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Study on Preparation and Properties of PVA-SA-PHB-AC Composite Carrier for Microorganism Immobilization

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ABSTRACT: Polyvinyl alcohol(PVA) bead crosslinked with boric acid has been widely utilized as a microorganism immobilization carrier. However, it has some disadvantages such as drastic cell viability loss, small adsorption capacity and mass transfer limitation. To improve upon these drawbacks, a new method to prepare PVA composite pieces with the addition of activated carbon (AC) and poly-3-hydroxybutyrate(PHB) was explored through a combination of freezing/thawing and the boric acid method and by using Tween-80 to improve the mass transfer performance of hydrophobic organics. *m*-Cresol and pyrene were used as representative compounds with benzene ring structures to model hydrophilic and hydrophobic organics in order to test the performance of PVA pieces. The results showed that, compared with the boric acid method alone, a combination of freezing/thawing and the boric acid method led to a decrease in total organic carbon(TOC) loss from 0.315 g g^{-1} to 0.033 g g^{-1} and increased the oxygen uptake rate(OUR) of microorganisms from 0.03 mg L^{-1}·min^{-1} to 0.22 mg L^{-1} min^{-1}. The *m*-cresol equilibrium adsorption amount of the PVA-SA(sodium alginate)-PHB-AC piece was 2.80 times that of the PVA-SA piece. The diffusion coefficient of pyrene in the PVA-SA-PHB-AC piece increased from 0.53×10^{-9} m^2 min^{-1} to 2.30×10^{-9} m^2 min^{-1} with increasing concentrations of Tween-80 from 1000 mg L^{-1} to 5000 mg L^{-1}. The PVA-SA-PHB-AC composite carrier demonstrated great scope for immobilizing microorganisms for practical wastewater bio-treatment. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39837.

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INTRODUCTION

A considerable amount of research has been carried out in recent years on the screening, isolation, purification and acclimation of highly efficient organics-degrading bacteria. Most of these bacteria can only grow optimally under suitable ecological conditions, while their biodegradation activity may be inhibited in a practical wastewater treatment system. To reduce the inhibitory effects and maintain continuous bacteria growth, it is necessary to construct a barrier between the bacteria and practical wastewater containing multiple compounds. Microorganism immobilization has the potential of being utilized to meet this requirement. Compared with suspended biomass, microorganism immobilization has several advantages, such as higher cell concentration, the protection of cells from toxic substrates, easier solid-liquid separation, shorter lag period in biodegradation and an increase in the biodegradation rate. Microorganisms imbedded in carriers can obtain an uninterrupted supply of nutrients without competing with other microorganisms and have protection against environmental stress, bacteriophages, toxins and UV irradiation, etc.[1]

Polyvinyl alcohol (PVA), a cheap synthetic polymer, has been widely used for cell immobilization due to its high durability in water and its nontoxicity in terms of microorganisms. PVA can be crosslinked in a variety of ways, like freezing, irradiation, and boric acid treatment (Figure 1).[2,3] Among the noted PVA immobilization methods, the boric acid method and the freezing/thawing method have been used most commonly. But each method has its advantages and disadvantages. For example, a PVA carrier prepared by the freezing/thawing method has little effect on cellular activity but is not stable. Also, the boric acid

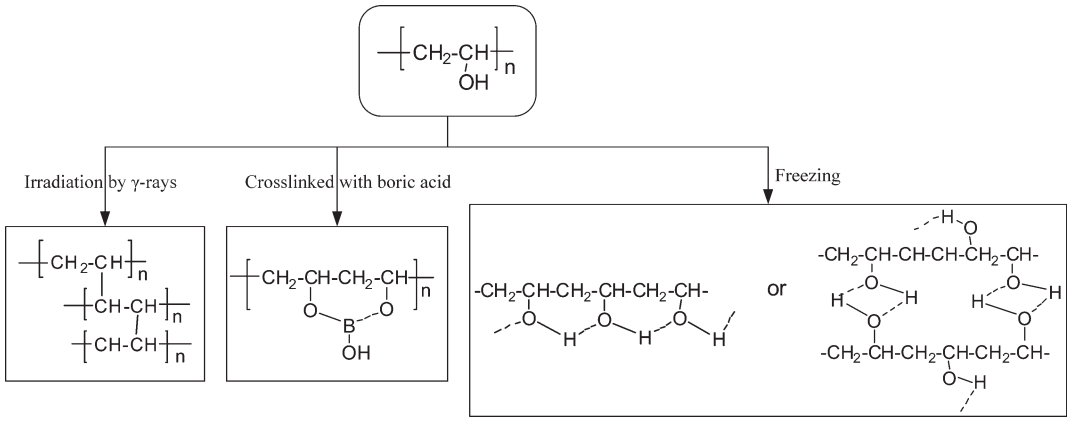

Figure 1. Cross-linking process of PVA with freezing, irradiation and boric acid treatments.

method may be used to prepare a stable carrier but causes drastic cellular activity loss.[4,5] To overcome these problems, several possible techniques have been reported on, such as treatment with sodium sulfate,[3,5,6] sodium orthophosphate,[1] or sodium nitrate[7,8] to reduce the loss in cellular activity, or using glutaraldehyde[9] and epichlorhydrin[10,11] to reduce hydration. However, little information can be found in the literature about the combination of the boric acid and the freezing/thawing methods.

