Immobilization of an enzyme from a *Fusarium* fungus WZ-I for chlorpyrifos degradation

Hui Xie, Lusheng Zhu,* Tingting Ma, Jun Wang, Jinhua Wang, Jun Su, Bo Shao

College of Resources and Environment, Shandong Agricultural University, Taian 271018, China. E-mail: huixie@sdau.edu.cn

Abstract

The free enzyme extracted from WZ-I, which was identified as *Fusarium* LK. ex Fx, could effectively degrade chlorpyrifos, an organophosphate insecticide. The methods of immobilizing this free enzyme and determined its degradation-related characteristics were investigated. The properties of the immobilized enzyme were compared with those of the free enzyme. The optimal immobilization of the enzyme was achieved in a solution of 30 g/L sodium alginate at 4°C for 4–12 hr. The immobilized enzyme showed the maximal activity at pH 8.0, 45°C. The maximum initial rate and the substrate concentration of the immobilized enzyme were less than that of the free enzyme. The immobilized enzyme, therefore, had a higher capacity to withstand a broader range of temperatures and pH conditions than the free enzyme. With varying pH and temperatures, the immobilized enzyme was more active than the free enzyme in the degradation reaction. In addition, the immobilized enzyme exhibited only a slight loss in its initial activity, even after three repeated uses. The results showed that the immobilized enzyme was more resistant to different environmental conditions, suggesting that it was viable for future practical use.

Key words: organophosphate pesticide; immobilized enzyme; degradation; fungus

DOI: 10.1016/S1001-0742(09)60341-7

Introduction

Because organophosphate pesticides have been used consistently all over the world, concerns regarding the relative safety of these chemicals with respect to the environment and living things are increasing.

Chlorpyrifos (*O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridin-yl phosphorothionate) is one of the most widely used organophosphate insecticides and is used in both agricultural and urban settings (US EPA, 2002). Chlorpyrifos residues were found in stored grains in Sonora, Mexico (Aldana et al., 2008). It also been detected in groundwater and surface water in USA (Echols et al., 2008). Concentrations of chlorpyrifos range from less than 1 to 5.5 ng/g in the Lower Missouri River, US (Echols et al., 2008).

Chlorpyrifos is a neurotoxin and a suspected endocrine disruptor. It associates with asthma, reproductive and developmental toxicity as well as acute toxicity (AOEC Exposure Codes), and is especially harmful to pregnant women and babies (Coony, 1999; Vidair, 2004). Chlorpyrifos is highly toxic to amphibians. The recent study by the United States Geological Survey found that its main toxic metabolite product 3,5,6-trichloro-2-pyridinol (TCP) is even more toxic to some animals (Ca′ceres et al., 2007).

Chlorpyrifos has a long residual period in soils, and the large quantity of use and high degree of persistence raise broad concerns about its biodegradation in the soil (Kumar et al., 2002).

It has been proved that bacteria *Flavobacterium* sp. ATCC 27551 (Mallick et al., 1999), *Enterobacter* strain B-14 (Singh et al., 2003, 2004), *Alcaligenes faecalis* (Yang et al., 2005), *Klebsiella* sp. (Ghanem et al., 2007) and fungal *Verticillium* sp. (Fang et al., 2008) can degrade and utilize chlorpyrifos as a nutritional source. *Serratia* sp. can transform chlorpyrifos to TCP and TCP-mineralizing fungal strain *Trichosporon* sp. co-cultures can completely mineralize chlorpyrifos (Xu et al., 2007). *Pseudomonas aeruginosa* can degrade chlorpyrifos and its metabolite TCP (Vidya et al., 2009). However, most previous investigations have focused on bacterial strains and only a few studies have focused on enzymes. Yu et al. (1999) showed that the immobilized enzyme could effectively degrade fenvalerate. Huang et al. (2008) studied the degradation of organic compounds in polluted water by immobilized enzymes. Zhao et al. (2008) investigated free and immobilized laccase for DDT remediation in soils. The information regarding to the immobilization and characterization of enzymes that can degrade chlorpyrifos is limited.

