that in a mixed liquor, due to the high population density (M) of the QQ bacteria (> 100,000 mg/l) but the relatively lower substrate available (F) in the vessel than in the broth (< 50 mg COD/l). Such an abnormally small F/M ratio could cause the growth inhibition of QQ bacteria, and eventually lead to the loss of their QQ activity.

In this study, a QQ microbial vessel was designed using a monolithic ceramic microporous membrane with seven lumens to augment the F/M ratio in the microbial vessel. Both air and fresh feed were directly supplied through one lumen located in the center of the ceramic microbial vessel (CMV), while the AHL-degrading QQ bacteria (*Pseudomonas* sp. 1A1) were entrapped inside the remaining six lumens in the CMV. Such a so-called "inner flow feeding mode" was expected to facilitate the mass transfer of air and nutrients and thus augment the local F/M ratio, eventually enhancing the QQ bacterial viability in the CMV. To achieve this goal, the overall experiments were carried out in three phases: (i) CMV preparation, (ii) evaluation of mass-transfer efficiency and cell viability in the CMV, and (iii) evaluation of its quorum quenching activity for the control of membrane biofouling in the MBR for wastewater treatment.

Materials and Methods

Bacterial Strains and Growth Conditions

An indigenous QQ bacterium, *Pseudomonas* sp. 1A1, was isolated from a lab-scale MBR using an enrichment culture method [2]. Strain 1A1 was cultured on Luria-Bertani (LB) medium at 30°C. *Agrobacterium tumefaciens* A136(Ti$^-$)(pCF218)(pCF372) was used as a reporter strain for the detection of *N*-acyl homoserine lactone (AHL), which is an autoinducer of gram-negative bacteria [5]. *A. tumefaciens* A136 was cultured on LB medium supplemented with spectinomycin (50 mg/l) and tetracycline (4.5 mg/l) to maintain the two plasmids that provide the AHL response system. Activated sludge obtained from a wastewater treatment plant (Sihwa, Korea) was inoculated into the MBR after being acclimated to synthetic wastewater with the composition previously described elsewhere [23].

Preparation of the Ceramic Microbial Vessel (CMV)

A monolithic ceramic microporous membrane with a nominal pore size of 0.45 μm (KERASEP, Novasep, France) was chosen as a supporting component of the CMV. The monolithic module consists of seven lumens, one located in the center and the other six in a circle (Fig. 1). The bottom sides of the lumens except for

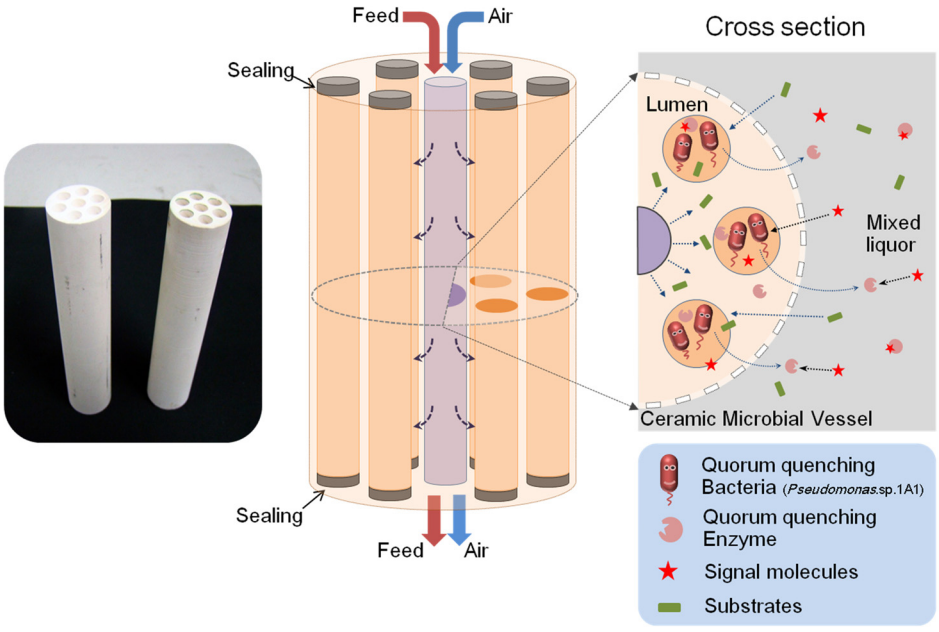

Fig. 1. Schematic diagram of the ceramic microbial vessel under the inner flow feeding mode.

