Quorum Quenching Cell Entrapping Bead by Polyvinyl Alcohol Method for Biofouling Mitigation in Lab-scale MBR

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Abstract

Quorum Quenching (QQ) bacteria such as *Rhodococcus* sp. BH4 and *Pseudomonas putida* have excellent anti-biofouling potential as they disrupt Quorum Sensing (QS) system and inhibit biofilm formation on membranes. Cell Entrapping Beads (CEBs) in which the QQ bacteria are immobilized is one of the most effective methods to mitigate membrane biofouling in MBR. The CEBs are crucial as they mainly protect QQ bacteria from the sludge's harsh environment for a better QQ effect and help in the physical cleaning of membranes in a submerged MBR. Previously simple sodium alginate (SA) beads were used, but it was found that their durability was very low in real wastewater. Polyvinyl Alcohol (PVA) is a better alternative due to its higher durability, chemical stability and low cost. Several brands of PVAs with different polymerization degrees were used here and small amount of SA was added to avoid agglomeration of PVA beads. Concentrations of SA/PVA were varied and different temperatures of cross-linking solution also was examined. Then the quality of the beads was evaluated on the physical and biological aspects. It was found that a PVA of 2,270 polymerization degree with 8% mixed in 1% SA makes the most stable CEBs. A specific brand of SA didn't prevent agglomeration of CEBs, while a particular brand of SA did even at lower concentrations. The temperature of cross-linking solution was also found to have a significant effect on beads' internal structure. The quality of CEBs made by the best method found in this research was confirmed through series of tests, i.e., freeze-drying, scanning electron microscopy, activity test after immobilization of QQ bacteria in the beads.

Keywords: Membrane biofouling, Quorum Sensing, Quorum Quenching, PVA-Alginate beads, Cross-linking solution, Freeze drying, Scanning electron microscopy.

Introduction

Biofouling is one of the main constraints associated with Membrane Bioreactors (MBR), leading to high energy consumption and operational costs. It is caused by the formation of a cake layer (biofilm) on the surface of membranes. It has been found that Quorum Sensing (QS) plays a crucial role in the development of biofilm by autoinducers called Acyl Homoserine Lactones (AHLs). QS is a bacterial cell-to-cell communication process that involves the synthesis, release and detection of AHLs [1]. The concentration of AHLs in the sludge increases with time, resulting in deposition of cake layer on membranes and rise in Transmembrane Pressure (TMP) until it reaches a point where filtration slows down or even stops. At this point the operation is stopped and membranes need to be regenerated or replaced.

Different methods have been used to mitigate membrane biofouling, including relaxation, standard backwash (back pulse with permeate in reverse direction of membrane filtration), and chemical backwash (back pulse with addition of certain chemicals chemically enhanced backwash). Physical cleaning such as standard backwash can remove only loosely attached cake layers from the membranes. Therefore it is not efficient alone and needs to be coupled with other methods. Chemical backwash from a strong oxidizing agent like sodium hypochlorite can remove irreversible fouling as it breaks the foulant-foulant bond. It was recently found that some bacteria in the cake layer become resistant to chemicals and remain attached to the membranes after backwash [2].

Quorum Quenching (QQ) is a relatively new and novel method as it disrupts cell to cell communication of bacteria by degrading AHLs in the sludge. The decrease in AHLs concentration slows down deposition of cake layer and delays TMP rise, resulting in increase of life span of membranes. Bacterial strain such as *Rhodococcus* sp. BH4 and *Pseudomonas Putida* were found to have excellent potential to inhibit membrane biofouling. Previously QQ method in MBR was done with the immobilization of QQ enzyme Acyl Homoserine Lactonases inside sodium alginate capsules. But this method had some practical drawbacks, including enzyme extraction, stability, and cost [1]. Sodium alginate beads are easily decomposed in natural wastewater [3,4] and the same phenomenon was reported by Ahmed and her colleagues [5].