The microorganisms and enzymes are both efficient and available for the removal of pesticide residue in the environment. Enzymes, however, are sensitive and unstable at different temperatures, in strong acids, strong alkaline

* Corresponding author. E-mail: lushzhu@sdau.edu.cn

solutions and organic solvents, and they can lose their ability to degrade substrates under certain circumstances. In the present study, we attempt to determine whether surface immobilization is able to solve these problems.

1 Materials and methods

1.1 Immobilization of free enzyme in embedding medium

WZ-I, which has been identified as *Fusarium* LK. ex Fx, was isolated in our laboratory (Wang et al. 2005), and preparation of the free enzyme has been described previously (Xie et al., 2005). The free enzyme was mixed with the embedding medium of polyvinyl alcohol or sodium alginate (SA) at the ratio of 1:10 (*V/V*) (Wang et al., 2007). Polyvinyl alcohol was added drop wise to 5% boric acid (*W/V*) to generate another type of embedding medium, and sodium alginate was added drop wise to 2% calcium chloride (*W/V*) to generate another type of embedding medium. The mixture of the free enzyme and the embedding medium was extruded through a syringe (2 mm diameter) and added into the solution for 12 hr gelation at approximately 4°C. After washing with 0.04 mol/L barbital sodium-HCl buffer (pH 7.0), the immobilized beads were used for chlorpyrifos degradation. Beads containing no enzyme served as a control.

Immobilized enzyme beads (2 g) with different immobilizing time of 4, 8, 10, 12, 16, 20, and 24 hr were added to 5 mL of a barbital sodium-HCl buffer (pH 7.0) containing chlorpyrifos (50 mg/L). They were then incubated for 10 min in a 40°C water bath. The resulting concentrations of chlorpyrifos were tested and compared.

1.2 Characteristics of immobilized chlorpyrifos degradation enzyme

Resistance of the immobilized enzyme to heat: 5-mL solution of 0.04 mol/L barbital sodium-HCl buffer (pH 7.0) and 2 g of immobilized enzyme were added to test tubes and incubated in a 50°C water bath for 1, 2 or 5 hr to test the residual activity of the immobilized enzyme.

Resistance of immobilized enzyme to pH: the buffer with differing pH (4, 5, 6 and 9) and 2 g of immobilized enzyme were added to test tubes and in cubated in a 30°C water bath for 2 hr. The activity of the immobilized enzyme was tested at pH 8.0 and 45°C.

Optimal reaction temperature: a buffer (pH 7.0) containing chlorpyrifos (50 mg/L) was added to test tubes and incubated at different temperatures (10, 20, 30, 40, 45, 50 and 55°C). The buffers were preheated for 10 min, and then 2 g of immobilized enzyme was added and allowed to react for 10 min. After the reactions were terminated by adding 0.2 mL HCl (1 mol/L), the concentrations of chlorpyrifos were measured. After determine the degradation activity of the immobilized enzyme at different temperatures, the activities could be compared to the immobilized enzyme with the highest activity (normalized to 100%). All of the enzyme activities listed in this study correspond to the relative enzyme activity.

Optimal reaction pH conditions: A buffer containing chlorpyrifos (50 mg/L) was added to test tubes and incubated at 45°C at different pH values (6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0). The buffers were preheated for 10 min, and then 2 g of immobilized enzyme was added and allowed to react for 10 min. Following the incubation, the enzyme activities were measured.

K_m **and** V_{max} **values of the immobilized enzyme**: buffers with different chlorpyrifos concentrations (1.43 $\times$ 10^{-4}, 2.28 $\times$ 10^{-4}, 2.85 $\times$ 10^{-4}, 3.42 $\times$ 10^{-4}, 4.28 $\times$ 10^{-4} and 5.71 $\times$ 10^{-4} mmol/mL) were added to test tubes and preheated in a 45°C water bath for 10 min. The immobilized enzyme (2 g) was added and allowed to react for 10 min. A buffer with added inactive immobilized enzyme was used as the control. By comparison, the impact of chlorpyrifos concentrations on the degradation rate could be determined.