In wastewater containing complicated composition, there are a lot of organic pollutants that can be degraded by microorganisms thermodynamically, but they are limited kinetically because their concentrations are lower than the thermodynamic limiting-concentration for microorganism biodegradation, i.e. the energy supplied by substrate utilization can not sustain microbial growth and metabolism. For these organics with low concentrations, adsorption is an effective pretreatment method. Activated carbon (AC) is the most popular adsorbent for the removal of dissolved organics in water. Besides this, our workgroup found that the innocuous and biodegradable poly-3-hydroxybutyrate (PHB) was a good lipophilic adsorbent for organochlorine compounds[12,13] and PAHs (polycyclic aromatic hydrocarbons).[14] Sometimes, the low concentration of organics, such as hydrophobic organic compounds (HOCs), was due to their low solubility in water, which could result in low bioavailability and limitations on biodegradation. Tween-80 is a nonionic surfactant that has been frequently used to enhance the solubility and bioavailability of HOCs.[15–19] For example, 13.1 mg L^{-1} of Tween-80 resulted in the most significant promotion of pyrene degradation with a maximal enhancement of 22.4% after an 18-day incubation period.[18]

Therefore, a new method, a combination of the freezing/thawing and boric acid methods, was developed in this study to prepare PVA composite pieces with the addition of AC and PHB. AC and PHB were selected as adsorbents to be added into PVA carriers and provided "hiding-sites" to enrich organic pollutants at low levels in wastewater, and the microorganisms immobilized in PVA carriers were thereafter able to degrade these organics easily. Mass transfer resistance between substrates and microorganisms immobilized in PVA carriers was a major impediment to the practical application of this method. So the research was focused on Tween-80 to improve the mass transfer performance of HOCs in PVA carriers. In order to evaluate cell viability, adsorption capacity, and the diffusion coefficient (De) of the PVA carrier, *m*-cresol and its efficient biodegrading bacteria, *Lysinibacillus cresolivorans*,[20] which has been isolated from aerobic sludge of a coking wastewater treatment plant in our laboratory, were used in this study. Additionally, pyrene was selected as a representative PAHs to model HOCs, and its solubility in water and log K_{ow} were 0.135 mg L^{-1}(25°C) and 4.88, respectively.

The purposes of the present study were to ① prepare a PVA-SA (sodium alginate)-PHB-AC piece that has good stability, little decrease in cell viability loss and an adsorption-enrichment effect in terms of objective pollutants through the combination of the freezing/thawing and boric acid methods and ② improve the mass transfer performance of HOCs in a PVA-SA-PHB-AC piece using surfactant Tween-80, which is able to enhance the solubility of HOCs. The results attempt to provide a greater scientific basis for the application of pure cultural microorganisms in practical wastewater treatment.

EXPERIMENTAL

Materials

PVA (average MW 4,441) with a grade of 99% saponification and degree of polymerization of 2000 was purchased from Aladdin Reagent Limited Company (Shanghai, China) and sodium alginate (aqueous solution at 20°C with a viscosity of 1.05–1.15 Pa S) was purchased from Fuchen Chemical Reagent Factory (Tianjin, China). PHB was purchased from the Northern Foods Limited Company (Tianjin, China). PHB was technical grade and was screened through a 20 meshes sieve before use. AC was produced by Xinhua Activated Carbon Limited Company (Taiyuan, China). All other chemicals were of analytical grade and purchased from qualified companies.

Preparation of the pyrene solution: Excess pyrene was dissolved by ultrasonic waves for 1 h in surfactant Tween-80 at a specific concentration. Then, a centrifuge was used (10,000 rpm for 15 min) for the mixing process and supernatant was taken for later use.

Preparation of PVA Carriers

PVA hydrogel carriers were prepared following the methods depicted in Figure 2. Totally, 10 g PVA, 2 g SA (eliminates the

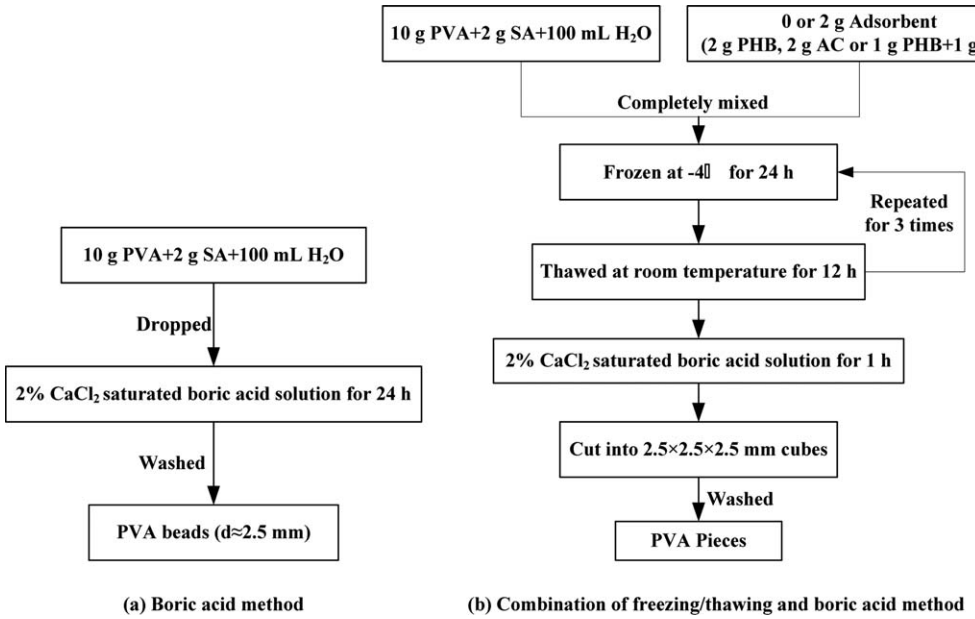

Figure 2. Flowchart of PVA carrier preparations.

agglomeration problem of PVA hydrogel[21]) and 100 mL H_2O were mixed, heated, and stirred by a magnetic stirrer at 85°C for 4 h until the mixture was completely homogenized.

During the application of the boric acid method, the mixture was extruded as droplets through a needle (18 G) into a 2% (w/v) calcium chloride-saturated boric acid solution. The spherical PVA beads were formed at room temperature through gentle stirring, and the resulting beads were about 2.5 mm in diameter.

In terms of the combination of the freezing/thawing method with the boric acid method, the mixture was firstly frozen at −4°C for 24 h and then thawed at room temperature for 2 h. After three cycles of the freezing/thawing process, the resulting PVA pieces were put into a 2% (w/v) calcium chloride-saturated boric acid solution for 1 h, and then were cut into 2.5 mm × 2.5 mm × 2.5 mm cubes.

The resulting PVA beads and pieces were completely washed with a large amount of distilled water three times to remove excess boric acid.

