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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.

was replaced with fresh synthetic water (COD 900 mg/L) for 14 days without sludge discharge. Every two days, a filter was taken out for filterability test and biomass measurement respectively. The components of fresh synthetic wastewater were as follows (mg/L): 900 mg/L Glucose, 45 mg/L Peptone, 45 mg/L Yeast extract, 450 mg/L $(NH_4)_2SO_4$, 270 mg/L KH_2PO_4, 270 mg/L K_2HPO_4, 8.1 mg/L $MgSO_4$, 3.564 mg/L $CaCl_2{\cdot}6H_2O$, 63 mg/L NaCl, 1350 mg/L $NaHCO_3$, 0.2 mg/L $FeCl_3{\cdot}6H_2O$, 0.2 mg/L $CoCl_2{\cdot}6H_2O$.

2.7. Measurement of permeability and biomass of membrane

Biofouling of membrane filter was quantitatively calculated by measuring the permeability level. To be specific, a membrane filter with seal removed was installed on a syringe, the biofilm sample was determined using the syringe with 4 mL distilled water under a constant pressure of 20 kPa. Concurrently, the time was recorded until the deionized water was completely squeezed out. The ratio of residual flux to initial flux for each membrane sample was defined as relative permeability at each time point. For biomass measurement, a membrane was taken out from the culture every other day and gently rinsed twice with deionized water to remove the media along with freely floating planktonic cells. Then, the biofilm was stained with 3 ml of 0.1% crystal violet (CV) solution for 30 min. After that, the CV solution was discarded completely and the biofilm was gently rinsed again, afterwards, it was immersed in 3 ml of 95% ethanol for 1 h to solubilize CV from the stained cells. Finally, the absorbance at OD 581 nm was measured to quantify the biofilm biomass on the membrane.

3. Results and discussion

3.1. Bacteria immobilization and characterization of PAC-PVA beads

In the preparation of PAC-PVA beads, a small amount of alginate was added to improve the surface properties of the beads so as to overcome their agglomeration trend. Moreover, in order to reduce the damage of the saturated boric acid to the activity of QQ bacteria, the crosslinking process was carried out in two steps. The beads were cross linked with the mixed solution of saturated calcium chloride for a short time firstly and then transferred to the sodium sulfate solution for further crosslinking. The concentrations of PVA and SA were 9.1% (w/v) and 0.9% (w/v) respectively. If the concentration of PVA is too high, the beads will adhere to each other. On the contrary, the elasticity of the beads will decrease and the beads will be damaged easily when the concentration of PVA is too low. Similarly, it is hard to form a sphere and the elasticity and hardness of the beads will reduced due to the SA concentration is too high. While too low concentration of SA will allow the aggregation tendency of beads. On the basis above, in order to further improve the performance of the beads such as permeability and mechanical strength, which is vital to the antifouling property of the PAV-PVA beads, the immobilized beads were modified by adding PAC. The PAC-PVA bead with a diameter of about 4 mm was obtained.

The microstructure of PAC-PVA bead was characterized by SEM. Entire exterior of the gel bead was shown as Fig. 1. The overall structure of the bead is sphere, and its outer surface is uneven with many wrinkles (Fig. 1a), which might be caused by dehydration during the freeze drying process that caused the collapse of the surface structure. In addition, it can be seen that a lot of activated carbon particles are inlaid evenly in the polymer carrier (Fig. 1b). The internal morphology of the carrier was shown as the figure Fig. 1c. An evident layered structure from the edge to the center can be seen. The outer layer is a crumpled surface, and layer near the surface is a compact reticulate structure, which plays a role in preventing the leakage of bacteria while substrates can be allowed in. Furthermore, there are many larger uplifts in the layer and the distance between the uplifts is about 40 microns. These big bulges play a key role in supporting, even under the impact and extrusion of external forces they can keep the carrier intact and not damaged. It is worth noting that there is a thin layer whose pore is larger than its adjacent layers and the width is about 60 microns (Fig. 1d). The porous structure of inner layer adjacent to the thin layer is looser than the near outer layer. The central layer, which has the most developed network structure and the largest pore size, can accommodate more quorum quenching bacteria and facilitate the penetration of substrates so as to ensure normal metabolism and reproduction of the bacteria. Magnified image (Fig. 1e) showed that a lot of QQ bacteria have distributed in the central layer, and densely attached to the reticular polymer.

