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Immobilization of activated sludge using improved polyvinyl alcohol (PVA) gel

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Abstract

The microbial immobilization method using polyvinyl alcohol (PVA) gel as an immobilizing material was improved and used for entrapment of activated sludge. The oxygen uptake rate (OUR) was used to characterize the biological activity of immobilized activated sludge. Three kinds of PVA-immobilized particles of activated sludge, that is, PVA-boric acid beads, PVA-sodium nitrate beads and PVA-orthophosphate beads were prepared, and their biological activity was compared by measuring the OUR value. The bioactivity of both autotrophic and heterotrophic microorganisms of activated sludge was determined using different synthetic wastewater media (containing 250 mg/L COD and 25 mg/L NH_4^+-N). The experimental results showed that the bioactivity and stability of the three kinds of immobilized activated sludge was greatly improved after activation. With respect of the bioactivity and the mechanical stability, the PVA-orthophosphate method may be a promising and economical technique for microbial immobilization.

Key words: immobilization; biological activity; oxygen uptake rate (OUR); polyvinyl alcohol (PVA)

Introduction

Immobilization of microbial cells has received increasing interest in the field of waste treatment. It offers a promising potential for the improvement of the efficiency of bioprocess. Compared with free cell, immobilized cell has several advantages (Wang, 2002): (1) it can increase the biodegradation rate through a higher cell loading; (2) the bioprocess can be controlled more easily; (3) the continuous process can take place at a high dilution rate without washout; (4) the catalytic stability of biocatalysts as well as the tolerance against toxic compounds can be improved.

Immobilized cell systems have the potential to degrade toxic chemicals faster than conventional wastewater treatment systems, because high densities of specialized microorganisms are used in immobilized cell systems. They have been widely applied for degradation of numerous toxic compounds, such as phenol (Wang *et al*., 1995), 4-chlorophenol (Wang *et al*., 1997a; Wang and Qian, 1999), 2,4-dichlorophenol (Quan *et al*., 2003), quinoline (Wang *et al*., 2001, 2002a), phthalic acid esters (Wang *et al*., 1997b, 2003) and so on. They are also applied to wastewater treatment, such as coke plant wastewater treatment (Wang *et al*., 2002b), nitrogen removal (Seo *et al*., 2002), heavy metal removal (Pan *et al*., 2005), decolorization of azo dye (Wu *et al*., 2005) and high-carbohydrate wastewater treatment (Zhang *et al*., 2005).

Entrapment of cells in polymeric matrixes is widely used for cell immobilization. Many natural and synthetic polymers have been used (Wang and Liu, 1996; Wang and Shi, 1998; Wang *et al*., 2000), but each has its drawbacks. Natural polymers (agar, agarose, alginate, kappa-carragenan) possess poor mechanical strength and durability, although they are not toxic to microorganisms. Conversely, synthetic polymers have strong mechanical strength and durability but are often toxic to microorganisms.

Polyvinyl alcohol (PVA) is a promising type of synthetic polymer, which is cheap and nontoxic to microorganisms. It is very suitable for microbial immobilization (Amanda and Wisecarver, 1992; Chen and Lin, 1994; Chen *et al*., 1996, 1998, 2003; Chang and Tseng, 1998; Chang *et al*., 2005; Cao *et al*., 2002; Hai and Syed, 1995; Li *et al*., 2002; Long *et al*., 2004; Lozinsky and Plieva, 1998; Preininger and Chiarelli, 2001; Szczesna and Galas, 2001; Szczesna *et al*., 2001; Wang *et al*., 1995; Wang and Shi, 1998). However, there are some problems emerged when using the PVA-boric acid method for immobilization of microbial cell, such as the agglomeration of PVA beads, the toxicity of boric acid and the swelling performance. We have done lots of research works to solve the problems of agglomeration and the swelling performance, and achieved satisfactory results.

The objectives of this research were to (1) improve the PVA immobilization method by using different gelating reagents, that is, boric acid, nitrate and orthophosphate,

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and (2) evaluate the bioactivity of immobilized activated sludge through measuring the oxygen uptake rate (OUR) to develop a suitable and practical immobilization method.