Table 1. Specifications of the ceramic microbial vessel.

Membrane material	TiO_2-Al_2O_3
Nominal pore size	0.45 μm
Number of lumens	7
Internal diameter of lumen	4 mm
External diameter of module	20 mm
Total length	100 mm
Packing density of QQ bacteria	26.5, 70.3 mg biomass/cm^3-lumen

the center one were completely sealed with silicon glue, and then the *Pseudomonas* sp. 1A1 culture was injected into the six lumens in a circle using a sterilized syringe. After cell encapsulation, the top sides of all six lumens were covered with a silicon cap, which can be opened in case of cell replacement. The bacterial population density in the CMV was set to 26.5 or 70.3 mg biomass/cm^3-lumen. *Pseudomonas* sp. 1A1 was inactivated with autoclaving and injected into a ceramic vessel in the same way as the CMV to prepare an "inactive CMV" as a control group. Detailed specifications of the CMV are given in Table 1.

AHL Bioluminescence Assay

The concentration of the AHL molecules was measured with the luminescence method using the reporter strain *A. tumefaciens* A136 (Ti$^-$)(pCF218)(pCF372) [12, 17, 22]. The reporter strain and the AHL sample were mixed and loaded onto a microwell plate. The microwell plate was placed in an incubator to maintain the temperature at 30°C for 1.5 h, and then the Beta-Glo Assay System (Promega, USA) was added to the solution for the luminescent reaction with β-galactosidase produced by the reporter strain. After 40 min, the luminescence was measured by a luminometer (Synergy 2; Biotek, USA). The amounts of AHL were calculated using relationship equations based on the calibration curve derived from standard samples of AHL.

Measurement of the QQ Activity of the CMV

The QQ activity was determined by the degradation rate of *N*-octanoyl-L-homoserine lactone (C8-HSL), which has been identified and reported as one of the major AHL molecules in a previous study [23]. To measure the QQ activity of the CMV, C8-HSL was added to 20 ml of Tris-HCl buffer (pH 7, 50 mM) for a final concentration of 200 nM. Then, the CMV was inserted into the buffer and the mixture was incubated at 30°C with orbital shaking (200 rpm) for 15, 45, 90, and 180 min. The remaining concentration of C8-HSL at each reaction time was measured using a bioluminescence assay.

Measurement of the Nutrient Transport Rate

Because the purpose of the inner flow feeding was to promote the mass transfer of nutrients to the inside of the lumens entrapping the QQ bacteria (*Pseudomonas* sp. 1A1), it was necessary to compare the mass transfer rate between the normal feeding and inner flow feeding modes. The six lumens in the circle of both ceramic vessels were filled with deionized water instead of QQ bacteria and then both ceramic vessels were immersed into two MBRs, respectively (Fig. 2). The normal feeding mode was applied to Reactor-1 in which fresh synthetic wastewater was fed into the reactor (*i.e.*, into the mixed liquor), whereas the inner flow feeding mode was applied to Reactor-2 in which the same wastewater was fed directly to the lumen that was in the center of the ceramic vessel. After the operation of both MBRs for 24 h, each ceramic vessel was taken out of the MBR to measure and compare the total nutrient concentrations inside the six lumens located in the circle of each vessel.

MBR Operation

Three laboratory-scale MBRs, each with a 3.0 L working volume were operated in parallel using synthetic wastewater (Fig. 3). The composition of the synthetic wastewater was as follows (g/l): glucose, 0.3; peptone, 0.1; yeast extract, 0.014; $(NH_4)SO_4$, 0.1; KH_2PO_4, 0.02; $MgSO_4$, 0.03; $MnSO_4$, 0.003; $FeCl_3$, 0.0002; $CoCl_2$,