Repeated use of the immobilized enzyme: the immobilized enzyme was put into a barbital sodium-HCl buffer (pH 8.0) at 45°C for 1 hr and then used to degrade chlorpyrifos for second and third time. Finally, the residual enzyme activity of the immobilized enzyme was tested and compared after each use.

1.3 Biodegradation of chlorpyrifos by the immobilized enzyme under different conditions

We assayed the biodegradation of chlorpyrifos by the immobilized enzyme under different conditions.

The optimal conditions for chlorpyrifos degradation were 40°C at pH 6.8 for the free enzyme and 45°C at pH 8.0 for the immobilized enzyme. The degradation of chlorpyrifos at different times (10, 20, 30, 40, 60 and 80 min) was compared for the free and immobilized enzyme.

A buffer (pH 7.0) and 0.2 mL of free enzyme or 2 g of immobilized enzyme were added to test tubes in a water bath at different temperatures (10, 20, 30, 40, and 50°C). After 1 hr, the residual activities of the free and immobilized enzymes were analyzed.

Buffers of different pH values (4, 5, 9 and 10) and 0.2 mL of the free enzyme or 2 g of the immobilized enzyme were put into test tubes in a 30°C water bath for 1 hr. The residual activities of the free and immobilized enzymes were tested at pH 8.0 and 45°C and compared.

1.4 Analysis of chlorpyrifos

Chlorpyrifos was qualitatively analyzed using an Agilent 1100 HPLC system equipped with an Agilent variable wavelength ultraviolet detector (USA). The separation was performed with a reversed-phase column with the column oven at 25°C. The system consisted of binary pump to control the flow rate of the mobile phase and an autosampler for automatic injection. The flow rate was 1.0 mL/min (methanol/water, 90:10, *V/V*), and the variable-wavelength UV detector was set at 300 nm. The detection limit for chlorpyrifos was 0.5 ng.

An external calibration method was used for measurement of chlorpyrifos at quantities between 0.5 $\times$ 10^{-10} and 2.5 $\times$ 10^{-7} g. The correlation coefficients of the calibration curves were > 0.999. The dose recovery of 0.01, 0.1,

1.0, and 5.0 mg/kg ranged from (91.4 ± 4.1)% to (105.1 ± 4.0)%. The results meet the requirement of pesticide residue analysis.

1.5 Calculation of degradation percent by immobilized enzyme

The degradation of chlorpyrifos can be calculated as Eq. (1):

$$X = \frac{(C_{ck} - C_x)}{C_{ck}} \times 100\% \qquad (1)$$

where, X is the degradation percentage of chlorpyrifos; C_x (mg/L) is the terminal concentration of the chlorpyrifos reaction after adding the immobilized enzyme; C_{ck} (mg/L) is the concentration of chlorpyrifos in the control.

After calculating the degradation percentage of chlorpyrifos, the activities of the degrading enzyme in different reactions were calculated. The activity in all reactions was normalized to the highest activity (set to 100%) allowing for the relative activities of the enzymes in different reactions to be compared.

The relative activities (RA) of the enzyme in different reactions can be calculated as:

$$RA = \frac{X}{X_{max}} \qquad (2)$$

where, X is the percentage of chlorpyrifos degraded in different reactions and X_{max} is the largest X in a relative group.

1.6 Statistical analysis

All degradation data were analyzed to determine the standard deviation. The SPSS/PC program (version 11.5) was used to determine the differences among all groups. Each treatment was performed in triplicates. The level of statistical significance was set at $P < 0.05$.

2 Results and discussion

2.1 Selection of embedding medium and immobilization time

The results of chlorpyrifos degradation by immobilized enzyme using different embedding media are listed in Table 1.