3.2. PAC effects on mechanical strength and permeability of PAC-PVA beads

Mechanical performance is of importance to QQ beads in the actual application of wastewater treatment. Excellent mechanical strength is favorable for beads to resist hydraulic impact in case of bacteria leakage during long time water treatment. In consequence, the quorum quenching effect of immobilized beads can be maintained as well as the biofouling of the membrane can be mitigated. After stirring at a constant speed for a period of time, the weight of beads was recorded and the mechanical properties of the beads were shown as the Fig. 2, the percentages of weight remaining for 4 groups of beads with PAC concentration from 0 to 1.5% are 82.1%, 87.2%, 93.4% and 88.9% respectively. Based on the result, the weight remaining of the beads added with PAC is better than that without PAC. Furthermore, the mechanical strength of beads increases with the concentration of PAC firstly, as the PAC concentration reaching to 1% the best mechanical strength of the beads is obtained. However, the mechanical strength of beads decreases when the PAC concentration continues to increase to 1.5%, this may be due to the appropriate concentration of PAC can enhance the gel structure of beads and excessive concentration of PAC can lead to the swelling of the beads.

The permeability of beads was evaluated by submerging beads in red ink. As shown in the Fig. 3, the permeation rate of four groups was very slowly in the first few minutes. At the 6th minutes, the permeation ratio of the beads without PAC was lowest, which was less than 20%. It may be attributed to the contacting with the crosslinking agent directly during the preparation of beads that made the gel structure near the surface of the beads more compact and decreased its permeation rate. Nevertheless, the following three kind of beads added with PAC were better than the beads without PAC, their permeation began to accelerate at about 4th minutes and their permeation ratio was higher over time. In addition, the higher the PAC concentration was, the faster it penetrated. Finally, the beads with 1.5% PAC had the fastest permeation, which was slightly better than that of beads with 1% PAC. Both of them were completely penetrated within 15 min, and the beads added with 0.5% PAC were slower than the previous two. The beads without PAC had the slowest penetration rate that took nearly 20 min to finish the permeation process. This result suggests that the addition of PAC can help to improve the permeability of the PVA gel beads, which can make it more convenient for the entry of nutrients and the excretion of metabolites in the beads so as to ensure the normal growth and reproduction of immobilized QQ bacteria. At the same time, it can help to degrade the signal molecules in the activated sludge mixture, and maintain the effect of quench quenching.

3.3. Synergic effect of adsorption and biodegradation on C8-HSL

The removal curve of C8-HSL was shown as the Fig. 4, compared to 0% PAC-QQ beads and 1% PAC-vacant beads, 1% PAC-QQ beads showed the highest removal efficiency. It removed 90.9% of C8-HSL within 120 min, while 0% PAC-QQ beads and 1% PAC-vacant beads only removed 75.4% and 80.9% of C8-HSL respectively. Three of them had the fastest degradation rate in the first 20 min. For control beads neither PAC nor QQ bacteria, an experiment was also conducted to