1 Materials and methods

1.1 Activated sludge

Activated sludge was taken from the Beijing Gaobeidian Sewage Treatment Plant.

1.2 Immobilization methods

Ten gram of polyvinyl alcohol (PVA, nominal degree of polymerization = 1750, approx. molecular weight 75000–80000) and 1 g of sodium alginate were dissolved in 50 ml of distilled water, the solution was cooled down to 40°C and then mixed thoroughly with 50 ml of concentrated activated sludge. The resulting mixture contained 10% (w/v) PVA, 1.0% (w/v) sodium alginate, and about 20 g/L of microorganisms. The following three different gelating solutions were used to form gel beads (about 2 mm in diameter): (1) the mixture was dropped into saturated boric acid and $CaCl_2$ (1% w/v) solution and kept for 1 h to form spherical beads (group B); (2) the mixture was dropped into saturated boric acid and $CaCl_2$ (1% w/v) solution and kept for 1 h to form gel beads, then transferring to 0.5 mol/L sodium orthophosphate solution and immersing for 1 h (group P); (3) the mixture was extruded as drops into a solution of sodium nitrate (50% w/v) and $CaCl_2$ (1% w/v), and then immersed for 1 h to form PVA-sodium nitrate beads (group N).

The formed particles were washed with physiological saline solution for 1 h and then stored in distilled water at 4°C until further use.

1.3 Experimental methods

1.3.1 Preparation of solutions

BOD solution: 1.031 g of glucose ($C_6(H_2O)_6{\cdot}H_2O$) was added into a 1.00-L volumetric flask containing distilled water to make the BOD standard solution. It was diluted according to the experimental requirement.

NH_4^+-N solution: 0.536 g of NH_4HCO_3, 0.380 g of $MgSO_4{\cdot}7H_2O$, 0.020 g of $CaCl_2{\cdot}2H_2O$ and 0.087 g of K_2HPO_4 was dissolved in distilled water, to make NH_4^+-N solution according to the required concentration. It was diluted according to the experimental requirement.

1.3.2 Activation of PAV immobilized activated sludge

Three kinds of immobilized particles stored at 4°C for one month was taken out and immersed in the solution containing 200 mg/L BOD and 20 mg/L NH_4^+-N and kept aerating for 2 h to activate the bioactivity.

1.3.3 Measurements of OUR

Eight gram of immobilized sludge was added into a flask containing 80 ml solution of different synthetic wastewater, and aerated to make the dissolved oxygen saturated, then stop aeration and monitor the DO variation with time by using Orion 850A DO meter (Thermo Electron Corporation, USA). The diagram of the OUR measurement system is shown schematically in Fig.1.

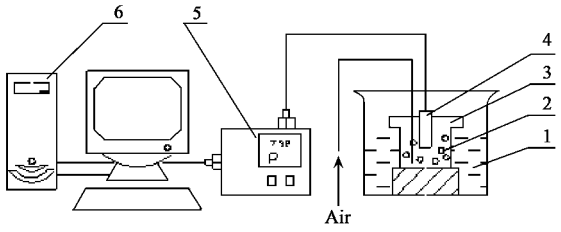

Fig. 1 Schematics of the OUR measurement system. (1) water bath; (2) immobilized microbial beads; (3) reactor; (4) oxygen electrode; (5) DO meter; (6) computer.

DO variation was monitored and recorded on time by a computer. The DO-*t* curve could be drawn according to the recorded data. The value of OUR was calculated according to the following equation:

$$\mathrm{OUR} = \frac{\mathrm{DO}_1 - \mathrm{DO}_2}{(t_2 - t_1)} \tag{1}$$

where, OUR is the oxygen consumed in unit time (mg O_2/(L·min)); DO_1 is the DO concentration at time t_1 (mg/L); DO_2 is the DO concentration at time t_2 (mg/L); t is measuring time, min.