Carrageenan, Chitosan, PVA and SA are the most commonly used embedding media (Wang et al., 2007). In the present study, PVA and SA were chosen for investigation of their embedding effects due to their low price and high availability. When PVA was used as the embedding medium at four different concentrations, no immobilized beads were produced (Table 1). The enzyme aggregated and large pieces of material were formed. Furthermore, the viscous mixture of embedding medium made the production process difficult. Therefore, PVA was not suitable for immobilizing this degradation enzyme.

Alternatively, immobilized beads were readily formed with four different SA concentrations, especially when the concentrations of SA were 2.5% and 3%. When comparing

the degraded amounts of chlorpyrifos using the immobilized enzyme prepared using the two concentrations of SA, we found that 3% immobilization resulted in the greatest degradation and was easy to manipulate. The comparison of the results from the different media is shown in Table 1. When 3% SA was used, the shape and elasticity of the immobilized beads were optimal in all tests, and the amount of chlorpyrifos degraded by the immobilized enzyme was the highest. Therefore, 3% SA was the optimal embedding medium. Wang et al. (2007) have reported that an atrazine degradation enzyme can also be embedded in 5% SA and the degradation ability can be retained at a high level.

In this experiment, when making immobilized beads, 4 hr was found to be the optimal immobilization time. For time between 4 and 12 hr, the immobilized enzyme beads formed ideal shapes. When the time of immobilization was prolonged beyond 12 hr, the inside structure of the immobilized beads became more and more compact, thus decreasing the contact chance between chlorpyrifos and the enzyme. A prolonged immobilization time also decreased the degradation activity of the immobilized enzyme. Wang et al. (2007) have investigated the immobilization of an atrazine degradation enzyme and found an optimal immobilization time of 4 hr. At the concentration of 5%, the immobilized beads formed easily and the degradation activity of the enzyme did not appreciably decrease.

2.2 Characteristics of the immobilized chlorpyrifos degradation enzyme

Resistance of the immobilized enzyme to heat: after heating at 50°C for 1, 2 and 5 hr, the relative residual activity of the immobilized enzyme remained at 92.7%, 68.8% and 63.1% of the initial activity, respectively.

Xie et al. (2005) have reported that the free enzyme from *Fusarium* LK. ex Fx has a good stability at 40°C. The immobilized enzyme was stable at higher temperatures, as evidenced by the small decrease in activity after heating at 50°C for 1, 2, and 5 hr. After immobilization, the degrading enzyme was more stable at even higher temperatures. The tolerance of higher temperatures after immobilization might be due to the protection of the enzyme by embedding medium.

Table 1 Chlorpyrifos degradation by the immobilized enzyme using different media

Embedding medium	Manipulation	Degradation percentage (%)
2.5% SA	Easy	30
3% SA	Easy	41.9
4% SA	Relatively difficult	35.4
5% SA	Difficult	32.1
10% PVA + 0.5% SA + 0.8% activated carbon	Very difficult	27.7
2.5% PVA	Easy conglutination	32.5
3% PVA	Conglutination	39.5
4% PVA	Difficult	14.4
5% PVA	Difficult large pieces of mass	13
10% PVA + 0.5% SA	Relatively difficult	25.7

SA: sodium alginate; PVA: polyvinyl alcohol.

Resistance of the immobilized enzyme at different pH: the relative activities of the immobilized enzyme at different pH levels were tested (Fig. 1). The relative residual activities of immobilized enzyme all remained over 75%.

It is known that pH 6.8 is the optimal pH for free enzyme (Xie et al., 2005), suggesting that the immobilized enzyme has a wider pH range than the free enzyme. This may be due to the protection offered by the embedding medium.