Stability Test of PVA Carriers

The stability of PVA carriers was tested through their total organic carbon (TOC) loss in water. The procedure was as follows: the prepared PVA carriers were immersed in 1 L of distilled water and agitated at 30°C in a rotary shaker at 150 rpm, and then the water was periodically sampled over time to monitor the TOC dissolved from the carrier into the water. Lesser amounts of TOC loss in water demonstrated greater stability in terms of the carrier.

Batch Sorption Experiments

PVA pieces of about 0.6 g were equilibrated with 80 mL of *m*-cresol solution (about 150 mg L^{-1}) in a 250 mL conical flask at 30°C in a rotary shaker rotating at 150 rpm. Adsorption of *m*-cresol was measured at a given contacting time, and an adsorption amount (q, mg g^{-1}) for the PVA piece was calculated. Each experiment was conducted in triplicate with the same conditions as noted above.

Determination of D_e in PVA Pieces

Diffusion Vessel. The diffusion vessel is shown in Figure 3. It was a plexiglas vessel consisting of two 113 mL half-cells separated by a PVA piece, of which the average thickness was 2.5±0.3 mm. The openings in the half-cell walls were two identical circular holes with diameters of 6 cm, the surface of which was much smaller than that of the PVA piece to avoid any leakage of liquid between the two chambers. One of the chambers was filled with 90 mL of *m*-cresol or pyrene aqueous solution, while an equal volume of sterile distilled water was placed in the other chamber. Both of half-cells were magnetically stirred at 30°C. The diffusion of solutes through the PVA piece was monitored by periodically removing 1 mL samples from the distilled water side.

Evaluation of D_e. Provided that chamber 1 and 2 in Figure 3 were sufficiently well-stirred to avoid mass transfer resistance

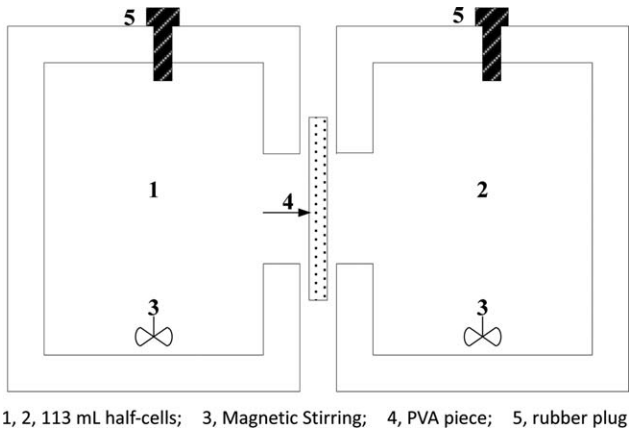

Figure 3. Sketch of diffusion vessel.

between the medium and PVA piece. The chambers were large enough to assume that the solute concentration in chamber 1 remained practically constant during the diffusion experiments, and chamber 2 was initially completely free of solute. The D_e of a solute could be obtained by plotting the total amount of solute transferred as a function of time. The amount of solute increased with time and the graph approached a straight line with the following equation:[22]

$$Q = A \times D_e \times K_P \times L^{-1} \times C_1 \times \left(t - \frac{L^2}{6D_e} \right) \quad (1)$$

where Q is the total amount of solute that has passed through a PVA piece in time t, A and L are the piece surface and thickness, respectively. D_e is the diffusion coefficient. K_P is the solute partition coefficient (very close to 1, so it is assigned to 1 in this paper), and C_1 is the initial solute concentration in chamber 1.

D_e could be calculated from the slope of the straight line along the time course of Q, i.e. $(dQ/dt)_{ss}$, corresponding to steady state (SS) and obtained by deriving eq. (1):

$$\left(\frac{dQ}{dt} \right)_{ss} = A \times D_e \times L^{-1} \times K_P \times C_1 \quad (2)$$

and

$$D_e = A^{-1} \times L \times K_P{}^{-1} \times C_1{}^{-1} \left(\frac{dQ}{dt} \right)_{ss} \quad (3)$$

Bioactivity of Microorganisms Immobilized in PVA Carriers

The bioactivity of microorganisms immobilized in PVA carriers was evaluated through measuring their OUR. *Lysinibacillus cresolivorans* immobilized in PVA carriers (10 g, wet weight) was immersed in a 100 mL culture medium supplemented with 150 mg L^{-1} *m*-cresol in a flask and aerated to make the dissolved oxygen (DO) saturated. The aeration was then ceased and the DO variation was monitored with time by using a DO meter (NeoFox, Ocean Optics, USA). At the same time, the solution was magnetically stirred at 30°C. The value of OUR was calculated according to the following equation:

$$OUR = \frac{DO_1 - DO_2}{t_2 - t_1} \quad (4)$$

Where OUR is the oxygen consumed per unit time (mg L^{-1} min^{-1}); DO_1 and DO_2 are the DO concentration at time t_1 and t_2 (mg L^{-1}); t is measuring time (min).

The culture medium used in this experiment contains the following ingredients (g L^{-1}): $(NH_4)_2SO_4$ 0.8, K_2HPO_4 1.5, KH_2PO_4 1.0, $MgCl_2 \cdot 2H_2O$ 0.2, NaCl 0.1, $MnSO_4 \cdot H_2O$ 0.03, $FeCl_3 \cdot 6H_2O$ 0.02, $CaCl_2$ 0.01. The pH value of the medium was adjusted to 7.0 before autoclaving, and then the medium was autoclaved for 20 min at 121°C and 10^5 Pa.

Characterization

PVA pieces were dried using freeze-drying equipment (VIRTIS Genesis, USA). The surface and interior structures of the prepared PVA pieces were examined using a scanning electron microscope (Carl Zeiss EVO LS10, Germany). A fully automated physical adsorption instrument (ASAP-2020N, Micromeritics, USA) was used to characterize the carrier's specific surface area and pore size. The pure water contact angle of PVA piece surface was determined using a surface contact angle analyzer (OCA15 Dataphysics, Germany) via the sessile drop technique.