Encapsulation (immobilization or entrapment) of QQ bacteria in freely moving beads is an innovative method as it allows the diffusion of nutrients to the bacteria and of products away from it. These Cell Entrapping Beads (CEBs) can control membrane biofouling by both physical and biological actions. Different types of polymers were used to make beads that include agar, agarose collagen, polyurethane, alginate, and cellulose [6]. Each of these materials have their drawbacks. Polymers such as agar, agarose, cellulose and alginate have poor mechanical strength and low durability. Similarly, polyurethane and polyacrylamide are highly toxic to bacterial cells and some have a high cost [6].

On the other hand, polyvinyl alcohol (PVA) is the synthetic water-soluble polymer used the most in the world. It was first used as an immobilizing matrix by Freeman and Aharonowitz and since then it has been used in wastewater treatment [7]. It is non-toxic to living cells and is produced economically. It has higher durability, mechanical strength and chemical stability [8].

PVA brand used in previous studies [3,4,6,8,9] to make PVA beads not available in the local market and recently the manufacturer discontinued the product. So, there is a need to investigate a brand that is easily accessible and can be used to prepare good quality polymer beads. Therefore, this research aims to find out the best bead-making method with available brands in different concentrations of PVA and other conditions for using the beads as immobilization media of QQ bacteria to mitigate biofouling and consequently improve the performance of MBR.

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Methodology

Chemicals

Four different PVA brands (with varying degrees of polymerization, i.e., 1,500, 1,640, 2,000 and 2,270) were used for this study. The PVA with polymerization degree 2000 was imported from Japan and the other three were locally purchased. Two brands of SA used in this study were Sigma and VWR Chemicals. Boric acid, calcium chloride dihydrate and sodium sulfate were also purchased locally. They are shown in Figure 1, Table 1 and 2.

Preparation and test of different PVA-alginate beads

- **Preparation of PVA-alginate beads**

PVA beads were prepared according to the method suggested by [8,9] with some modification. Takei reported that PVA hydrogel beads were successfully made with 10w/v% PVA with 0.8%w/v SA by using boric acid and calcium chloride first and sodium sulfate later as cross-linking solution. He used PVA of polymerization degree 2,000 of Wako pure chemical, Japan. Van suggested 10~12w/v% PVA with 1%w/v SA would be the best when he used PVA of polymerization degree 1,750 of Kuraray Co. Singapore. But these two PVAs were not available in Pakistan, four brands of PVAs with different polymerization degree were tested for various concentration of PVA (0.5~10w/v%), three mixing methods (heating at 121°C in the autoclave, heating at 105°C in a dry oven, and heating and stirring at 120°C on hotplate-stirrer), two cross-linking temperatures (room temperature and 40°C) and different cross-linking times. In order to avoid agglomeration of PVA beads due to insufficient linkage of PVA and boric acid [10], two different brands of SA were tested.

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a) Polyvinyl alcohols with different polymerization degree

b) Sodium alginate with different brands

c) Calcium chloride and boric acid

Figure 1: Chemicals used in making PVA beads

Table 1 Polyvinyl alcohols used in the study

Chemical	Brand	Polymerization Degree (p.d.)	Appearance
PVA #1	KANTO	2,000	White fine powder
PVA #2	DUKSAN REAGENTS	1,500	White crystals
PVA #3	APPLI Chem	1,640	White fine powder
PVA #4	UNI-CHEM	2,270	White small pallets

Table 2 Sodium alginate and cross-linking chemicals

Chemical	Brand	Density g/cm^3	Appearance
Sodium Alginate #1	VWR Chemicals	1.6	White to yellowish brown powder
Sodium Alginate #2	Sigma	-	White to yellowish brown powder
Calcium Chloride	UNI-CHEM	2.15	White granules
Boric Acid	SAMCHUN	1.44	White fine powder
Sodium Sulfate	SAMCHUN	2.66	White fine powder

When PVA-alginate solution was dissolved and mixed homogeneously then dropped into first cross-linking solution (saturated boric acid 4w/v% and calcium chloride 7w/v%) solution through nozzle in the form of small drops by peristaltic pump. And then, the beads were dipped into a second cross-linking solution (sodium sulfate 0.5M). Selected independent variables that may affect the quality of PVA-alginate beads are summarized in Table 3.