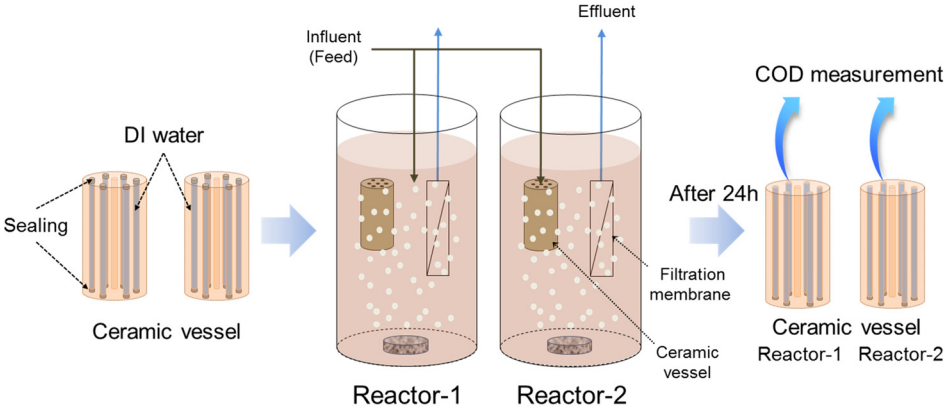

Fig. 2. Experimental set-up for the measurement of the nutrient transfer rate under two different feeding modes.
Reactor-1: CMV with distilled water under the normal feeding mode. Reactor-2: CMV with distilled water under the inner flow feeding mode.

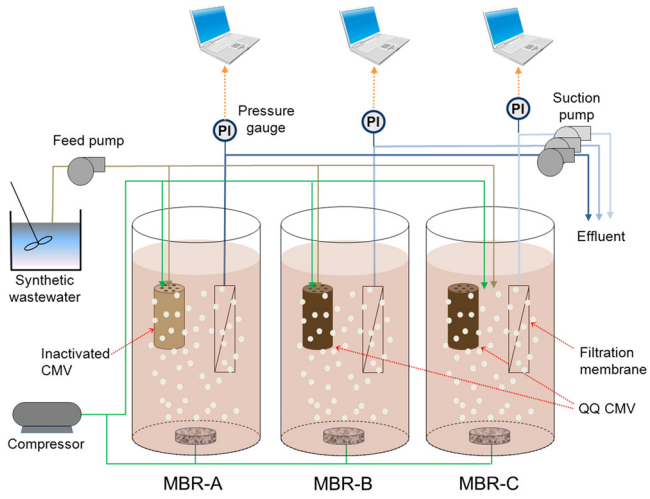

Fig. 3. Schematic diagram of the three MBRs in parallel operation.
MBR-A: CMV with inactivated QQ bacteria under the inner flow feeding mode. MBR-B: CMV with QQ bacteria under the inner flow feeding mode. MBR-C: CMV with QQ bacteria under the normal feeding mode.

0.002; $CaCl_2$, 0.004, and $NaHCO_3$, 0.26.

MBR-A was operated under the inner flow feeding mode with the CMV filled with inactivated *Pseudomonas* sp. 1A1. MBR-B was operated under the inner flow feeding mode with the CMV filled with active *Pseudomonas* sp. 1A1. MBR-C was operated under the normal feeding mode with the CMV filled with active *Pseudomonas* sp. 1A1. The filtration membranes with an effective membrane area of 0.021 m^2 were prepared using hydrophilic polyvinylidenefluoride (PVDF) hollow fiber (ZeeWeed 500; GE-Zenon, USA) with a nominal pore size of 0.04 μm. Other operating parameters are described in Table 2.

Analytical Methods

The LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probe, USA) was used to determine the active state of the biomass in the CMV according to the manufacturer's instructions. The biocake that formed on the membrane surface during MBR operation was stained with SYTO 9 (Molecular Probe), which has specificity for nucleic acids from bacterial cells [8, 16]. All of these fluorescently stained samples were immediately observed with a confocal laser scanning microscope (Nikon C1 plus, Japan). Images were recorded in the green channel (excitation 488 nm and emission 515/30 nm) and red channel (excitation 543 nm and emission 600/50 nm). A Z-section image stack for each green and red channel was constructed using the IMARIS software (Bitplane AG, Zurich, Switzerland).

Mixed liquor suspended solids (MLSS) and chemical oxygen demand (COD) were determined according to standard methods. Soluble microbial products (SMP) were obtained by centrifuging the mixed liquor (5,000 ×*g*, 5 min) and filtering the supernatant through a glass microfiber filter (pore size of 1.2 μm). Extracellular polymeric substances (EPSs) were extracted from the biocake using the heat extraction method [13]. The proteins and polysaccharides in the SMP and EPS were quantitatively analyzed using the modified Lowry method and phenol-sulfuric acid method [4, 11], respectively.