Analytical Method

TOC was measured using a TOC analyzer (TOC-V, Shimadzu, Japan). Total carbon(TC) and inorganic carbon(IC) were oxidized into CO_2 by catalytic combustion method and acidifying with hydrochloric acid, respectively, then TOC=TC−IC. 150 mg·L^{-1} TC standard solution was prepared by adding 0.3187 g potassium hydrogen phthalate into 1 L ultrapure water and 150 mg L^{-1} IC standard solution was prepared by adding 0.6615 g Na_2CO_3 and 0.5250g $NaHCO_3$ into 1 L ultrapure water.

The concentrations of *m*-cresol and pyrene in aqueous phase were analyzed via HPLC system (Shimadzu LC-20AT, Japan) equipped with a Prominence SPD-20A UV/Vis Detector (detecting at 270 nm), a Prominence SIL-20A/20AC, and a Diamonsil C18 reverse-phase column (250 × 4.6 mm, 5 μm). The mobile phase involved ultrapure water and methanol with a volume ratio of 10:90, and the flow rate was 1 mL min^{-1}. The column temperature was set at 40°C. The linearly dependent coefficient (R^2) of standard curve for the determination of *m*-cresol and pyrene were 0.9994 and 0.9993, respectively.

RESULTS AND DISCUSSION

Comparison of Stability and Cell Viability Between PVA-SA Beads and Pieces

The PVA-SA beads were prepared with the boric acid method and the PVA-SA pieces were prepared with a combination of the freezing/thawing and boric acid methods (Figure 4). PVA-SA beads were broken into pieces, while PVA-SA pieces remained intact after being shaken for several hours in water. The TOC loss in water of PVA-SA beads and pieces was 0.315 g g^{-1} and 0.033 g g^{-1} after 99 h, respectively. This phenomenon could be explained by a low crosslinking rate of PVA with boric acid. When the PVA solution was extruded into the saturated boric acid solution as droplets, the gelling reaction occurred immediately on the surface of the beads formed by the crosslinking of the hydroxyl groups of PVA with borate ions. Subsequently, the gelling reaction inside was accomplished with further diffusion of the boric acid into the beads. If the PVA didn't react with the borate ions completely, the beads would not be stable. However, freezing could make PVA, a kind of hot water soluble polymer, transform into a stable macro-porous hydrogel from outside to inside. The macro-porous hydrogel was immersed into boric acid for 1 hour to make the surface of the PVA pieces more stable. As noted above, the results showed that the water stability of the PVA-SA pieces was superior to that of the PVA-SA beads.

The OUR of microorganisms immobilized in PVA-SA beads and pieces were 0.03 and 0.22 mg L^{-1} min^{-1}, respectively, during the 15 min experimental period, which indicated that the combination of the freezing/thawing and boric acid methods could reduce cell viability loss in the immobilization process because of the short crosslinking time (1 h) with the saturated boric

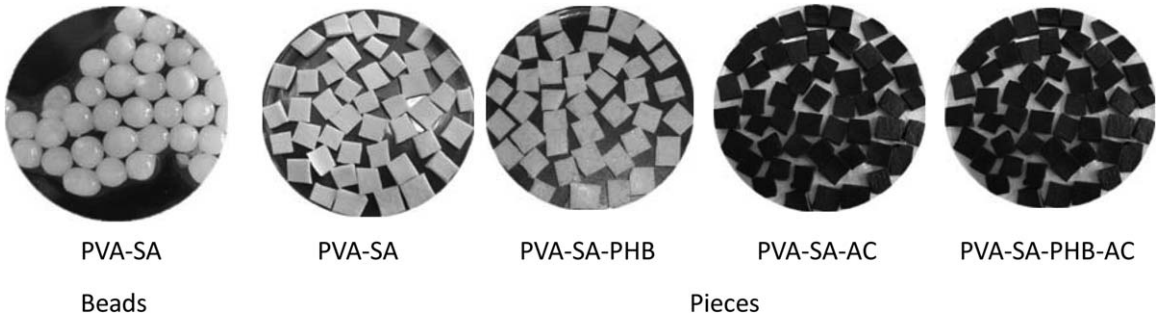

Figure 4. Pictures of PVA beads and pieces.

acid solution. The saturated boric acid solution was highly acidic (pH< 4) and caused a drastic decrease in the viability of the immobilized cells. For example, the viability of *Saccharomyces cerevisiae* in saturated boric acid solution was found to decrease to <1% after 3 h of incubation.[5] PVA beads prepared with 5% boric acid possessed at least 18% higher enzyme activity than those prepared with 7% (w/v) boric acid.[6] Both decreasing the concentration of boric acid and shortening the crosslinking time with boric acid could lessen the loss of cell viability. Moreover, during the method involving a combination of the freezing/thawing and boric acid methods, initially PVA was frozen to form a macro-porous hydrogel structure, which could protect the immobilized microorganisms from decreasing cell viability by creating a stable microenvironment. All in all, compared with the boric acid method, the application of the method involving a combination of the freezing/thawing and boric acid methods resulted in a more stable PVA carrier with higher cell viability.

Comparison of Stability, Adsorption Capacity, and Mass Transfer Performance of Different PVA Pieces

Surface Morphology and Pore Structure Analysis of PVA Pieces. Four kinds of PVA pieces were successfully prepared (Figure 4). The density of these PVA pieces ranged from 1.0141 to 1.0893 g cm^{-3}, which is close to that of water and suitable for suspended carriers in water treatment.[23]

The morphology and structure of the PVA pieces were characterized by images obtained through a scanning electron microscope (SEM) and a fully automated physical adsorption instrument. SEM images of the surface and interior parts of the PVA pieces are distributed on the left and right sides in Figure 5, respectively. It is evident that the PVA pieces (except PVA-SA-AC) exhibited porous structures. From Figure 6, it can be seen that the dominant pore sizes of the PVA pieces were all macro-pores (>50 nm), smaller than the diameter of bacteria (0.5-5 μm) and larger than the molecular diameter (order of magnitude was 10^{-10} m[24]), which allowed the objective pollutants to get into the inside of the pieces and prevented the immobilized microorganisms' exudation. It was beneficial for the growth of microorganisms and for protection against detrimental conditions when they were used as carriers. The addition of both PHB and AC into PVA-SA pieces made the surface area larger and pore size smaller, which might increase the adsorption capacity and reduce the mass transfer performance. Among the four kinds of PVA pieces, the PVA-SA piece had the greatest pore size and the smallest surface area, while the PVA-SA-AC piece had the smallest pore size and the greatest surface area (see table I). However, the PVA-SA-PHB-AC piece had uniform size distribution, the BET surface area and BJH desorption average pore diameter of which were 15.2999 $m^2\ g^{-1}$ and 33.6789 nm. Compared with the PVA-SA piece, its surface area was 5.27 $m^2\ g^{-1}$ greater and its pore diameter remained constant. It was relatively good for adsorption and mass transfer for organics.