Table 3 Selected independent variables tested in this study

Independent variable	Description
Polyvinyl alcohol	4 brands with different polymerization degree (1,500, 1,640, 2,000, 2,270) 0.5~10w/v%
Sodium alginate	2 brands, 0.2~2w/v%
Meting and mixing method	autoclave(121°C), dry oven (60°C, 105°C), hotplate-stirrer (125°C)
1st cross-linking time	0.5~2 hours
2nd cross-linking time	2~8 hours
1st cross-linking temperature	Room temperature (20~25°C), 40°C

- **Test of PVA-alginate beads**

During and after making the beads, the quality of beads was evaluated in six aspects (Table 4). They include a) melting and mixing property of PVA-alginate solution, b) beads formation by 1st and 2nd cross-linking, c) agglomeration of the beads, d) physical appearance of the beads, e) mechanical strength of the beads by centrifugation, f) swelling of the beads in the distilled water. Total scores then evaluated the overall quality of PVA-alginate beads, the sum of assigned points (0: bad, 1: not good, 2: good) to each aspect of the test (a~f) mentioned above.

Preparation and test of QQ-CEBs

- ***Rhodococcus* sp. BH4 strain**

Rhodococcus sp. BH4 (Accession no. CP014941) plates were obtained from Yonsei University, South Korea. To confirm the strain, 16S rRNA sequencing was conducted by Cosmogenetech, South Korea.

- **Immobilization of Rhodococcus in the PVA beads**

After finding out the best method to prepare PVA-alginate beads (without immobilization of bacterial cells), *Rhodococcus* cells were added into the PVA-alginate solution prepared by the best method. As a summary, PVA and SA were mixed in 90 mL distilled water and placed in dry oven for four hours. When the solution was adequately mixed, it was cooled to about 40°C.

Properly prepared LB broth with *Rhodococcus* strains was transferred into falcon tubes and the tubes were centrifuged at the speed of 10,000rpm for 10min. After discarding the supernatant in the falcon tubes, the LB broth with the strains was again added into the same falcon tubes and centrifuged to accumulate the same tube's strains. This process was repeated until all the LB broth finished. The addition of 10mL deionized water then resuspended the pellet in the tubes. This resuspension was added into the PVA-alginate solution and thoroughly mixed. The final concentration of cells in the PVA-alginate-cell mixture solution was 5mg/mL (mg dry cells per mL mixture solution). The temperature of the mixture solution was maintained at 40°C. It was then dropped into the first cross-linking solution through a nozzle by a peristaltic pump. Once the beads were formed, they were transferred to the second cross-linking solution after rinsing with tap water and distilled water (sodium sulfate). After the set time, they were washed and stored in deionized water at 4°C before use. These beads are called QQ-CEBs (Quorum Quenching Cell Entrapping Beads). This process was depicted in Figure 2.

Table 4 Evaluation category of PVA-alginate beads

Test category	Description
Melting and mixing	Whether the mixture of PVA and SA is well dissolved in deionized water and mixed well homogeneously or not.
Bead formation	Whether drops of the PVA-alginate solution are formed as beads in the 1st and 2nd cross-linking solution or not
Agglomeration	Whether the beads formed in cross-linking solutions remain discretely without agglomeration
Physical appearance	Whether the shape of beads is uniform, and spherical, oval, tailed or irregularly shaped
Swelling	How much the beads get swollen in distilled water. Diameter of the swollen beads are monitored along with time interval.
Mechanical strength	How much the beads withstand against centrifugal force (500~15,000rpm). Number of beads broken at specific rpm was recorded to determine relative strength of each beads

- **Activity test of QQ bacteria after immobilization**

Rhodococcus can degrade AHLs as a carbon source in both sludge and wastewater. The activity of bacteria can be observed by checking soluble COD at a regular interval of time. If the immobilization method used in this research is effective, then soluble COD should be decreasing by the activity of living cells entrapped in the beads. 10% QQ-CEBs prepared at 30°C cross-linking solutions and 10% QQ-CEBs prepared at 40°C cross-linking solutions were introduced into each batch reactor 500mL of synthetic wastewater (approximately 250mg/L COD). They were aerated at an air flow rate of 1L/mim. COD was then observed after 24, 48, 72 and 96 hours.