1.3.4 Scanning electron microscopy (SEM) observation

The samples were fixed in 0.1 mol/L phosphate buffer (pH = 7.3) containing 2.5% glutaraldehyde for 12 h at 4°C. After fixation, samples were rinsed three times in 0.1 mol/L phosphate buffer (pH = 7.3) and dehydrated gradually after successive immersions in ethanol solutions of increasing concentration (50%, 70%, 80%, 90% and 95%). Each rinsing and dehydrating step took 10 min. The samples were then washed three times in 100% ethanol before in hexamethyldisilazane for 30 s. Drying was completed by incubating the samples for 2 h at 30°C. The particles were then coated with gold powder and attached on to the microscope supports with silver glue. SEM photographs were taken at 10 kV under Scanning Electron Microscope (Hitachi S-570, Japan).

2 Results and discussion

2.1 DO consumption by PVA beads after activation

Three kinds of synthetic solutions, that is, solution A (containing 250 mg/L BOD), solution B (containing 25 mg/L NH_4^+-N) and solution C (containing both BOD and NH_4^+-N), were used to determine the bioactivity of immobilized beads of activated sludge. Three different kinds of PVA immobilized beads designated group B, group N and group P were prepared and their bioactivity before and after activation was measured and compared.

The DO consumption by activated sludge may be divided into two parts: the oxidation of organic compounds by heterotrophic microorganisms and the nitrification by autotrophic bacteria. In the solution A which contained BOD only, the DO was consumed only by heterotrophic microorganisms, while in the solution B which contained

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NH_4^+-N the DO consumption was contributed only by the nitrification of autotrophic bacteria, in the solution C containing both BOD and NH_4^+-N, not only the heterotrophic microorganisms but autotrophic bacteria consumed DO, therefore the DO consumption was caused by both heterotrophic and autotrophic bacteria.

The DO variation with time of three different PVA immobilized beads in different solutions is depicted in Fig.2. The OUR is calculated and shown in Table 1.

OUR is an important parameter to express the microbial activity of activated sludge in the wastewater treatment process. The OUR of three different PVA immobilized beads in different solutions was calculated and given in Table 1.

Table 1 Comparison of oxygen uptake rate (OUR) of three kinds of PAV beads in different solution

Solution	PVA beads	DO_1 (mg/L)	DO_2 (mg/L)	t_2-t_1 (min)	OUR (mg O_2/(L·min))
Solution A	Group B	8.33	7.83	15	0.033
	Group N	7.83	6.20	15	0.109
	Group P	7.50	3.73	15	0.251
Solution B	Group B	8.35	8.04	15	0.021
	Group N	7.77	6.83	15	0.063
	Group P	7.70	5.48	15	0.148
Solution C	Group B	8.43	7.89	15	0.036
	Group N	7.82	6.08	15	0.116
	Group P	7.54	3.46	15	0.272

Solutions A, B, and C are the same as that in Fig.2. Groups B, N, and P are explained in Section 1.2.

Figure 2 and Table 1 show that the OUR of the phosphorylated PVA beads (group P) was the highest either in the solutions A, B, or C during 15 min experimental period, which indicated that among the three different PVA immobilization method, PAV-orthophosphate method could maintain a high bioactivity of activated sludge because of the low toxicity of gelation solution.

2.2 DO consumption by PVA beads before activation

The PVA particles was prepared and stored at 4°C for one month, their DO variation with time was measured before activation and OUR was calculated. Fig.3 demonstrates the DO consumption of the three different kinds of PVA immobilized particles.

Figure 3 indicates that the bioactivity and the biostability of immobilized activated sludge were poor before activation, in comparison with that after activation. The DO variation was low for a long time. The DO variation before activation was much smaller than that after activation for all the three solutions, that is to say, for either heterotrophic or autotrophic bacteria, their bioactivity could be greatly enhanced by activation after storage for a long time.