Comparison of Stability of PVA Pieces

The stability of PVA carriers was tested through the TOC loss in water. Figure 7 shows the TOC loss of the four kinds of PVA pieces. The results revealed that the stability of the PVA pieces was in the following order from the most to the least stable: PVA-SA-AC> PVA-SA-PHB-AC > PVA-SA-PHB > PVA-SA. This phenomenon indicated that adding AC and PHB into PVA could reduce the TOC loss and increase the stability of PVA pieces in water, which can be explained by the steric hindrance of AC and PHB particles within the PVA hydro-gels.[25] AC and PHB particles could intercalate into PVA molecules and effectively block the hydrogen bonding between water and hydroxyl groups in PVA molecules.[26] A study showed that dispersion of Fe_3O_4 nanoparticles in the poly-urethane foam could slightly increase its thermal stability.[27] Additionally, the equilibrium TOC loss of PVA pieces doped with AC and PHB was about 3% (w/w) after 99 hours, and we also found that a PVA-SA-PHB-AC piece could be used stably for more than 60 days.[28]

Comparison of Adsorption Capacity and Mass Transfer Performance of PVA Pieces

Figure 8 represents the adsorption amount of *m*-cresol over time. It can be seen that the adsorption capacity of PVA pieces followed the following order from the most to the least: PVA-SA-AC>PVA-SA-PHB-AC>PVA-SA-PHB>PVA-SA, which is in accordance with the order of their specific surface areas. The *m*-cresol equilibrium adsorption amounts by PVA-SA-PHB, PVA-SA-AC and PVA-SA-PHB-AC pieces were 1.38, 5.84 and 2.80 times that of PVA-SA pieces, respectively. The increases in adsorption amount could be attributed to the increased numbers of accessible adsorption sites supplied by PHB and AC. The BET surface area of PHB and AC used in this study were 1.36 and 882.67 $m^2 \cdot g^{-1}$, respectively. PHB's ester and hydrocarbyl groups, similar to the functional groups of the lipid, had a lipophilic and hydrophobic nature and also had a good adsorption of organochlorine compounds[12,13] and PHAs.[14] The diameter of the bacteria in general is greater than the pores diameter that they

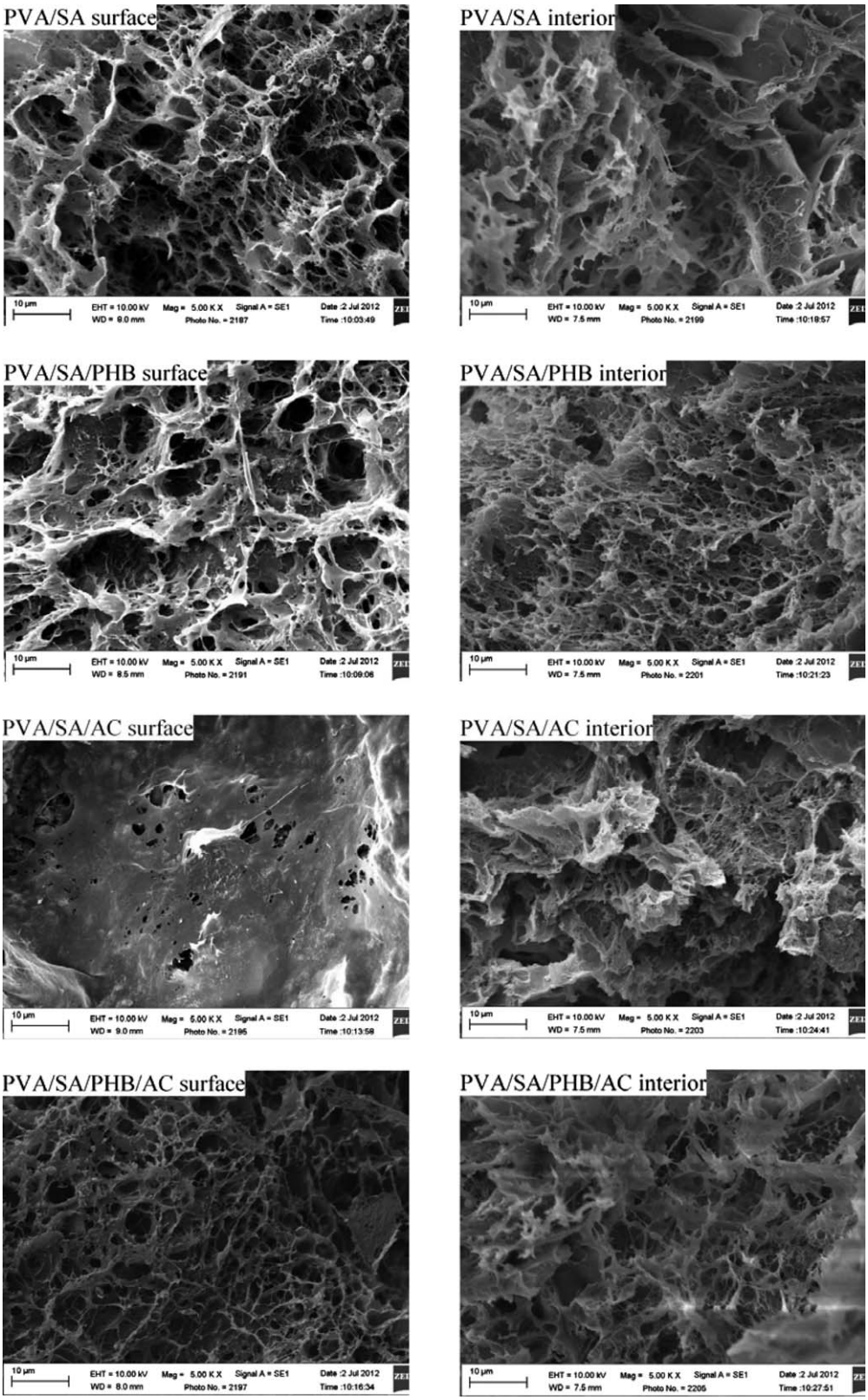

Figure 5. SEM images of PVA pieces at a magnification of 5000×.

