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Quorum quenching bacteria encapsulated in PAC-PVA beads for enhanced membrane antifouling properties

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ABSTRACT

In order to improve the antifouling properties of quorum quenching (QQ) bacteria immobilized beads, the mechanical strength and permeability of QQ beads were modified by adding powdered activated carbon (PAC) based on traditional polyvinyl alcohol (PVA)-boric acid method. Optimal PAC concentration was investigated through measuring the mechanical strength, permeability and *N*-octanoyl-DLhomoserine lactone (C8-HSL) removal ratio of the PAC-PVA beads. Particularly, the enhanced antifouling effects of the optimal PAC-PVA beads were compared with those of original QQ beads through a membrane filtration experiment under constant pressure. The optimal concentration of PAC was 1% (w/v), under that PAC concentration, the mechanical strength, permeability and removal ratio of C8-HSL increased by 11.3%, 29.3% and 12.4% respectively. Synergistic effect between adsorption and biodegradation of 1% PAC-PVA beads was also observed. In membrane filtration experiment, membrane permeability with the 1% PAC-QQ beads decreased to 55.4% after 14 days, while the membrane permeability with 0% PAC-QQ beads decreased to 39.9%. The addition of PAC (1%) increased the antifouling efficiency of the QQ beads 15.5%. This paper demonstrated PAC-PVA bead as a QQ bacteria immobilized method had a great potential for biofouling control in membrane bioreactors (MBRs).

1. Introduction

Membrane bioreactors (MBRs) have been widely used in commercial applications for several decades. However, biofouling in membrane separation process is still the main obstacle that handle its continued development [1–5]. Membrane biofouling caused by the deposition of mixed liquor suspended solids (MLSS) and biofilm growth [6,7] can reduce the membrane performance, increase the cost of operation and maintenance and even cause the irreversible damage of the membrane [8–10].

There are many physical and chemical methods to mitigate membrane biofouling, such as using additives [11], changing the internal structure of MBR [12], optimizing operating conditions [13] and chemical cleaning [14]. Although the traditional measures have played a certain effect in membrane biofouling control, the problem of biofilm re-formation on membrane surface still existed.

Recently, a novel concept of membrane biofouling control based on quorum sensing (QS) has been studied [15,16]. QS is a bacterial density-dependent cell-to-cell communication, in which some particular signaling molecules are produced and identified by bacteria [17–20]. QS has been proved to regulate the bacterial gene expression and stimulate some microbial behaviors, for instance, the regulation of bioluminescence, synthesis of antibiotics, production of soluble microbial products (SMP), extracellular polymeric substances (EPS) and biofilm formation [21–24]. We can disrupt the QS process by controlling the signal molecules such as *N*-acylhomoserine lactone (AHL), namely quorum quenching, so as to prevent or mitigate membrane biofouling at molecular level. In general, the feasible QQ methods are carried out through AHL-acylase or QQ bacteria, which is either directly purchased [25–27] or separated from a wastewater treatment plant [28]. However, some practical issues such as high cost with enzyme extraction and purification as well as enzyme instability are yet to be solved [29]. On the contrary, QQ bacteria, as a feasible and economical technology, has received extensive attention. Cheong et al. isolated an indigenous QQ bacteria, *Pseudomonas* sp. 1A1, using *N*-hexanoyl-DL-homoserine lactone (C6-HSL) as the sole carbon source for

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membrane biofouling control in MBR [30]. Kim et al. isolated diverse AHL-degrading bacteria from the sludge of real wastewater treatment plants such as *Microbacterium* sp. and *Rhodococcus* sp. Strains [31].

Even though the bacteria with high QQ activity have been obtained in some ways, their directly application in wastewater treatment is not appropriate because they have to compete with other microorganisms and are also affected by the adverse surroundings, such as toxic substances in the mixture [32–35]. Therefore, cells immobilization is necessary to solve these problems. For many years, a variety of cells immobilization techniques have been developed rapidly. One of the most wide and basic method is cells entrapment, where the living cells are enclosed in a matrix which is porous enough to allow the diffusion of substrates to the cells and of metabolites away from the cells [36]. Various natural polymers (gelatin, agarose, alginate, carrageenan and pectate) and synthetic polymers (polyurethane, polyacrylamide and poly (ethylene glycol) prepolymer) have been used for cell immobilization [37–41]. However, each polymer has its shortcomings such as poor mechanical strength, biodegradability, microbial toxicity, poor durability and high cost [42,43], while the synthetic polymer polyvinyl alcohol (PVA) shows a non-toxic property to microorganism, good mechanical strength and low cost [38], especially for high durability in water, making it have great potential in the field of water treatment as cell immobilization material [43,44].

In this study, for more efficient antifouling properties of QQ beads, a modified matrix, polyvinyl alcohol (PVA) gel added with powdered activated carbon (PAC), was crosslinked with boric acid and sodium sulfate successively to immobilize the QQ bacteria (*Rhodococcus* sp. BH4). The PAC effects on mechanical strength and permeability of immobilized QQ beads were studied. The optimal PAC concentration and the synergism between biodegradation and adsorption of QQ beads were investigated. Particularly, with the addition of PAC at an optimal concentration, the PAC effects on the antifouling efficiency of immobilized QQ beads were explored through a membrane filtration system.