2.3 SEM photograph of PVA-orthophosphate beads

For the PVA-orthophosphate immobilized activated sludge, the bioactivity was the highest among three different PVA particles, so the microorganisms of the group P after activation were observed by using scanning electron

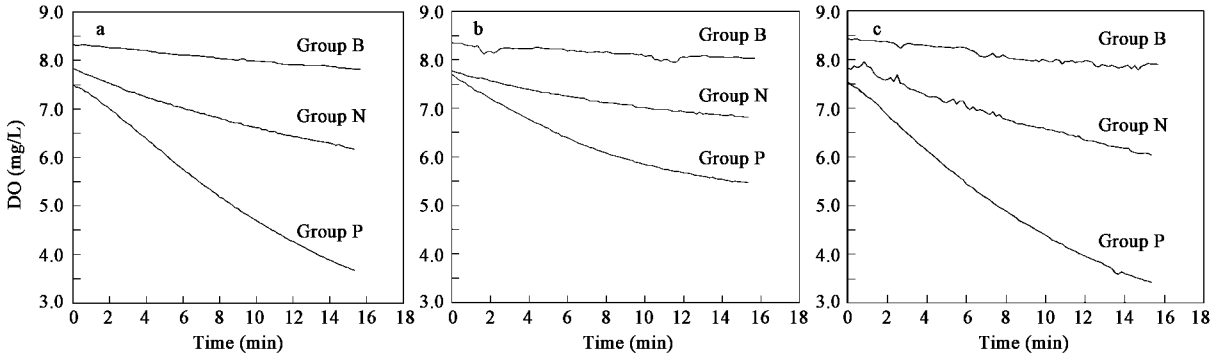

Fig. 2 DO–*t* curve of newly prepared immobilized cells in solution A containing 250 mg/L COD (a), solution B containing 25 mg/L NH_4^+-N (b) and solution C containing 250 mg/L COD and 25 mg/L NH_4^+-N (c). Groups B, N, and P are explained in Section 1.2.

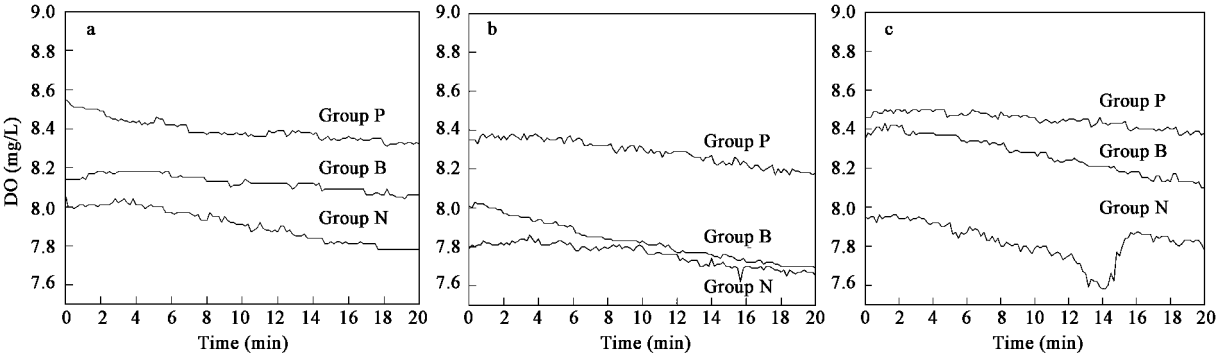

Fig. 3 DO–*t* curve of immobilized cells stored for one month in solution A containing 250 mg/L COD (a); solution B containing 25 mg/L NH_4^+-N (b) and solution C containing 250 mg/L COD and 25 mg/L NH_4^+-N (c). Groups B, N, and P are explained in Section 1.2.

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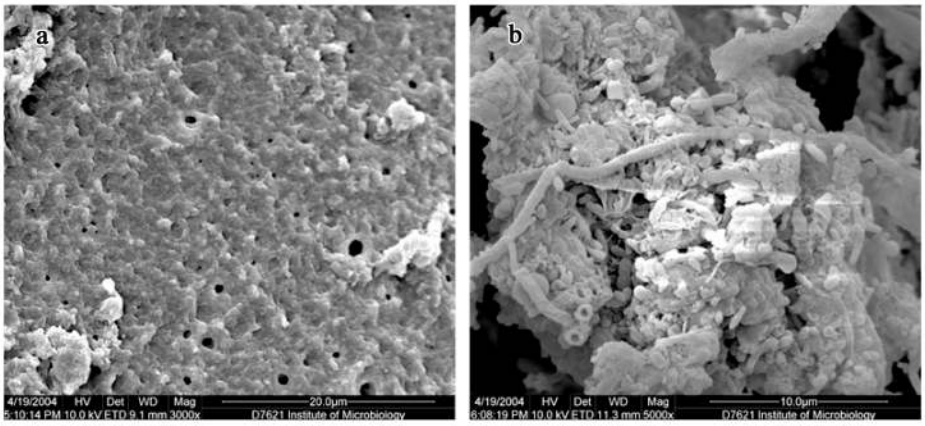