couldn't get into the transitional pores of the PVA pieces. But the enzymes and coenzymes secreted by microorganisms was able to penetrate into the transitional pores and come into contact with the adsorbed organics, and then the organics were degraded by the immobilized microorganisms, which constitutes the synergistic effect of immobilization material and microbial degradation.

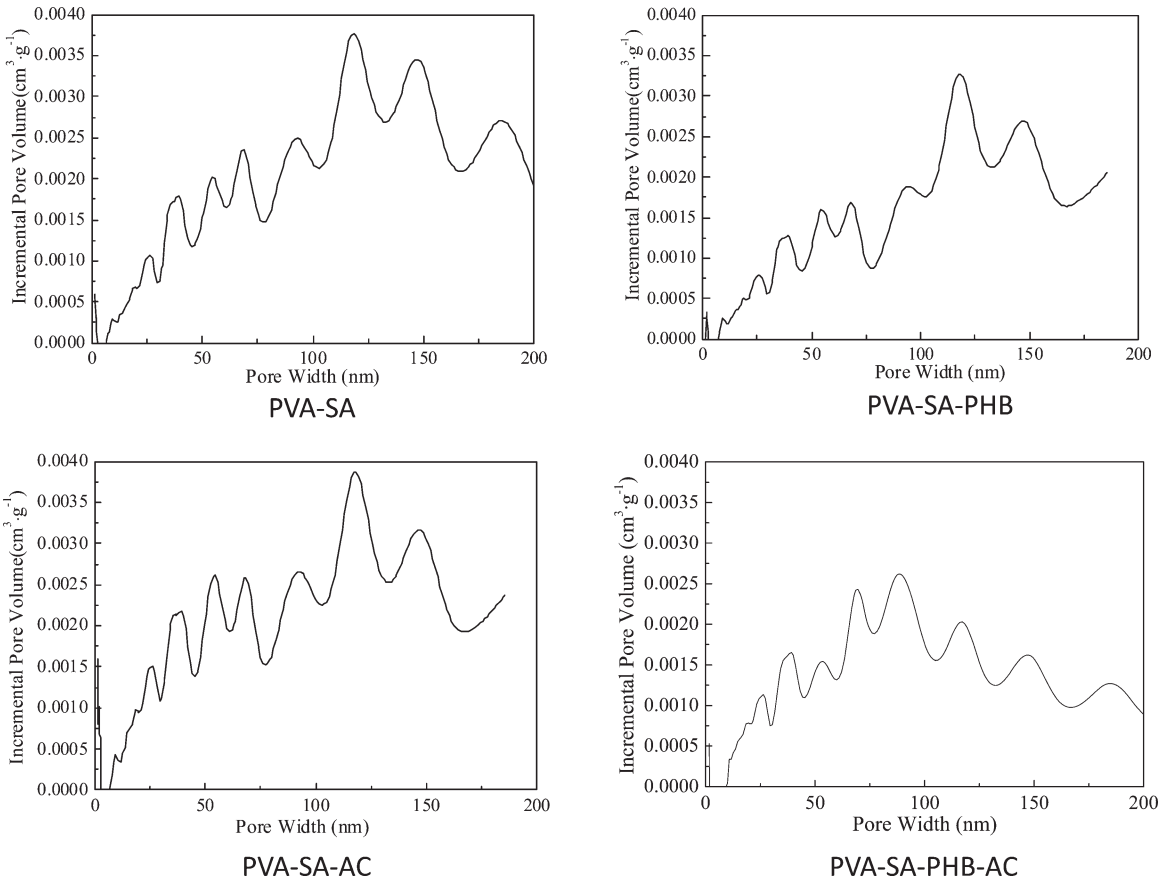

Figure 6. Pore distribution of PVA pieces.

Mass transfer performance of the PVA pieces was of critical importance for the total biodegradation efficiency of bacteria immobilized in the PVA pieces. The D_e of *m*-cresol in the PVA pieces was calculated by eq. (3). The average values of the effective D_e of *m*-cresol in the PVA-SA, PVA-SA-PHB, PVA-SA-AC, and PVA-SA-PHB-AC pieces were 6.18, 6.11, 4.58, and 5.62×10^{-8} m^2 min^{-1}, respectively. Compared with the D_e of *m*-cresol in the PVA-SA pieces, the D_e of the PVA-SA-PHB, PVA-SA-AC, and PVA-SA-PHB-AC pieces was 1.13%, 25.89%, and 9.06% lower, respectively. The lower D_e is caused by AC or PHB particles that occupied the carriers' micro-porous channels formed during the freezing/thawing preparation process and also the increased tortuosity of the gel matrix that increased the time required for the *m*-cresol to reach the internal pores. This is shown by the results noted in (a) and (b) in Figure 8. Ten hours was required to reach absorption equilibrium for the PVA-SA-AC piece, while only several minutes was needed for adsorbent AC. Additionally, because of the coverage of the accessible adsorption sites of AC by PVA in the piece, the *m*-cresol adsorption capacity of the PVA-SA-AC piece was about 10% of that of AC.