Table 2. MBR operating conditions.

Working volume	3.0 L
SRT	60 days
HRT	6 h
Flux	25, 30, 35 L/m^2/h
MLSS	11,000–13,000 mg/l
Feed COD	400–570 mg/l
F/M ratio	0.12–0.21
pH	6.8–7.0
COD removal efficiency	95–99%

Results and Discussion

Quorum Quenching Activity of the Ceramic Microbial Vessel

Two CMVs with different biomass packing densities (26.5 or 70.3 mg biomass/cm^3-lumen) were prepared and the AHL degradation activity of each was evaluated using C8-HSL as a signal molecule. One control CMV with inactivated *Pseudomonas* sp. 1A1 was also prepared to check the potential adsorption of C8-HSL by a ceramic vessel and the inactivated bacteria. As shown in Fig. 4, the CMV with the inactivated QQ bacteria did not show a

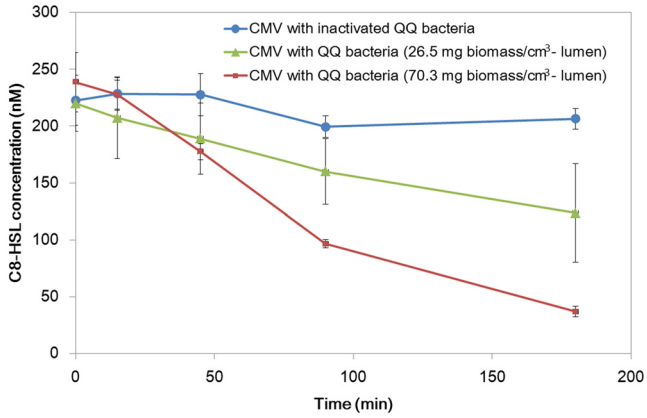

Fig. 4. Quorum quenching activity of the ceramic microbial vessels with different microbial packing densities.
Error bar: standard deviation (n = 3).

substantial decrease in the C8-HSL concentration, suggesting that its adsorption of C8-HSL was negligible. However, the AHL degradation rates of the two CMVs were 0.013 and 0.032 nmol C8-HSL/min for a 90-min reaction time depending on the QQ bacterial weights in the CMVs, proving that the CMV has AHL quenching activity.

Effect of the CMV on MBR Biofouling

Two MBRs with different CMVs were run in parallel to investigate the effect of the CMV on membrane biofouling (MBR-A & -B in Fig. 3): (i) MBR-A, CMV with inactivated QQ bacteria under the inner flow feeding mode; and (ii) MBR-B, CMV with QQ bacteria under the inner flow feeding mode. Membrane biofouling was monitored by TMP rise-up in the operation of the submerged MBRs. In the 1st cycle, the two MBRs were operated without the CMV at constant flux of 30 LMH to confirm there was little difference in the membrane fouling tendency among them (Fig. 5A). The two MBRs had similar rates of TMP rise-up, suggesting the population dynamics of the microorganisms in the mixed liquors were identical for the two MBRs.

In the 2nd cycle, two CMVs with a biomass packing density of 70.3 mg biomass/cm^3-lumen were installed into each reactor. MBR-A with inactivated QQ bacteria showed the same trend in TMP rise-up as in the 1st cycle, indicating QQ was not carried out because of the inactivation of the QQ bacteria. MBR-B with the QQ bacteria, however, showed a delay in the TMP rise-up compared with MBR-A. This proves the quorum quenching effect of the CMV with QQ bacteria on membrane biofouling under the inner flow feeding mode. When the flux was decreased to 25 LMH from 30 LMH at the 3rd cycle, the rate of TMP rise-up slowed down for both MBRs, but a large gap in TMP between MBR-A and -B was still maintained at the end of 10 days of operation. To elucidate the discrepancy in TMP between the two MBRs, the used filtration membranes were taken out at points 1 and 2 in the 2nd cycle and the total attached biomass (TAB) accumulated on the surface of the filtration membrane was measured. The TAB in MBR-A was 119 (±5.0) mg, whereas that in MBR-B was only 44 (±1.0) mg. The difference in the TAB implies that the development of the biocake on the membrane surface was retarded in MBR-B by the QQ bacteria in the CMV. It was also confirmed visually with CLSM photography of the used membranes at the same points (Fig. 5B). In detail, the reconstructed CLSM images of the used filtration membranes at points 1 and 2 clearly show that the biocake on the membrane surface was developed much less in MBR-B than in MBR-A.