- **Scanning Electron Microscopy**

Before taking SEM images, to improve the quality of the images, different beads were dehydrated by freeze dryer

(Lyophilization machine, BIOBASE to remove moisture content from the beads. Vacant PVA-alginate beads and QQ-CEBs at 30°C cross-linking solution and 40°C cross-linking solution were placed in the freezer (4°C) 48 hours and then placed separately in lyophilization machine for 8 hours. Beads that retained their size and shape were then examined using NOVA Nano Scanning Electron Microscope 450 of Department of Physics, Lahore University of Management and Sciences. Immobilization of *Rhodococcus* cells inside PVA-alginate beads was visually observed.

- **An additional test of the quality of QQ-CEBs (structure restoration test)**

This test was done to check if QQ-CEBs could restore their original shape and size in distilled water after being dried. For this purpose, 10 beads were placed on dry surface for 24 hours. After they became dry and shrunk, they were dipped in distilled water to check if their original structures are recovered.

Performance of QQ-CEBs in MBR

The best QQ-CEBs were introduced in a lab-scale MBR and their performance was monitored. Basic operational conditions of MBR were kept consistent throughout operations as shown in Table 4. Each operation's performance was evaluated based on length of operational duration until MBR gets fouled by monitoring transmembrane pressure (TMP) profile. TMP was monitored by data logging manometer (Sper Scientific, Model# 840099). Removal efficiencies of BOD, COD and ammonia were determined by comparing influent and effluent permeate. Analyses were conducted according to Standard Method for the Examination of Water and Wastewater 22nd Edition.

Results and discussions

Evaluation results of different PVA-alginate beads

- **Melting and mixing property of PVA-alginate solution**

Because previous studies used PVA of Wako pure chemical (p.d.=2,000, [8] and Kuraray Co. (p.d.=1,750, [6]) to make good quality of beads, PVA #1 of KANTO (p.d.=2,000) was expected to be the best to make good quality beads. But various concentrations of from 0.5% to 10% with 1% SA #1 were not mixed at all. It was challenging to make it homogeneous when heated by autoclave and by a dry oven. So, beads couldn't be made from PVA #1 when tried to melt and mix by autoclave or dry oven. It was possible to melt and mix the PVA-alginate mixture to be homogeneous by heating and stirring it on the hotplate-stirrer. But the other PVAs (#2 p.d.=1,500, #3 p.d.=1,640, #4 p.d.=2,270) with 1% SA #1 were melted and mixed well homogeneously by any three methods (by autoclave, dry oven or hotplate-stirrer).

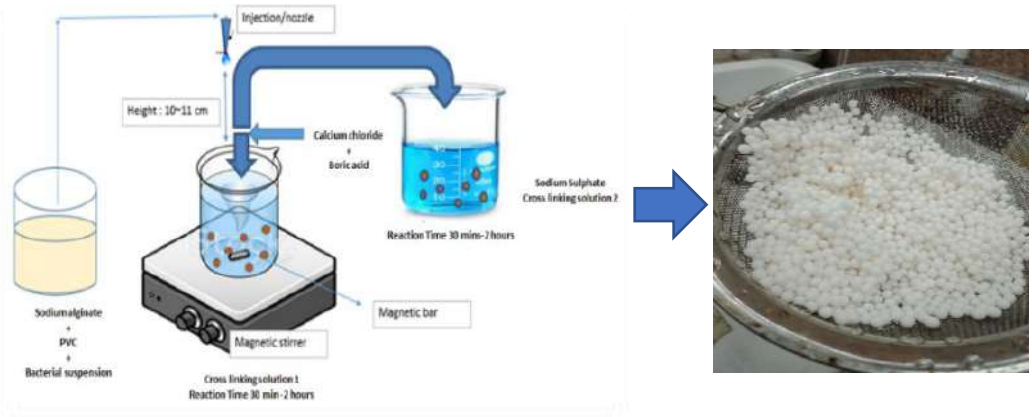


Figure 2 CEB preparation by a dripping method

Table 5 Basic operational conditions of MBR and membrane information

MBR information		Membrane information	
Type of MBR	Single-stage, submerged	**Manufacturer**	PHILOS Korea
Working volume	4L	**Membrane material**	Hydrophilic PVDF
Permeate flow rate	13mL/min	**Supporting material**	Polyester
Backwash mode	1min after 10min filtration	**Inner / outer diameter**	1.0 / 2.3mm
HRT	5.1hr	**Pore size**	0.1µm
SRT	20~30 days	**Module design**	U-shape
MLSS	8,000mg/L	**Effective length**	50cm/fibre
Flux	27LMH	**Effective surface area**	289cm^2/reactor