Optimal reaction temperature: The optimal temperature for the immobilized enzyme reaction was 45°C (Fig. 2). Between 40 and 55°C, the relative activity of immobilized enzyme exceeded 80%, whereas below 30°C, the activity of the immobilized enzyme decreased. This might be the reason that at lower temperatures, the enzyme is not active enough and thus cannot carry out the reaction. Therefore, the immobilized enzyme is more suitable for higher temperatures with 45°C as the optimal temperature for degradation of chlorpyrifos. According to Xie et al. (2005), the optimal temperature for degradation of chlorpyrifos by the free enzyme is 40°C. Immobilization has increased the adaptability of the enzyme to higher temperatures.

Optimal reaction pH conditions: the relative activities of the immobilized enzyme under different pH conditions are shown in Fig. 3. The optimal pH was 8. It is known that the optimal pH for the free enzyme is pH 6.8 (Xie et
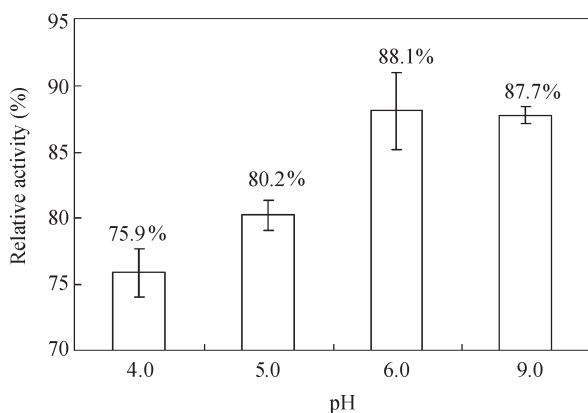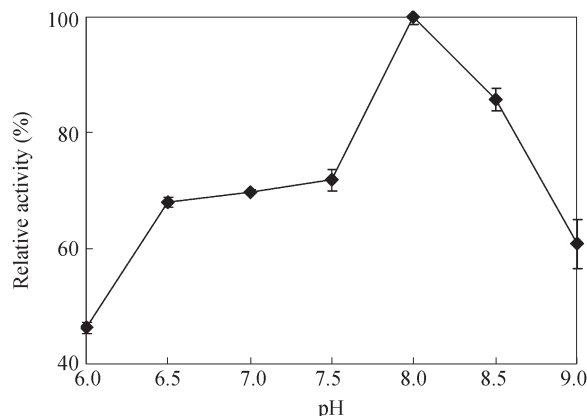

Fig. 3 Effect of pH on initial rate of catalysis by the immobilized enzyme.

al., 2005). This difference is likely because sodium alginate is a cation embedding medium and stabilize immobilized enzyme at basic pH.

K_m **and** V_{max} **values for the immobilized enzyme**: the maximum initial rate (V_{max}) and the substrate concentration (K_m) values for chlorpyrifos degradation by the immobilized enzyme were calculated using the double reciprocal Lineweaver-Burk plot in Fig. 4 (Ghanem et al., 2007). The K_m and V_{max} values were 0.1672 mmol/L and 50.246 nmol/min, respectively. Both values were less than those of the free enzyme, which are K_m 1.04926 mmol/L and V_{max} 253.5 nmol/min (Xie et al., 2005).

According to Michaelis-Menten theory of enzyme action, V_{max} is the maximum initial rate of an enzyme catalyzed reaction, i.e., when virtually all the enzyme present in the reaction, the mixture is present as enzyme-substrate complex. K_m is the substrate concentration at which the initial reaction rate is half maximal. Each enzyme has a specific characteristic K_m value and the K_m value corresponding to the structure and the enzyme catalyzed substrate. It is unrelated to the enzyme concentration. K_m is also an indicator of the affinity of the enzyme for its substrate. The higher the K_m, the lesser the affinity is, although this actually depends upon the mechanism of catalysis. The smaller K_m indicates an increased affinity

Fig. 1 Resistance of immobilized enzyme at different pH values.