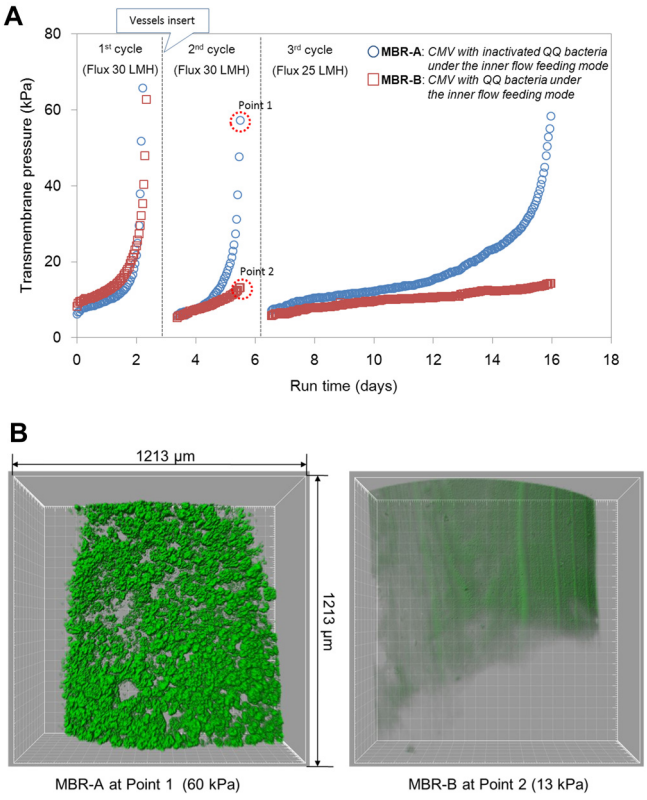

Fig. 5. (**A**) TMP profiles during 30 days of MBR runs, and (**B**) CLSM images of the biocakes on the surface of the used filtration membranes at points 1 and 2 in the 2nd cycle (CMV with a packing density of 70.3 mg biomass/cm^3-lumen).

Effect of the Inner Flow Feeding Mode on TMP Rise-Up in QQ MBR at Constant Flux

The main purpose of the "inner flow feeding mode" through the CMV was to enhance the growth of the QQ bacteria by ensuring the nutrient supply, thereby mitigating membrane biofouling. In order to compare the biofouling mitigating effect of the inner flow feeding mode to that of the normal feeding mode, MBR-C, the MBR with the CMV under the normal feeding mode, was operated together with MBR-A and -B (Fig. 3). After confirming that all three MBRs were in the same biological environment based on the TMP profile (the 1st cycle in Fig. 6), the three CMVs with a biomass packing density of 26.5 mg biomass/cm^3-lumen were installed into each reactor before starting the 2nd cycle. The profiles of the TMP rise-up during three cycles for MBR-A and -B (Fig. 6) were quite similar to those in the previous run (Fig. 5A). In the 2nd cycle, MBR-C with the QQ bacteria in the CMV under the normal feeding mode

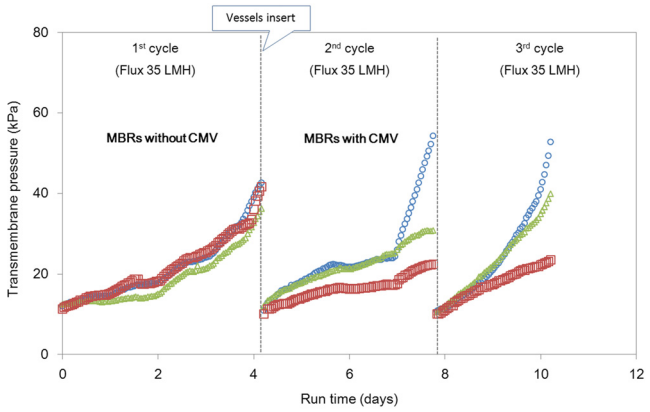

Fig. 6. Effect of the CMV and its inner flow operation mode on MBR filterability (CMV with a packing density of 26.5 mg biomass/cm^3-lumen).