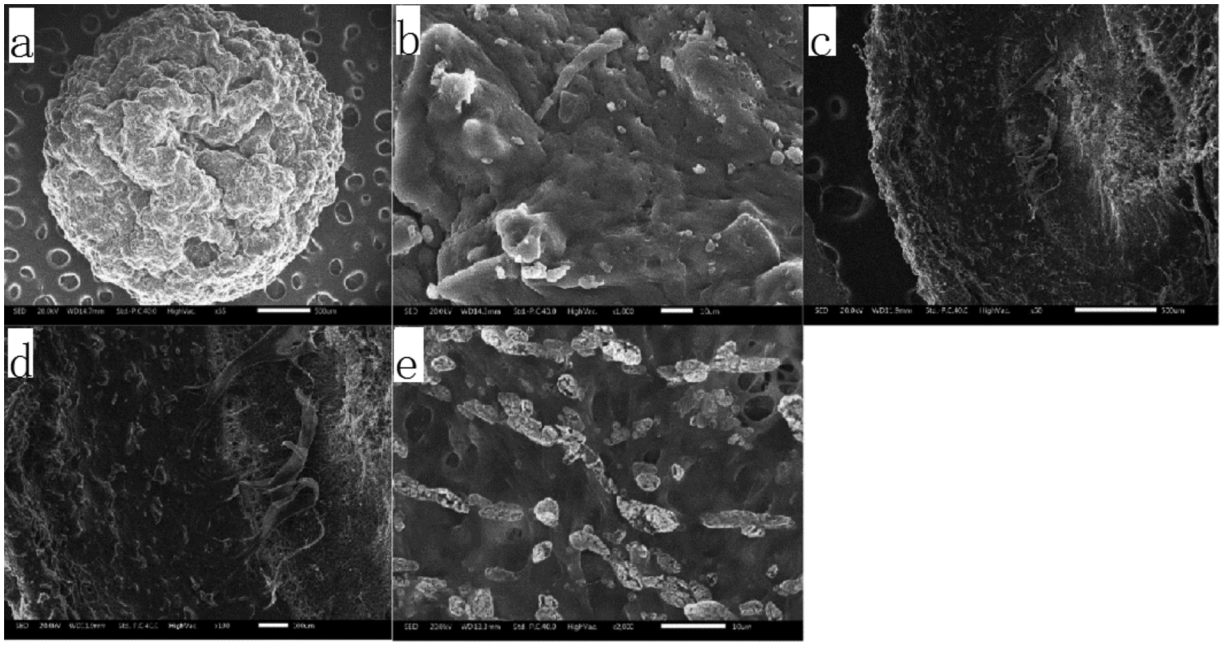

Fig. 1. SEM images of PAC-PVA beads: (a) entire exterior of gel bead with 35× of magnification; (b) peripheral surface with 1000× of magnification; (c) cross-sectional morphologies of gel bead with 50× of magnification; (d) cross-section with 100× of magnification; (e) biological attachments to reticular structure of bead with 2000× of magnification.

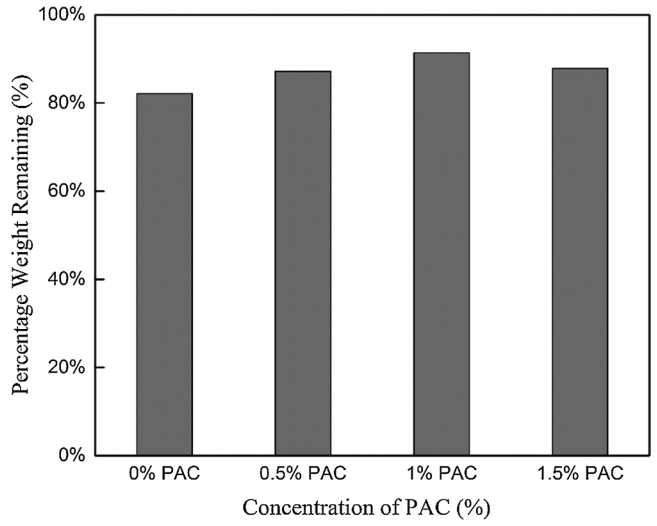

Fig. 2. Effect of PAC concentration on mechanical strength of PAC-PVA beads. The weight ratio of residual beads to initial beads after stirring was considered as the mechanical strength of PAC-PVA beads.