Fig. 2 Effect of temperature on initial rate of catalysis by the immobilized enzyme.
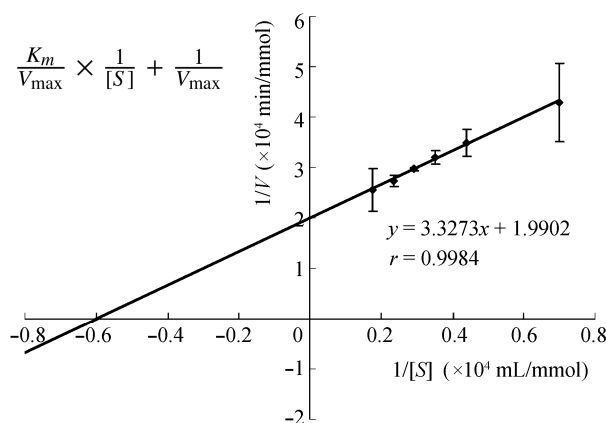

Fig. 4 Lineweaver-Burk plots of chlorpyrifos degradation by the immobilized enzyme.

between the enzyme and chlorpyrifos, which contributes to chlorpyrifos degradation. A smaller V_{max} was obtained when the free enzyme was immobilized in the embedding medium. This is likely due to the unlikeliness of the enzyme and chlorpyrifos coming into direct contact with each other, thus decreasing the chance of a reaction and consequently slowing the reaction rate.

Effect of repeated use of the immobilized enzyme for chlorpyrifos degradation: the results of assays for the repeated use of the immobilized enzyme show that the immobilized enzyme was able to degrade about 86.7% of chlorpyrifos for 1 hr, which decreased to 78.2% after the second use and to 69.4% after the third use. These data show that the immobilized enzyme could be repeatedly used.

We found that the degradation ability of immobilized enzyme decreased by approximately 17% after three uses. The free enzyme, however, could not be used repeatedly. This might be because the immobilized enzyme has a slower contact with the pesticide. Only a part of the enzyme is exposed to the pesticide. The inside part of the immobilized beads require time to be released. Therefore, after repeated use, the immobilized enzyme still has a good degradation ability. The free enzyme could only be used once before its degradation activity disappeared.

2.3 Biodegradation of chlorpyrifos by immobilized enzyme under different conditions

The chlorpyrifos degradation at different time by the free and immobilized enzyme under optimal temperature and pH were determined. The results showed that the amount of chlorpyrifos degraded by the immobilized enzyme increased quickly with a degradation rate reaching 86.7% after 60 min. In comparison, the degradation by the free enzyme increased slowly, resulting in less than 50% degradation after 80 min. The data also showed that the optimal test time for chlorpyrifos degradation by the immobilized enzyme was 1 hr; whereas, it was 10 min for the free enzyme.

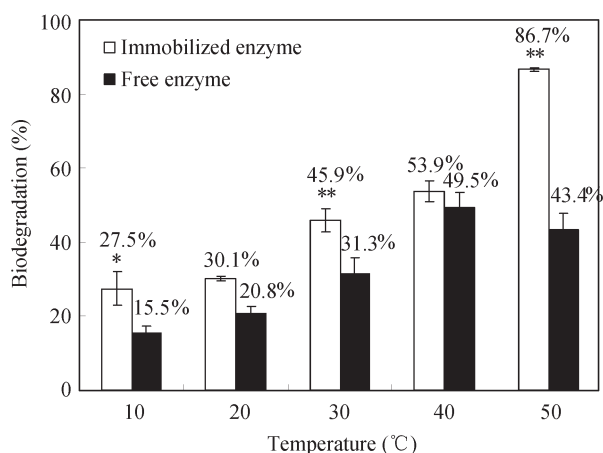The comparison between the free and immobilized enzyme to degrade chlorpyrifos are shown in Fig. 5.