○ MBR-A: CMV with inactivated QQ bacteria under the inner flow feeding mode. □ MBR-B: CMV with QQ bacteria under the inner flow feeding mode. △ MBR-C: CMV with QQ bacteria under the normal feeding mode.

showed retardation in the TMP rise-up compared with MBR-A (green color in Fig. 6), but the extent of retardation was less than that in MBR-B under the inner flow feeding mode (red color in Fig. 6). This result was attributed to the greater viability of the active QQ bacteria (*Pseudomonas* sp. 1A1) in the CMV under the inner flow feeding mode. The 3rd cycle of operation for the three MBRs further confirmed the reproducibility of the discrepancy in the rate of the TMP rise-up.

Of interest is that the MBR-C with the CMV under the normal feeding mode gradually lost most of its quorum quenching activity in the 3rd cycle. Because it was thought that the loss of QQ activity might be related to the deterioration of cell viability with the operation time due to the low F/M ratio in the CMV, the CMVs were taken out of MBR-B and MBR-C, respectively, right after the 3rd cycle of operation, and then the staining of live/dead cells inside the CMVs was conducted to check the cell viability in both CMVs. The portion of live cells (green color) in the CMV in MBR-B under the inner flow feeding mode (Fig. 7A) was observed to be much higher than that in MBR-C under the normal feeding mode without the inner flow (Fig. 7B). Quantitatively, the live/dead ratios in the CMVs of MBR-B and MBR-C were calculated as 4.8 (±0.5) and 1.3 (±0.3). It was concluded that the QQ MBR with the CMV could be more effective as a membrane biofouling control technique by adopting the inner flow feeding mode, which enabled the CMV to maintain a greater bacterial QQ activity through the facilitated nutrient transfer.

Effect of the Inner Flow Feeding Mode on the Rate of Nutrient Transfer to the CMV

To further clarify the greater mass transfer in MBR-B under the inner flow feeding mode than that in MBR-C under the normal feeding mode, the mass transfer rate of nutrients into the six lumens of each ceramic vessel was estimated in terms of the COD level (Fig. 2). After the operation of both MBRs for 24 h as shown in Fig. 2, each ceramic vessel was taken out of Reactor-1 and Reactor-2, and then the total nutrient concentration inside the six lumens located in the circle of each vessel was measured and compared (Fig. 8). Under the normal feeding mode, the soluble COD in lumen 1 (25.4 mg/l) became almost the same as that of the bulk phase (26.3 mg/l) after the operation of the MBR for at least 24 h, indicating the

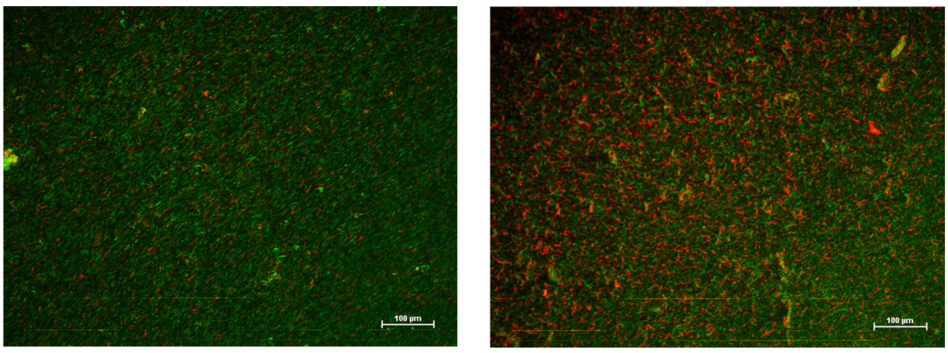

Fig. 7. Images of live/dead quorum quenching bacteria from the lumens of the used CMVs.

(**A**) MBR-B with the CMV under the inner flow feeding mode, (**B**) MBR-C with the CMV under the normal feeding mode. Green color: live cell; red color: dead cell.