2. Material and methods

2.1. Materials and microorganism

Polyving akohol 124 (PVA), sodium alginate (SA), powdered activated carbon (PAC), boric acid, sodium sulfate, calcium chloride and crystal violet were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *N*-octanoyl-DLhomoserine lactone (C8-HSL) was purchased from Sigma-Aldrich, US. *Rhodococcus* sp. BH4 (QQ bacteria) and *Agrobacterium tumefaciens* A136 (reporter strain for AHL) were obtained from school of Chemical and Biological Engineering (Seoul National University, Republic of Korea).

2.2. Preparation of PAC-PVA beads

Rhodococcus sp. BH4 was inoculated in 400 ml Luria-Bertani medium at 30℃ for 24 h. Subsequently, the culture was centrifuged (12,000*g*, 15 min), washed with saline and resuspended in 10 mL of deionized water for further use.

9.1 g PVA and 0.9 g SA were dissolved in 90 ml deionized water. The PAC (0–1.5 g) and 10 ml QQ bacteria suspension (140 mg/ml) were added into the mixture and stirred evenly, the concentration of PVA, SA, PAC and QQ bacteria were 9.1% (w/v), 0.9% (w/v), 0–1.5% (w/v) and 1.4% (w/v). After that, the mixture of PVA, SA, PAC and *Rhodococcus* sp. BH4 was dripped into a 500 mL mixed solution of saturated boric acid and calcium chloride (4%, w/v) using a syringe to form beads and agitated for 1.5 h. Afterwards, the gel beads were further treated with 0.5 M sodium sulfate solution for another 4 h and rinsed with deionized water for three times. The PAC-PVA beads with *Rhodococcus* sp. BH4 content about 14 mg/g beads were obtained.

2.3. Measurement of mechanical strength and permeability

A beaker with a diameter of 67.4 mm and height of 107.7 mm was divided into four equal regions by baffles with width 11 mm. 2 g PAC-PVA beads were added to the beaker with 200 mL of deionized water in. The agitation speed was controlled at 1200 rpm by digital display electric blender. The beads were stirred for 48 h in the beaker and dried out in a desiccator until no more change with weight. Finally, the weight of beads was recorded. The weight ratio of residual beads to initial beads was considered as the mechanical strength of PAC-PVA beads.

The permeability of PAC-PVA beads was defined as the ratio of permeability thickness to radius of PAC-PVA beads. 50 immobilized beads morphologically intact and with the same particle size were immersed in inert red ink (Yingxiong, China) and 3 of them were taken every 2 min to cut and determined the average depth of penetration of red ink until the immobilized beads were completely permeated.

2.4. Removal efficiency of C8-HSL with PAC-PVA beads

The QQ activity of PAC-PVA beads was calculated by the removal rate of standard *N*-octanoyl-DL-homoserine lactone (C8-HSL), which has been proved one of the main signal molecules (AHL) in the MBR for wastewater treatment [16] and the relative activity of PAC-PVA beads was calculated as the ratio of residual activity to the initial activity for each bead. In detail, C8-HSL was dissolved in 50 mM Tris-HCL buffer (pH 7.0, 50 mL) to a final concentration of 200 ng/ml. 2 g of beads were added to the Tris-HCl buffer containing C8-HSL and cultured in 150 rpm at 30℃. Next, the method of Bioassay [16] was used to detect residual concentration of C8-HSL, which was based on the calibration curve acquired from the color zone sizes on indicating agar plate in accordance with each standards concentration of C8-HSL. The indicating agar plate was made of LB agar and X-gal and overnight culture of *Agrobacterium tumefaciens* A136.

2.5. Morphological observation

The PAC-PVA beads swelled in deionized water and then were sliced quickly. After that, they were freeze-dried using vacuum freeze dryer (FD5-2.5, Gold-SIM, USA) and sputter-coated with gold for observation by a scanning electron microscopy (SEM, JSM-IT300LA).

2.6. Biofilm formation on membrane filter

For the growth of biofilm on the membrane, 7 poly (vinylidene) fluoride (PVDF) membrane filters with a pore size of 0.22 μm (Xingya, China) that outlet side sealed with resin and 8 g immobilized beads were placed into a 500 ml conical flask. Then, 200 ml of activated sludge obtained from the lab-scale MBR (parameters described as Table 1) where sludge was derived from a real wastewater treatment plant (Changsha, China) was poured into the conical flask and incubated under shaking condition (130 rpm) at 30 ℃. To maintain the biofilm growth on each membrane filter, the used synthetic wastewater

Table 1
Operating conditions of the lab-scale MBR system.

Working volume	5 L
Flux	15 L/m^{2}h
HRT	16.67 h
SRT	No sludge discharge during this experiment
Membrane type	PVDF; 0.2 μm (Motimo, China)
Membrane area	0.02 m^2
MLSS	7000-7500 mg/L
Dissolved oxygen	2-4 mg/L
Influent COD	900 mg/L

PAC-QQ beads and 1% PAC-QQ beads.