Fig. 5 Biodegradation of chlorpyrifos by immobilized enzyme and free enzyme at different temperatures.
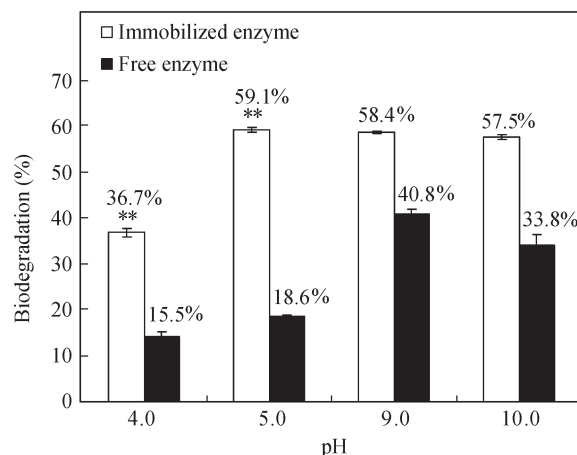
Fig. 6 Biodegradation of chlorpyrifos by immobilized enzyme and free enzyme at different pH values.

After immobilization, the degradation of chlorpyrifos by the enzyme increased with temperature, especially at 10 and 50°C. When the reaction was conducted at 10°C for 1 hr, the amount degraded by the immobilized enzyme was about 30%; however, it was only 15.5% for the free enzyme. At 50°C for 1 hr, the amount degraded by the immobilized enzyme was about 86.7%, but it was only 43.4% for the free enzyme. Comparing the free and immobilized enzyme for chlorpyrifos degradation at 10, 30 and 50°C, the P values were 0.017, 0.005 and 0.0, respectively ($P < 0.05$), therefore significant differences exist.

Differences between the ability of the free and immobilized enzyme to degrade chlorpyrifos at different pH values is shown in Fig. 6. After immobilization, the amount of chlorpyrifos degraded by the immobilized enzyme was approximately 60% at pH 5.0, 9.0, and 10.0, whereas the highest degradation rate achieved by free enzyme was about 40%.

At pH 4.0 and 5.0, P values were 0.02 and 0.01 ($P < 0.05$), respectively, therefore significant differences exist.

We found that the immobilized enzyme showed superior activity comparing to free enzyme when the reactions were carried out at different times at optimal temperature and pH. Although the free enzyme had a high degradation rate for short times, the immobilized enzyme had a higher degradation rate than the free enzyme over time.

At different temperatures and pH values the immobilized enzyme is more durable than the free enzyme. This suggests that the immobilized enzyme is better able to adapt to difference conditions. We found that the degradation ability of immobilized enzyme was superior to that of the free enzyme. The immobilized enzyme had a better degradation ability and is more suitable for future practical application in chlorpyrifos remediation under different environments.

3 Conclusions

To increase the repetitive use of the degrading enzyme and catalyzation characteristics, the parameters

for immobilizing the free enzyme of WZ-I have been examined. The degradation differences between the free enzyme and the immobilized enzyme were compared. The optimum parameters for enzyme immobilization were 3% sodium alginate (*W/V*) with an immobilization time of 4 hr at 4°C. The optimal conditions for chlorpyrifos degradation by the immobilized enzyme were 45°C and pH 8.0. The K_m and V_{max} values of the immobilized enzyme are 0.1672 mmol/L and 50.246 nmol/min, respectively, which are both less than the values obtained for the free enzyme. These results indicate that the immobilized enzyme had a better affinity with the chlorpyrifos molecule. The immobilized enzyme was also robust at different temperatures and under different pH conditions. After three repeated uses, the immobilized enzyme degraded about 69.4% of the substrate, and the physical characteristics had no visible change.

Acknowledgments

References

Aldana-Madrid M L, Valdez-Hurtado S, Vargas-Valdez N D, 2008. Insecticide residues in stored grains in Sonora, Mexico: Quantification and toxicity testing. *Bulletin of Environment Contamination and Toxicology*, 80: 93–96.