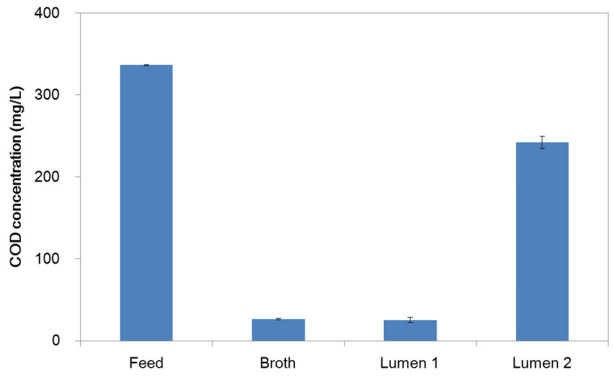

Fig. 8. COD concentrations in the feed, broth, and lumens under different feeding modes.
Error bar: standard deviation (n = 3). Lumen 1: COD in the lumen under the normal feeding mode. Lumen 2: COD in the lumen under the inner flow feeding mode.

soluble COD in the feed (336 mg/l) was equally distributed in the ceramic vessel and in the mixed liquor of the activated sludge. However, the soluble COD in lumen 2 under the inner flow feeding mode (242 mg/l) was much higher than that in the mixed liquor. This implies that the inner flow feeding mode makes the COD level in the ceramic vessel approach close to that in the fresh feed. Consequently, it was anticipated that the inner flow feeding mode would help compensate the extremely low F/M ratio inside the CMV in the QQ MBR by forced feeding through the center lumen.

Effect of the CMV on SMP and EPS Production in the MBR

Polymeric substances, which are well known to be related to membrane biofouling, were analyzed in terms of soluble microbial products in the mixed liquor and extracellular polymeric substances in the membrane-biocake [3, 10]. It was reported that the QQ in MBR can reduce polysaccharides and proteins in both the mixed liquor and the biocake [10]. In order to investigate the effect of the CMV on SMP and EPS production in MBRs, polysaccharide and protein concentrations in the mixed liquor and biocakes were measured from the three MBRs at the end of the 2nd cycle in Fig. 7. The CMV with QQ bacteria reduced substantially polysaccharides in the mixed liquor regardless of the feeding mode (Fig. 9A): from 23.6 (±0.6) to 8.9 (±1.1) for MBR-B or to 11.5 (±0.9) mg/l for MBR-C. However, the proteins in the mixed liquor from the three MBRs had a similar level.

In the case of EPS in the biocake, the CMV with QQ

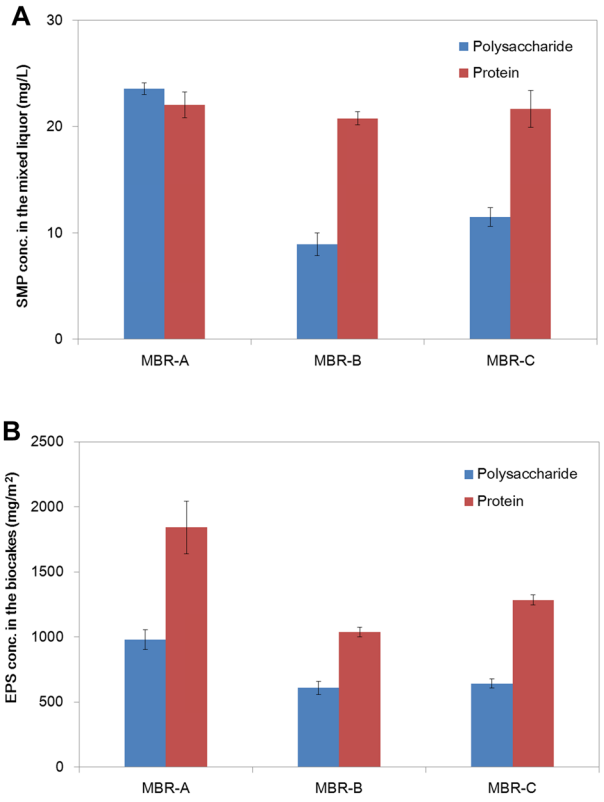

Fig. 9. (**A**) SMP in the mixed liquor, and (**B**) EPS in the biocakes on the membrane surface with the insertion of CMV with QQ bacteria.
Error bar: standard deviation (n = 3). MBR-A: CMV with inactivated QQ bacteria under the inner flow feeding mode. MBR-B: CMV with QQ bacteria under the inner flow feeding mode. MBR-C: CMV with QQ bacteria under the normal feeding mode.

bacteria reduced both polysaccharides and proteins (Fig. 9B): polysaccharide from 980 (±80) to 610 (±50) for MBR-B or to 650 (±40) mg/m^2-membrane for MBR-C; protein from 1,840 (±200) to 1,040 (±40) for MBR-B or to 1,290 (±40) mg/m^2-membrane for MBR-C. Taking into account that EPS and SMP are closely associated with microbial physiology, the QQ bacteria in the CMV are presumed to have regulated gene transcription for the production of EPS and SMP by inhibiting quorum sensing between microorganisms in the MBRs.