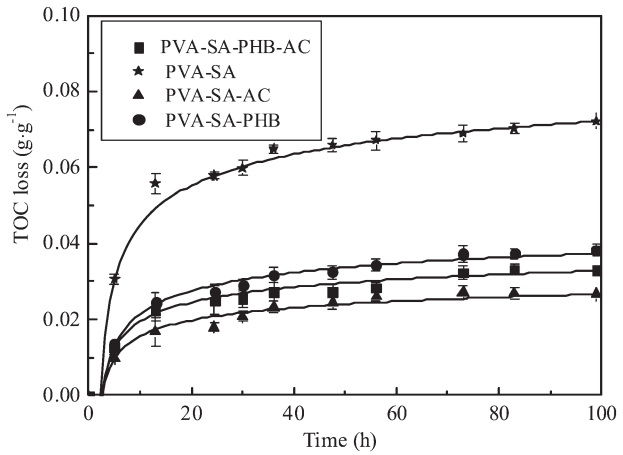

Figure 7. TOC loss of the different PVA pieces in water.

On the basis of these results, adding adsorbents (PHB and AC) into PVA gels could improve the PVA carrier's stability and adsorption capacity but present a barrier to the transportation of oxygen and substrates. Compared with PVA-SA pieces without the addition of any adsorbent, the PVA-SA-PHB-AC piece showed the best comprehensive performance, of which the *m*-cresol equilibrium adsorption amount was 2.80 times greater and the D_e was only 9.06% lower. Also, it could be used stably for more than two months.[28] A large number of studies have shown that materials with a 40°~60° surface water contact angle are helpful for the adhesion and growth of microorganisms.[29–31] The pure water contact angles on the PVA-SA and PVA-SA-PHB-AC piece surfaces were 26.6° and 37.5°, respectively, which increased with the addition of inorganic AC and hydrophobic PHB. Therefore, the PVA-SA-PHB-AC piece showed relatively

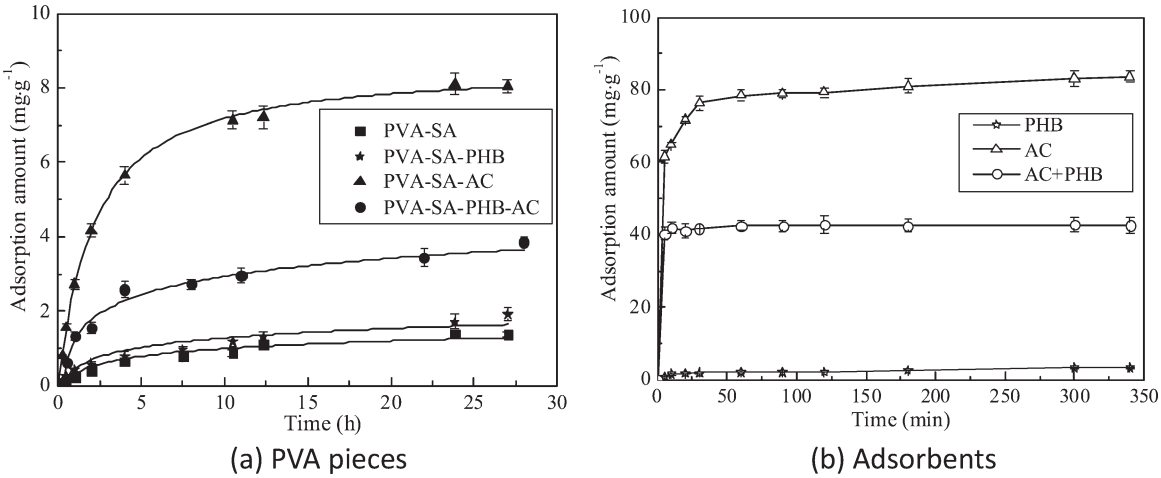

Figure 8. Comparison of *m*-cresol absorption amounts by PVA carriers and adsorbents.

suitable stability, adsorption capacity, mass transfer performance, and hydrophilicity.

Effect of Tween-80 Concentration on the D_e of Pyrene in the PVA-SA-PHB-AC Piece

Comparison Among Solubilization of Pyrene by SDS, Tween-20, and Tween-80. The bioavailability of an organic material has a strong relationship to its solubility. Surfactants have a solubilization effect on hydrophobic organic compounds. Thus, it was expected that adding surfactants into a carrier during its preparation process could improve the solubility and diffusion rate of organics with a high K_{ow}.

Three kinds of surfactants, sodium dodecyl sulfonate (SDS), Tween-20, and Tween-80, were used to increase the solubility of pyrene in distilled water (Figure 9). The degree of solubility enhancement of pyrene by the surfactants occurred in the following order from the most to the least: Tween-80>Tween-20>SDS. Tween-80 has lower toxicity to microorganisms and is often used to improve the bioavailability of HOCs,[16,18,19] of which the critical micelle concentration(CMC) is 13–15 mg L^{-1}. So, Tween-80 was used in the following experiments.

Effect of Tween-80 Concentration on the D_e of Pyrene. Tween-80 can increase solubility, mobility and bioavailability of hydrophobic or insoluble organics. Thus, different Tween-80 concentrations (of 0–5000 mg L^{-1}) were added into chamber 1 (Figure 3). As shown by the increase in the slope of the steady state straight line in Figure 10, the D_e of pyrene in the PVA-SA-PHB-AC piece was an increasing function of the increasing Tween-80 concentration. When the Tween-80 concentration was 1000, 3000, and 5000 mg L^{-1}, the D_e was 0.53, 1.28, and 2.30×10^{-9} m^2 min^{-1}, respectively.