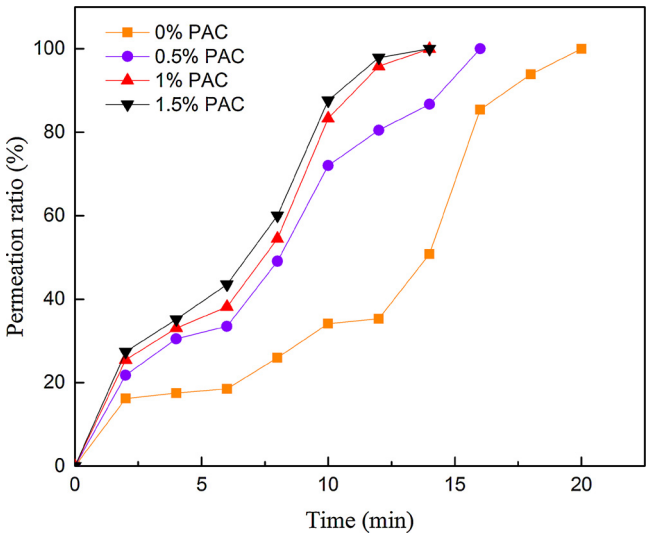

Fig. 3. Effect of PAC concentration on permeability of PAC-PVA beads. The permeability of PAC-PVA beads was expressed as permeability in ink.

check its potential removal of C8-HSL. The C8-HSL removal with control beads was only attributed to its physicochemical adsorption because they had neither quorum quenching bacteria nor quorum quenching enzyme, but the adsorption was not significant, only 12.7% of C8-HSL was removed within 120 min. The results indicated that 1% PAC-vacant beads had a good adsorption of C8-HSL in the pure solution with low concentration of C8-HSL, and its removal efficiency was even better than that of 0% PAC-QQ beads, which mainly relied on biodegradation. This might be attributed to the larger specific surface area of PAC, which strengthened the adsorption capacity of beads. The result also showed that QQ bacteria still remained excellent quorum quenching activity after immobilization by this novel method. More than that, due to the addition of PAC, the removal ability of QQ beads on C8-HSL was obviously improved, which was mainly attributed to the synergistic effect of adsorption and biodegradation.

3.4. Optimization of PAC concentration

In order to explore the optimal concentration of PAC, the immobilized beads with PAC concentration ranging from 0 to 1.5% were prepared for C8-HSL removal experiments. As shown in the Fig. 5, the beads without PAC obtained the minimum C8-HSL removal ratio 80.9% within 120 min. With concentration of PAC increasing to 1%, the C8-HSL removal ratio reached 90.9%. However, when we continued to increase the PAC concentration to 1.5%, the removal ratio only increased to 91.2%. These results indicated that increasing the concentration of PAC properly in immobilized beads was conducive to the removal of C8-HSL and the improvement of removal ratio was not obvious if the PAC concentration was more than 1%, it might be because the high concentration of PAC affected the uniform distribution of QQ bacteria in beads which influenced the QQ efficiency. From the

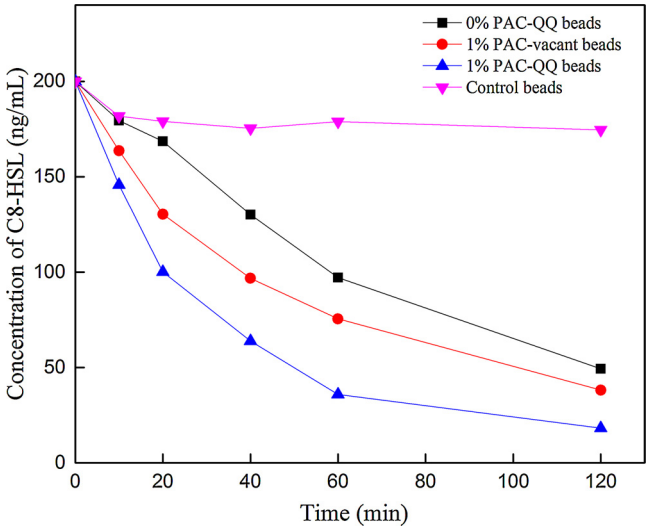

Fig. 4. Comparison of AHL (C8-HSL) removal rate among the 0% PAC-QQ beads, 1% PAC-vacant beads, 1% PAC-QQ beads and Control beads (neither PAC nor QQ bacteria).