So, it was found that the melting and mixing property of PVA-alginate mixture is not dependent on polymerization degree but another factor, e.g., the perhaps different chemical-manufacturing process of PVAs of different brands. Therefore, the easiest way to make a homogeneous PVA-alginate solution, i.e., using a dry oven, was selected to prepare PVA-alginate solution for further process.

- **Bead formation**

When homogenized PVA-alginate solution prepared from PVA #1 (heated on hotplate-stirrer) was dropped into 1st cross-linking solution, spherical beads were formed well. But when the formed beads were dipped into the 2nd cross-linking solution, the beads were dissolved back. PVA was leaking from the beads and lost their shapes. It was considered because cross-linking of PVA with boric acid was weak. So dipping time was increased from 30min to 2hr, 4hr, 6hr, 8hr and 24hr. It was found that a longer dipping time in 1st cross-linking solution increased the physical strength of beads. But the beads formed through 1st cross-linking started leaking of PVA immediately from the beads and got dissolved in the 2nd cross-linking solution. Increasing the dipping time of beads in the 1st cross-linking solution did not prevent the dissolution of beads in the 2nd cross-linking solution. The molarity of 2nd cross-linking solution was also changed from 0.5M to 0.25M and 1M, but it was useless to prevent the problem. So PVA #1 was considered to be "not suitable" for making PVA-alginate beads. Similarly, when well homogenized 10% PVA #2 and 1% SA #1 mixture was dropped into the 1st cross-linking solution, beads were formed well, but they become softer and softer in the 2nd cross-linking solution. The beads were slowly dissolved back. When 10% PVA #3 and 1% SA mixture was used, stable beads were formed through 1st and 2nd cross-linking. However, they were dissolved back in distilled water. Cross-linking of PVA #3 with sodium sulfate was not strong enough. When PVA #4 (5~10%) was used with 1% SA #1, beads were formed well through 1st and 2nd cross-linking. They were not dissolved back in distilled water after bead formation through 1st and 2nd cross-linking. So PVA #4 was considered better than other PVAs.

- **Agglomeration**

When PVA-alginate solution with 10% PVA #4 and 1% SA #1 was prepared by heating at 60°C in dry oven, the solution was too sticky and agglomeration happened. In all the other combinations with SA #1, agglomeration was prevented. When SA #2 (instead of SA #1) was used with PVA #4 to check if there is any effect of quality of different brands of SA, beads were formed in the 1st cross-linking solution. But when they were rinsed with distilled water before 2nd cross-linking, agglomeration happened. So, SA #2 was considered "not suitable" for making PVA-alginate beads as it did not prevent agglomeration.

- **Physical appearance**

It was observed that PVA of high polymerization degree (PVA #4, p.d.=2,270) becomes too sticky to form the spherical shape of drops through the nozzle when the concentration is greater than 9% no matter it was melted and mixed at 60°C or 105°C. So, when 10% of PVA #4 and 1% of SA #1 was used, regardless of the other condition (melting temperature, cross-linking time), the shape of beads was irregular or oval or tailed. When 8~9% of PVA #4 and 1% of SA #1 were used, relatively strong and spherical shape beads were formed without leakage of PVA (dissolving of beads) agglomeration. The shape of the beads made of PVA #1, #2 and #3 was also spherical but they had a problem of leakage of PVA in 2nd cross-linking solution or in distilled water after 2nd cross-linking. So 8~9% of PVA #4 and 1% of SA #1 was considered better combination than others.

- **Swelling of the beads**

When mixture of 5~8% of PVA #4 and 1% of SA #1 was heated at 60°C in dry oven to melt and mix them, it was observed that spherical beads were formed without problem of agglomeration and dissolving of beads, however, the beads got swollen in the distilled water. When mixture of 8~9% of PVA #4 and 1% of SA #1 was heated at 105°C, spherical beads were formed without problem of agglomeration, dissolving of beads as well as swelling. So, heating the PVA-alginate mixture at 105°C in dry oven was considered better than other melting/mixing methods.