Effect of the CMV on the Biodegradation of Organics in MBRs

Considering that quorum sensing regulates microbial physiology [10], possible side effects of the CMV should be checked. As shown in Table 2, the biodegradation of organics in terms of COD removal was 95%–99% for all

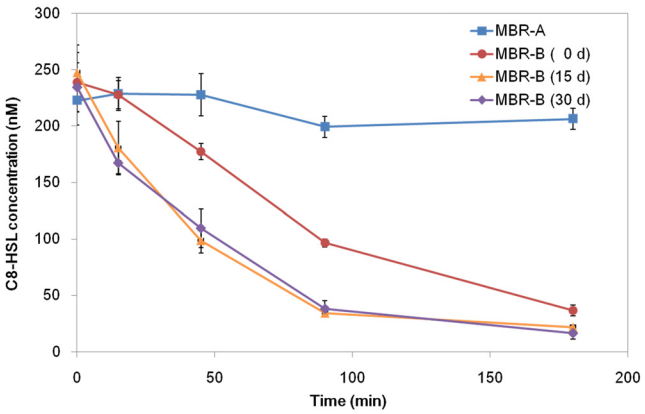

Fig. 10. Quorum quenching activity of the CMV with C8-HSL as a standard during 30 days of MBR operation.
Error bar: standard deviation (n = 3). MBR-A: CMV with inactivated QQ bacteria under the inner flow feeding mode. MBR-B: CMV with QQ bacteria under the inner flow feeding mode.

MBRs, suggesting that the insertion of the CMV does not affect the microbial activity of the activated sludge for the biodegradation of organics.

Stability of the QQ Bacteria in the CMV

Maintaining the QQ bacterial activity is one of the most important factors to be considered in QQ MBRs with CMV. Consequently, the activity of the CMV in the QQ MBR under the inner flow feeding mode was periodically monitored during the operation of MBR-A and MBR-B for 30 days. For each time point at 15 and 30 days, the CMVs were taken out of the MBRs and then the activity of the CMV was measured using C8-HSL as a standard signal molecule for the bioluminescence assay (Fig. 10). The degradation rate of C8-HSL with a fresh CMV was 0.032 nmol C8-HSL/min in a 90-min reaction time, whereas that at 15 days was 0.047 nmol C8-HSL/min, which was about 47% higher than the initial rate. This increase in activity was attributed to the good adaptation of the QQ bacteria to their new environment of the mixed liquor in the MBR as well as to their growth inside the CMV. Moreover, the activity of the CMV at 30 days was 0.044 nmol C8-HSL/min, indicating little loss of its activity. As a result, it was concluded that the CMV has the potential of facile and effective biofouling control in MBRs because it can maintain its quorum quenching activity at least during the test period without any further inputs.

In summary, a quorum quenching MBR with a ceramic microbial vessel was designed so as to augment the cell activity in the CMV, which was prepared using a monolithic ceramic microporous membrane and AHL-degrading QQ bacterium *Pseudomonas* sp. 1A1. The following conclusions were drawn: firstly, the inner flow feeding mode under which fresh feed is supplied to the MBR only through the center lumen in the CMV compensated for the extremely low F/M ratio inside the CMV, and thereby enabled the CMV to maintain greater bacterial QQ activity through the facilitated nutrient transfer. Secondly, the quorum quenching effect of the CMV on the control of membrane biofouling was more pronounced when the feeding mode was changed from the normal mode to the inner flow mode. Thirdly, the QQ bacteria in the CMV are presumed to have inhibited the quorum sensing between the activated sludge in the MBR, which regulates gene transcription for the production of EPS. Lastly, the CMV showed little loss in its initial AHL degradation activity over 30 days of MBR operation, leading to the expectation for its potential in effective biofouling control in long-term MBR operations.

Acknowledgments

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