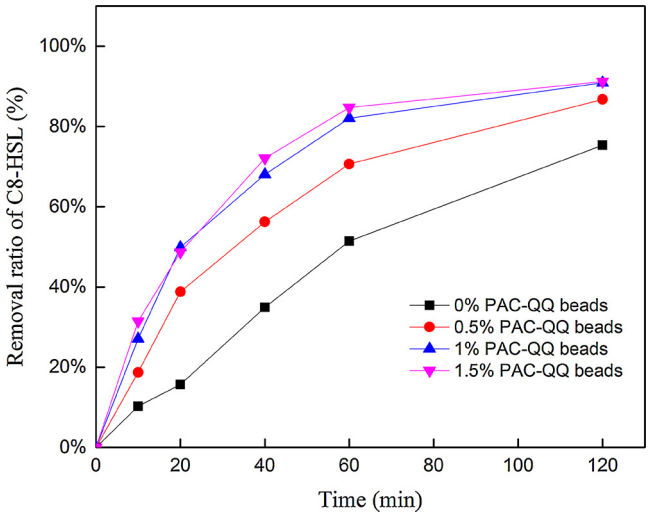

Fig. 5. Effect of PAC concentration on AHL (C8-HSL) removal rate of PAC-PVA beads.

practical point of view, reducing the use of PAC as much as possible while ensuring the removal effect of C8-HSL, at the same time, taking the mechanical and permeability of the immobilized beads into consideration, 1% PAC-PVA beads were selected as the optimum immobilized beads for the subsequent study of antifouling.

3.5. Antifouling of PAC-PVA beads in membrane filtration system

A membrane filtration system for wastewater treatment was designed to study the potential efficiency of PAC-PVA beads in antifouling. PVDF membrane, the most widely used in the commercial membrane processes, was selected as a model membrane in this membrane filtration system, because of its excellent resistance to chemicals, such as chlorine. One side of the membrane filter was sealed with resin, which made the biofilm grow only on the other side of the membrane filter. The PAC-PVA beads were added into the conical beaker with filters, and the activated sludge obtained from the lab-scale MBR device was cultured in synthetic wastewater with high concentration of organic loading. This system was designed to observe the growth of biofilms within 14 days. Every two days, a membrane filter

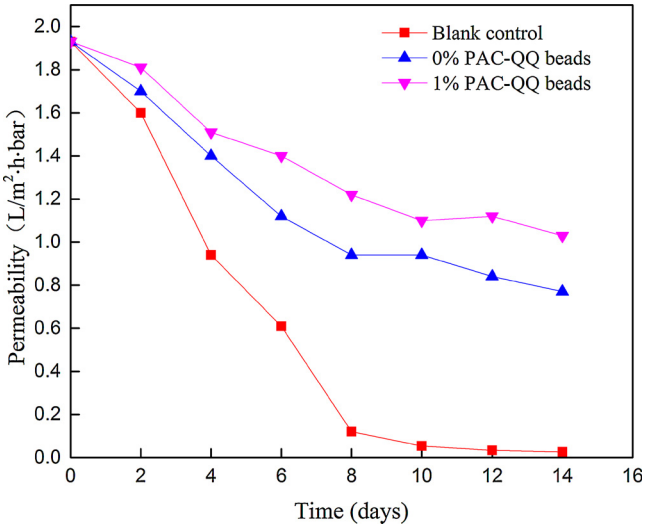

Fig. 6. Antifouling in constant pressure membrane filtration system by 0% PAC-QQ beads, 1% PAC-QQ beads and blank control (activated sludge mixture without QQ beads). The original permeability of filter with distilled water was 1.93×10^3 L/m^2·h·bar. The experimental period was 14 days without sludge discharge.

was taken out for membrane permeability and membrane biomass tests.