Ca'ceres T, He W X, Naidu R, Megharaj M, 2007. Toxicity of chlorpyrifos and TCP alone and in combination to *Daphnia carinata*: The influence of microbial degradation in natural water. *Water Research*, 41: 4497–4503.

Coony C M, 1999. EPA struggles to implement pesticides law. *Environmental Science and Technology*, *News*, 33: 8–9.

Echols K R, Brumbaugh W G, Orazio C E, 2008. Distribution of pesticides, PAHs, PCBs, and bioavailable metals in depositional sediments of the Lower Missouri River, USA. *Archives of Environment Contamination and Toxicology*, 55: 161–172.

Fang H, Xiang Y Q, Hao Y J, 2008. Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pak-choi. *International Biodeterioration and Biodegradation*, 61: 294–303.

Ghanem I, Orfi M, Shamma M, 2007. Biodegradation of chlorpyrifos by *Klebsiella* sp. isolated from an activated sludge sample of waste water treatment plant in Damascus. *Folia Microbiology*, 52: 423–427.

Huang G T, Guo C H, He J, 2008. Preliminary study on degradation of organics and inhibition of algae in polluted water by immobilized enzymes. *Chinese Journal of Water Purification Technology*, 27(5): 37–39.

Kumar S B, Walker A, Wright D J, 2002. Persistence of chlorpyrifos, fenamiphos, chlorothalorill and pendimethalin in soil and their effects on soil microbial characteristics. *Bulletin of Environment Contamination and Toxicology*, 69: 181–188.

Mallick K, Bharati K, Banerji A, 1999. Bacterial degradation of chlorpyrifos in pure cultures and in soil. *Bulletin of Environment Contamination and Toxicology*, 62(1): 48–54.

Singh B K, Walker A, Morgan J A W, Wright D J, 2003. Effects of soil pH to the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. *Applied and Environmental Microbiology*, 69: 5198–5206.

Singh B K, Walker A, Morgan J A W, Wright D J, 2004. Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. *Applied and Environmental Microbiology*, 70: 4855–4863.

US EPA (United States Environmental Protection Agency), 2002. Interim reregistration eligibility decision for chlorpyrifos. EPA 738-R-01-007. Final/Technical Report. U.S. Environmental Protection Agency, Washington DC.

Vidair C A, 2004. Age dependence of organophosphate and carbamate neurotoxicity in the postnatal rat: extrapolation to the human. *Toxicology and Applied Pharmacology*, 196: 287–302.

Vidya L C, Kumar M, Khanna S, 2009. Biodegradation of chlorpyrifos in soil by enriched cultures. *Current Microbiolgy*, 58(1): 35–38.

Wang J H, Zhu L S, Wang J, Qin K, 2005. Degrading characters of 3 chlorpyrifos degrading fungus. *Chinese Journal of Applied and Environmental Biology*, 11(2): 211–214.

Wang J, Wang X G, Zhu L S, Xie H, 2007. Atrazine degrading enzyme's immobilization in sodium alginate. *Chinese Journal of Agro-Environment Science*, 26(5):1733–1737.

Xie H, Zhu L S, Wang J, 2005. Enzymatic degradation of organophosphorus insecticide chlorpyrifos by fungus WZ-I. *Chinese Journal of Environmental Science*, 26(6): 164–168.

Xu G M, Li Y Y, Zheng W, 2007. Mineralization of chlorpyrifos by co-culture of *Serratia* and *Trichosporon* spp. *Biotechnology Letters*, 29(10): 1469–1473.

Yang L, Zhao Y H, Zhang B X, 2005. Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. *FEMS Microbiology Letters*, 251: 67–73.

Yu Y L, Shi F, Fan D F, 1999. Immobilization of a fenvalerate degrading enzyme and its degrative characteristics. *Chinese Journal of Pesticide Science*, 1(1): 74–77.

Zhao Y C, Fu R, Mo C H, 2008. Effect of Cd on remediation of DDT contaminated soil using different laccase forms. *Chinese Journal of Environmental Science*, 29(8): 2231–2235.