Fig. 4 SEM photographs of PVA-orthophosphate immobilized activated sludge. (a): 3000×; (b): 5000×.

microscopy. The results are shown in Fig.4.

The SEM photographs show that structure of phosphorylated PVA beads (group P) had lots of micropores inside the gels, which was suitable for the immobilization of microbial cells. The photograph also indicated that activated sludge could be successfully entrapped in PVA beads by the PVA-orthophosphate methods.

3 Conclusions

Three different kinds of PVA biocatalyst beads labeled as group B, group N and group P were prepared and their biological activity was compared. The results showed that the formation of spherical gel beads without agglomeration for all three types was successful during gelation process.

The PVA-orthophosphate method can reduce the toxicity caused by saturated boric acid solution, compared to the PVA boric acid method. This new method could maintain the higher bioactivity of the activated sludge immobilized in the beads of PVA-orthophosphate gel. The PVA-orthophosphate method is a cheap, low toxic and easy to operate, it may be a promising and economical technique for microbial immobilization.

References

Amanda K Y, Wisecarver K D, 1992. Cell immobilization using PVA crosslinked with boric acid[J]. Biotechnol Bioeng, 39: 447–449.

Cao G M, Zhao Q X, Sun X B *et al*., 2002. Characterization of nitrifying and denitrifying bacteria coimmobilized in PVA and kinetics model of biological nitrogen removal by coimmobilized cells[J]. Enzyme and Microbial Technology, 30: 49–55.

Chang C, Tseng S K, 1998. Immobilization of *Alcaligenes eutrophus* using PVA crosslinked with sodium nitrate[J]. Biotechnology Techniques, 12: 865–868.

Chang I S, Kim C I, Nam B U, 2005. The influence of poly-vinyl-alcohol (PVA) characteristics on the physical stability of encapsulated immobilization media for advanced wastewater treatment[J]. Process Biochemistry, 40: 3050–3054.

Chen K C, Lin Y F, 1994. Immobilization of microorganisms with phosphorylated polyvinyl alcohol (PVA) gel[J]. Enzyme Microbe Technol, 16: 79–83.

Chen K C, Chen S J, Houng, J Y, 1996. Improvement of gas permeability of denitrifying PVA gel beads[J]. Enzyme and Microbial Technology, 18: 502–506.

Chen K C, Lee S C, Chin S C *et al*., 1998. Simultaneous carbon-nitrogen removal in wastewater using phosphorylated PVA-immobilized microorganisms[J]. Enzyme and Microbial Technology, 23: 311–320.

Chen K C, Wu J Y, Huang C *et al*., 2003. Decolorization of azo dye using PVA-immobilized microorganisms[J]. Journal of Biotechnology, 101: 241–252.

Hai T, Syed Q, 1995. Poly (vinyl alcohol) hydrogels: 2. Effects of processing parameters on structure and properties[J]. Polymer, 36: 2531–2539.

Li H Z, Wang J L, Wen X H *et al*., 2002. Improvement of PVA-H_3BO_3 immobilization method[J]. Environmental Sciences Research, 15(5): 25–28.

Long Z, Huang Y H, Cai Z L *et al*., 2004. Immobilization of *Acidithiobacillus ferrooxidans* by a PVA-boric acid method for ferrous sulphate oxidation[J]. Process Biochemistry, 39: 2129–2133.

Lozinsky V I, Plieva F M, 1998. Poly (vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments[J]. Enzyme and Microbial Technology, 23: 227–242.