Membrane permeability was measured by a self-made dead-end filtration system under a constant pressure of 20 kPa. The dead-end filter system was composed of constant weight fixed on the syringe. The filter was mounted on a syringe loaded with 4 ml water and then began to filter under the constant weight, namely constant pressure. Permeability was related to the degree of membrane biofouling and the initial membrane flux with deionized water was 1.93×10^3 L/m^2·h·bar. Shown as Fig. 6, the permeability of the blank control where there was activated sludge mixture but no QQ beads decreased slowly in the first 2 days, then declined rapidly after 4 days incubation. There was no permeability observed after 10 days, indicating the membrane biofouling was very serious. Compared with the former, the membrane permeability of 1% PAC-QQ beads and 0% PAC-QQ beads declined slowly throughout the process. However, it was obvious that membrane flux with 1% PAC-QQ beads was larger than that with 0% PAC-QQ beads, after 14 days, their permeability decreased to 55.4% and 39.9% respectively, which demonstrated the antifouling performance of 1% PAC-QQ beads was better than 0% PAC-QQ beads. This might be due to the addition of PAC not only increased the adsorption capacity of beads but also improved the permeability of beads.

To determine the growth of biofilm, the membrane was taken out regularly and stained with crystal violet, the biomass on membrane was quantified by absorbance (OD 581 nm). The general trend of biofilm growth was consistent with the decrease of permeability as could be seen in Fig. 7, that growth of biofilm in the group added with 1% PAC-QQ beads was slowest during the 14 days of culture while the membrane biofilm with the 0% PAC-QQ beads grew faster than the former. For blank control (only activated sludge mixture), the biofilm grew relatively slowly in the first three days but grew pretty rapidly after that. It indicated that biofouling on membrane could not be inhibited without QQ immobilized beads, moreover, it was confirmed again that the addition of PAC promoted the antifouling efficiency of the QQ beads.

4. Conclusions

A modified matrix, PVA added with PAC, was prepared for QQ bacteria immobilization. The addition of PAC increased the mechanical strength and permeability of PVA beads significantly, which was not

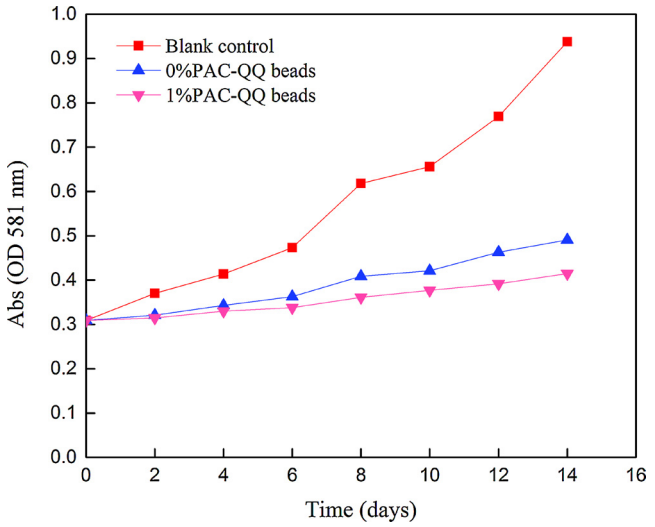

Fig. 7. Biofilm formation on the membrane surface with blank control (activated sludge mixture without QQ beads), 0%.

only conducive to prolonging their service life but also improving the efficiency of membrane antifouling in wastewater treatment. The synergism between biodegradation and adsorption was observed in the hybrid PVA-PAC beads. The optimal concentration of PAC was 1%, taking into account the C8-HSL removal efficiency of the beads and their previous research results. A filtration experiment under constant pressure demonstrated that PAC-PVA beads had a better membrane antifouling effects due to the existence of PAC, the effects including maintaining membrane permeability and inhibition of biofilm formation. As a result, PAC-PVA bead as a novel bacteria immobilized material, has superior application prospects for antifouling in the field of membrane filtration systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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