When the concentration was higher than its CMC, Tween-80 could form micelle. These micelles interacted with pyrene, making pyrene wrapped in its hydrophobic center and then its hydrophilic surface distributed uniformly in the whole aqueous phase and apparently causing pyrene to dissolve in water. The dissolved pyrene could transfer into the PVA-SA-PHB-AC piece and it was available to the pyrene-degrading consortium immobilized in the PVA-SA-PHB-AC piece. Additionally, the sorption of Tween-80 on the surface of the PVA-SA-PHB-AC piece increased its surface hydrophobicity by occupying its hydrophilic sites. This facilitated

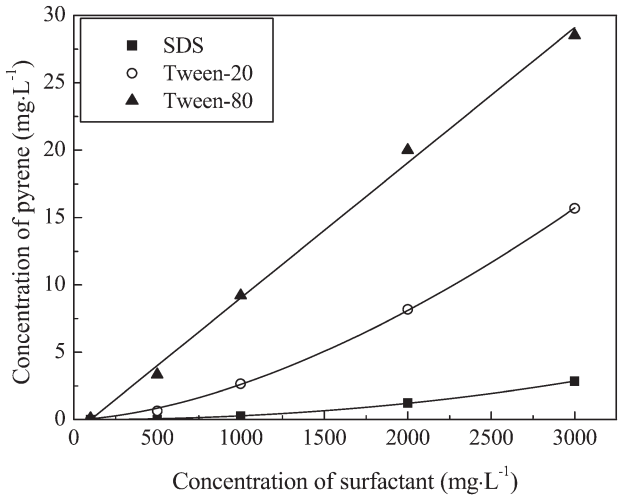

Figure 9. Water solubility enhancements of pyrene by surfactant solutions.

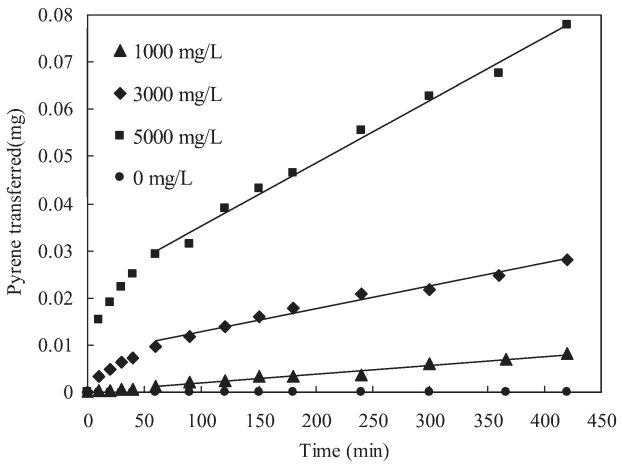

Figure 10. Effect of Tween-80 concentrations on pyrene transferred.

Table I. Properties of PVA Pieces

Performance parameter	Value			
	PVA-SA	PVA-SA-PHB	PVA-SA-AC	PVA-SA-PHB-AC
BET Surface Area/$m^2 \cdot g^{-1}$	10.0307	10.6650	42.9825	15.2999
Langmuir Surface Area /$m^2 \cdot g^{-1}$	14.1217	15.3347	59.7651	20.8734
Micropore Area/$m^2 \cdot g^{-1}$	1.7748	—	8.5908	8.0329
External Surface Area/$m^2 \cdot g^{-1}$	8.2559	10.7304	34.3917	7.2670
BJH Desorption Cumulative Micropore Volume/$cm^3 \cdot g^{-1}$	0.0660	0.0563	0.0915	0.0583
BJH Adsorption Average Pore Diameter/nm	47.1086	28.9556	17.3666	47.0666
BJH Desorption Average Pore Diameter/nm	34.9351	29.3879	16.4327	33.6789

the sorption of pyrene and affected the uptake process of pyrene from the aqueous solution.[18]

Although Tween-80 was able to promote the mass transfer of pyrene across the PVA-SA-PHB-AC piece, its concentration had to be kept at a suitable level, owing to the need to limit the concentration for the sake of microorganism growth. For example, Tween-80 concentrations higher than 500 mg L^{-1} restrain fungal growth and the degradation of decabromodiphenyl ether.[16] At the applied levels of 0–900 mg L^{-1} of Tween-80, the growth of *Mycobactrium* spp. KR2 increased.[32] Dibenzothiophene was converted to a maximum of 2-hydroxybiphenyl by a desulfurization strain at a Tween-80 concentration of 4000 mg L^{-1}.[15] The decomposition rates of hemicellulose and cellulose were increased about 8.0% and 11.6%, respectively, by 1500 mg L^{-1} of Tween-80.[33] Therefore, it was necessary to conduct a single-factor experiment to determine an appropriate Tween-80 concentration for pyrene biodegradation.

When the PVA-SA-PHB-AC piece contained 0.3% (w/v) of Tween-80, namely the PVA-SA-PHB-AC-Tween piece, the D_e of pyrene decreased significantly. The D_e of pyrene in the PVA-SA-PHB-AC-Tween piece was 0.22×10^{-9} m^2 min^{-1}, and was about 82.8% lower compared with 1.28×10^{-9} $m^2 \cdot min^{-1}$ in the PVA-SA-PHB-AC piece. It is speculated that the PVA-SA-PHB-AC-Tween piece was able to trap lots of pyrene in the process of determining the D_e of pyrene in the PVA-SA-PHB-AC-Tween piece because of the addition of Tween-80. But for *m*-cresol, there was no effect on its D_e when filling chamber 1 with 3000 mg L^{-1} of the Tween-80 solution or adding Tween-80 in the PVA piece. This is because the solubility of *m*-cresol in water is 22,700 mg L^{-1} (25°C), much higher than the concentration used in the experiment (less than 500 mg L^{-1}), and Tween-80 has no solubilization in it. Moreover, because of the differences of molecular weight, space structure, electro-negativity and hydrophilcity between *m*-cresol and pyrene, the D_e of *m*-cresol was one order greater in magnitude than that of pyrene in PVA piece.

CONCLUSIONS

1. Compared with the boric acid method, the combination of the boric acid method and freezing/thawing method could improve the water stability and cell viability of PVA carriers.
2. Adding PHB and AC into a PVA carrier improved its stability and adsorption capacity but decreased its D_e. Compared with the PVA-SA piece, the PVA-SA-PHB-AC piece's *m*-cresol equilibrium adsorption amount was 2.80 times greater and its D_e was 9.06% lower, and it offered great scope for immobilizing microorganisms for practical wastewater biotreatment.
3. With increasing concentrations of Tween-80 from 1000 mg L^{-1} to 5000 mg L^{-1}, the D_e of pyrene in the PVA-SA-PHB-AC piece increased from 0.53×10^{-9} $m^2 \cdot min^{-1}$ to 2.30×10^{-9} m^2 min^{-1}. Tween-80 has the potential benefit of improving mass transfer performance of HOCs.

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