Pan X L, Wang J L, Zhang D Y, 2005. Biosorption of Pb(II) by *Pleurotus ostreatus* immobilized in calcium alginate gel[J]. Process Biochemistry, 40: 2799–2803.

Preininger C, Chiarelli P, 2001. Immobilization of oligonucleotides on crosslinked poly (vinyl alcohol) for application in DNA chips[J]. Talanta, 55: 973–980.

Quan X C, Shi H C, Wang J L *et al*., 2003. Biodegradation of 2,4-dichlorophenol in sequencing batch reactors augmented with immobilized mixed culture[J]. Chemosphere, 50(8): 1069–1074.

Seo J K, Jung I H, Kim, M R *et al*., 2002. Nitrification performance of nitrifiers immobilized in PVA (polyvinyl alcohol) for a marine recirculating aquarium system[J]. Aquacultural Engineering, 24: 181–194.

Szczesna M, Galas E, 2001. *Bacillus subtilis* cells immobilised in PVA-cryogels[J]. Biomolecular Engineering, 17: 55–63.

Szczesna M, Galas E, Bielecki S, 2001. PVA-biocatalyst with entrapped viable *Bacillus subtilis* cells[J]. Journal of Molecular Catalysis B: Enzymatic, 11: 671–676.

Wang J L, Hou W H, Qian Y, 1995. Immobilization of microbial cells using polyvinyl alcohol (PVA)-polyacrylamide gels[J]. Biotechnology Techniques, 10: 203–208.

Wang J L, Liu P, 1996. Comparison of citric acid production

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by *Aspergillus niger* immobilized in gels and cryogels of polyacrylamide[J]. Journal of Industrial Microbiology, 16: 351–353.

Wang J L, Shi H C, Qian Y *et al*., 1997a. Immobilization of microorganisms using carrageenan gels coated with chitosan and application to biodegradation of 4-chlorophenol[J]. J Environ Sci, 9: 283–287.

Wang J L, Liu P, Qian Y, 1997b. Biodegradation of phthlic acid esters by immobilized microbial cells[J]. Environmental International, 23: 775–782.

Wang J L, Shi H C, 1998. The research and development of microbial immobilization using polyvinyl alcohol (PVA) gel[J]. Journal of Industrial Microbiology, 28(2): 35–39.

Wang J L, Qian Y, 1999. Microbial degradation of 4-chlorophenol by microorganisms entrapped in carrageenan-chitosan gels[J]. Chemosphere, 38: 3109–3117.

Wang J L, Wu L B, Qian Y, 1999. Characterization of nitrification activity of activated sludge by oxygen uptake rate (OUR)[J]. Acta Scientiae Circumstantiae, 19(3): 225–229.

Wang J L, Horan N, Qian Y *et al*., 2000. The radial distribution and bioactivity of *Pseudomonos* sp. immobilized in calcium alginate beads[J]. Process Biochemistry, 35: 465–469.

Wang J L, Han L P, Shi H Ch *et al*., 2001. Biodegradation of quinoline by gel immobilized *Burkholderia* sp.[J]. Chemosphere, 44(5): 1041–1046.

Wang J L, 2002. Microbial immobilization techniques and water pollution control[M]. Beijing: Science Press.

Wang J L, Quan X C, Han L P *et al*., 2002a. Microbial degradation of quinoline by immobilized cells of *Burkholderia pickettii*[J]. Water Res, 36: 288–296.

Wang J L, Quan X C, Wu L B *et al*., 2002b. Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater[J]. Process Biochemistry, 38: 777– 781.

Wang J L, Ye Y C, Wu W Z, 2003. Comparison of biodegradation of di-*n*-methyl phthalate by free and immobilized microbial cells[J]. Biomedical and Environmental Science, 16: 126–132.

Wu J Y, Hwang S C, Chen C T *et al*., 2005. Decolorization of azo dye in a FBR reactor using immobilized bacteria[J]. Enzyme and Microbial Technology, 37: 102–112.

Zhang Y M, Rittmann B E, Wang J L *et al*., 2005. High-carbohydrate wastewater treatment by IAL-CHS with immobilized *Candida tropicalis*[J]. Process Biochemistry, 40: 857–863.

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