- **Mechanical strength**

Beads prepared from PVA of polymerization degree 1,500, 1,640 and 2,000 showed fragile mechanical strength. It was found that these beads maintain their shape and structure to the speed of 1,000~2,500rpm. Beyond this speed, beads started rupturing. On the other hand, beads prepared from PVA #4 (p.d.=2,270) showed excellent mechanical strength. Even at the speed of 15,000rpm, they did not rupture and maintained their structure. The temperature of 1st cross-linking solution did not have any effect on the mechanical strength of beads. PVA beads prepared at 30°C and 40°C showed the same strength.

- **Effect of cross-linking time**

The physical structure of beads was checked by dipping them in cross-linking solutions for varying time periods. Only PVA found stable in both the first and second cross-linking solution was PVA #4 (p.d.=2,270). The first cross-linking solution has boric acid and can damage cells' viability if its exposure is increased. So, if all the other beads' properties maintain well enough, a shorter cross-linking time is better to avoid possible damage on the living cell entrapped in the QQ-CEBs. When 30min~2hr of dipping time was applied for 1st cross-linking, spherical beads were formed well. Agglomeration did not happen regardless of the length of the dipping time. The same was observed for 2nd cross-linking. Dipping in the 2nd cross-linking solution for 2~8hr did not show the difference in the quality of beads. When the proper concentration of PVA and SA of specific brands was used, which can form regular shape of spherical beads, shorter cross-linking time than previous studies did not degrade the quality of beads. When the improper concentration of PVA and SA of specific brands was used, longer cross-linking time than previous studies did not improve the quality of beads. Any relationship between cross-linking time and the beads' quality was not observed under the range of time (0.5~2hr for 1st cross-linking, 2~8hr for 2nd cross-linking) applied in this study. So, shorter cross-

linking times, i.e., 30min and 2 hours, were selected as the best for 1st and 2nd cross-linking, respectively.

- **Effect of cross-linking temperature on the internal structure of beads**

Increasing the temperature of 1st cross-linking temperature from room temperature (20~25°C) to 40°C to be similar to the temperature of the PVA-alginate mixture solution showed a significant effect on the internal structure of beads. The beads prepared at room temperature had a hollow space in the beads and they got shrunk when placed in a lyophilization machine. However, the beads prepared at 40°C of 1st cross-linking solution do not have a hollow space in the beads and maintained their shape and size throughout the whole freeze-drying process. The hollow space in the beads looking like entrapped air bubble was thought to be generated when the warm PVA-alginate mixture solution dropped in the cool cross-linking solution. While the warm solution got solidified by cross-linking from the drop's surface, cooling caused negative pressure to generate air bubble inside the bead. The warm cross-linking solution might also have a positive effect on the stronger linkage of PVA. So, maintaining the 1st cross-linking solution at 40°C while cross-linking (to be the same with a temperature of PVA-alginate mixture solution) is considered better than using room temperature (i.e. cooler than temperature of PVA-alginate mixture solution).

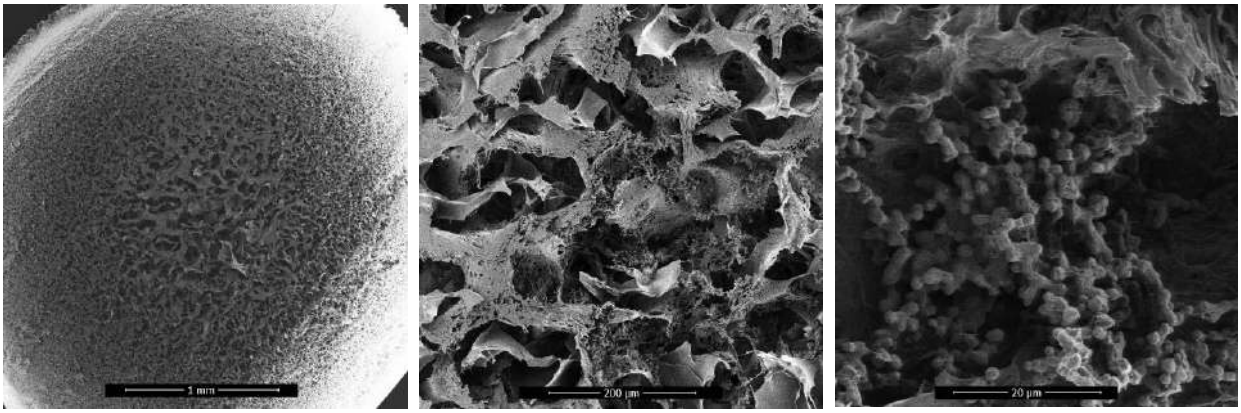
- **The best condition to make PVA-alginate beads**

It was found that the best conditions for making PVA beads were 1% Sodium Alginate #1 of VWR Chemical with 8% PVA #4 (p.d.=2,270), mixed at 105°C melting temperature in dry oven, dipped in first cross-linking solution (Calcium Chloride and Boric Acid) for 30 minutes at 40°C and in second cross-linking solution (Sodium Sulfate) for 2 hours at room temperature. The finding was summarized in Table 6.

Figure 3: Irregularly shaped, tailed, agglomerated and swollen beads

Table 6 Summary of the problem of each condition of PVA-alginate beads making methods

Variables	Description
Polyvinyl alcohol	• PVA #1: Melting/Mixing is difficult. It can be melted and mixed by hotplate stirrer but not easy and has a problem of bead formation in 2nd cross-linking
	• PVA #2, #3: Meting/mixing is easy (by dry oven) but has a problem of bead formation after 2nd cross-linking in distilled water (leakage of PVA from the bead, dissolving back in the water)
	• PVA #4: Meting/mixing is easy (by dry oven), with no leakage of PVA from the bead • ≤6%: good spherical beads are formed but low mechanical strength • 8%: good mechanical strength, good spherical beads are formed • ≥10%: good mechanical strength, but irregularly shaped (tailed, oval)
Sodium alginate	• SA #1: Agglomeration is prevented at 1% • SA #2: Agglomeration is not prevented at 1~2%
1st cross-linking temperature	• Room temperature (colder than PVA-alginate mixture solution): shrunk during freeze-dry process, hollow space in the bead • 40°C (the same temp as that of PVA-alginate mixture solution): better inner structure of the bead, no shape change during freeze-dry process, no hollow space in the bead
Cross-linking time	• No significant effect on bead quality when proper concentration and brand of PVA and SA are used • Shorter cross-linking (0.5hr and 2hr for 1st and 2nd cross-linking, respectively) is recommended



a) cross section of bead (x52) b) internal structure (x250) c) immobilized bacteria (x2500)

Figure 4 SEM Images of the beads

a) Original b) After drying c) After dipping in water

Figure 5 Structural restoration property of QQ-CEBs

Table 7 Comparison of operational duration and effluent production

Operation name	Operational conditions [a]	Operational duration (hr)	Effluent production (L)	Improvement (%)
A	No bead (control group)	153	87	-
B	1% [b] vacant beads	532	302	250
C	1% [b] QQ-CEBs	887	503	480

a: All operations were with standard backwash

b: Filling ratio of the volume of beads to the working volume of the reactor

Evaluation results of QQ-CEBs

- **Activity test of *Rhodococcus* sp. BH4 after immobilization**

The activity test showed that CEBs prepared at room temperature and 40°C cross-linking solutions degraded soluble COD of synthetic wastewater without a significant difference between the two groups. The speed of degradation was high during the first 48 hours and became slow over time. Increased temperature of the 1st cross-linking solution (40°C) than room temperature to improve the physical structure of bead did not negatively affect Rhodococcus sp activity. BH4 entrapped in the bead. These results showed that the immobilization method of QQ bacteria in PVA-alginate beads is good enough to make QQ-CEBs degrade the AHL signal molecule in the MBR.

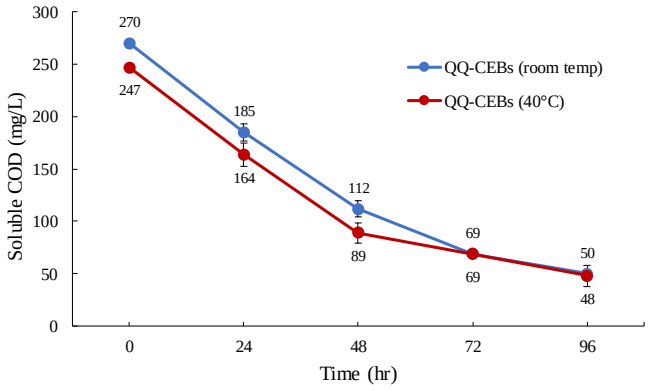


Figure 6 Degradation of soluble COD by QQ-CEBs

- **Scanning Electron Microscopy images**

PVA beads prepared at 40°C cross-linking solutions maintained their structure during the freeze-drying process. QQ-CEBs were cut into two halves, and snapshots were taken. SEM images clear showed rod-shaped *Rhodococcus* inside beads confirming the successful immobilization method.

- **Structural restoration test**

QQ-CEBs showed the ability to regain their size and shape. After they were dried and shrunk, they restored their original size and shape when they were dipped again in distilled water. This property of PVA beads is helpful as they can be preserved for a longer time.

Performance of QQ-CEBs in MBR

QQ-CEBs showed excellent performance in delaying the biofouling of membranes in the lab-scale MBR.

Transmembrane pressure (TMP) profiles of Figure 7 showed that the operational duration was extended by 379 hours (250%) with the introduction of vacant beads in MBR and by 734 hours (480%) with QQ-CEBs when compared to that of no bead operation (control). The extension of operational duration (532-153=379 hours) was contributed to the physical cleaning effect of the beads against biofilm on the surface of the membrane. The extra extension (887-532=355 hours) was contributed to the Quorum Quenching effect of QQ-CEBs in MBR. The combined effect of physical cleaning and QQ on MBR performance was a 734 hours extension of operational duration (480% improvement).

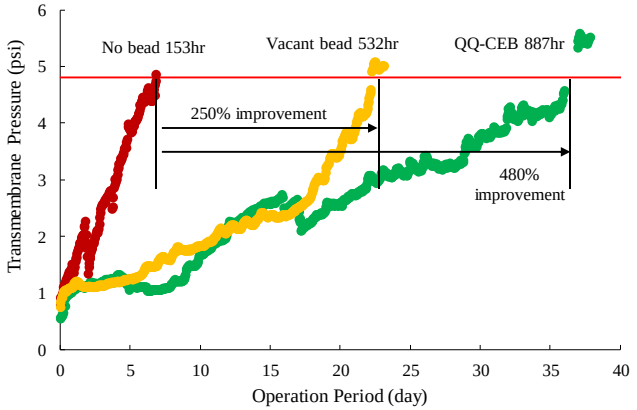


Figure 7 TMP profiles of each MBR operations

Water quality parameters analyses (BOD, COD and ammonia) showed greater than 90% removal efficiencies.

Conclusion

Excellent quality of PVA-alginate beads was made from a locally available PVA with a polymerization degree of 2,270. The ideal concentration for making PVA-alginate beads was found to be 1% SA and 8% PVA. It was also found that the stability of PVA-alginate beads is related to polymerization degree and a specific brand. This was evident as various other brand concentrations of PVAs did not make good quality beads. Sodium alginate of a specific brand was suitable for making PVA-alginate bead as it prevented agglomeration even at low concentration while the other brand did not. It was found that maintaining the temperature of the 1st cross-linking solution at 40°C to be similar to the temperature of the PVA-alginate mixture solution had a positive effect on the inner structure of beads. This increase in temperature did not harm the viability of QQ bacteria. SEM images confirmed the immobilization of *Rhodococcus* sp.BH4 inside beads. It was also found that QQ-CEBs (QQ bacteria entrapped PVA-alginate beads) showed great potential in improving the performance of MBR by the combined effect of biological and physical cleaning. Operation with 1% real volume QQ-CEBs and periodic standard backwash at MLSS 8000mg/L showed longer operational duration and more effluent production (480% improvement compared to control operation). Water quality parameter (BOD, COD and ammonia) analyses showed that QQ by *Rhodococcus* did not have any negative effect on the quality of effluent.

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