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SUPPLEMENTARY MATERIAL TO

Degradation of chlorpyrifos in contaminated soil by immobilized laccase

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DETAILS ABOUT LACCASE

The laccase can survive in ambient air[1] and the only product of the reaction is water, which makes it an environmentally friendly type of enzyme. Due to its particular extracellular degradation properties, this enzyme has been used in several industrial applications, such as dye effluent decolorization,[2,3] pulp and paper industry[4,5] and removal of herbicides.[6,7] Recently, laccase was also used as a cathode for reducing of oxygen to water in enzymatic biofuel cells.[8] During this process a stable current could be produced by using sustainable and renewable resources. The application of enzymes in the industry has greatly improved industrial processes by reducing the requirements for chemical treatments, limiting water usage and energy consumption.[9] In the decolorization of textile dyes, Vásquez *et al*.[10] used an enzyme that could reduce the high costs associated with the enzyme purification, and enhance the enzyme stability and recovery.

Laccasae immobilization

Sakai *et al.*[11] immobilized the *Pseudomonas cepacia* lipase on electrospun polyacrylonitrile fibers through physical adsorption. The results showed that the rate of the reaction conducted in the presence of adsorbed lipase was 23-fold higher than the one obtained by the use of the initial material, and that the immobilized *Pseudomonas cepacia* lipase could be employed for transesterification in non-aqueous solvent. A large number of enzymes have been used in the analysis technologies, recovery of enzymes from reaction solutions, and separation of the enzymes from substrates and products.[12] Mulagalapalli *et al.* adopted the method of immobilization of urease by pigeonpea fixed on agar to form a common cem-

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entitious material. There was an increase in the Michaelis constant (*Km*) from 3.23 to 5.07 mM after the immobilization.[13]

Noureddini *et al*.[14] immobilized *Pseudomonas cepacia* lipase by sol–gel entrapment, and the results showed that the immobilized lipase was consistently more active and stable than the free enzyme. Also, the enzyme retained more than 95% of its initial activity after twelve hours. The authors also applied these results in the hydrolysis of soybean oil. In addition, some studies paid attention to the development of immobilized enzyme entrapped within inorganic matrix, and the results indicated that the thermal stability of the immobilized lipases was enhanced by the entrapment.[15–18]

Glutaraldehyde possesses unique characteristics, which make it one of the most effective protein crosslinking reagents for immobilization by crosslinking.[19,20] Abelyan[21] linked aldehyde groups by various bifunctional aromatic diamines and then produced immobilized cells by glutaraldehyde, which was proved available in biocatalysis with high-molecular-weight substrates. Babu and Panda[22] cross-linked whole *Escherichia coli* containing penicillin amidase with surface modified precipitated silica and chitosan, and the chitosan obtained a catalyst with good mechanical stress stability. Yang and co-workers[23] studied a series of silica-supported macroporous chitosan membranes, which were prepared by silica particles. They used different crosslinking agents for covalent immobilization of biological macromolecules, especially enzymes. The immobilized enzyme possessed good operational stability and reusability properties, which could support its potential for practical applications. Another study was focused on a biofilm, which was treated with glutaraldehyde thus leading to an approximately five-fold increase in its bioadhesive strength.[24]

CHLORPYRIFOS AS PESTICIDE

Recently, China has been playing a more important role in the global chlorpyrifos (CPF) pesticide industry in terms of production, capacity and output.[25] Nearly 1,000 kinds of CPF-related pesticide products were registered in China, and almost 18,000 t of CPF were consumed annually.[26] CPF is moderately toxic, and its chronic exposure has been linked to neurological effects[27] and reproductive toxicology[28] in animals and human beings. In 2014, according to the statistics by CSICC, 367 pesticide enterprises reached a total production of 3.7 million t, generating a slight annual increase of 1.4 %. The extensive applications of chlorpyrifos in agriculture and the presence of their residuals in the environment have raised public concerns and demands for safe technologies to overcome the pollution and toxicity problems.

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EXPERIMENTAL DETAILS

Chemicals and equipment

The specific white-rot fungi was domesticated and trained in the microbiological laboratory of Shenyang University of Technology. The soils without Chlorpyrifos were obtained from the campus of the university. The chlorpyrifos was purchased from Shandong Rongbang Pesticide Chemical Co., Ltd. (China). Sodium alginate was purchased from Tianjin Bo Di Chemical Co., Ltd. (China). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Ruibio (Germany). Glutaraldehyde (AR) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

An ultraviolet–visible spectrophotometer (EOK UV721, China) was used for determination of laccase activity. High performance liquid chromatography (HPLC, Agilent 1260, Singapore) was used for determination of the degradation rate of carbofuran.

Illustration of the immobilization

Fig. S-1. Immobilized laccase by the method of embedding–crossliniking.

Preparation of chlorpyrifos contaminated soil

After sampling, the soil for use was filtered through 20 mesh at room temperature. The accurately weighted chlorpyrifos was placed into volumetric flask with volume of 500 ml, the flask was filled to the mark with water and shaken thoroughly. Then a certain volume of the above solution was placed into 300 g soil with zero background and then water was used in order to fully mix the solution and the soil. After the mixture was completely dry, the artificially chlorpyrifos contaminated soil was prepared. For each of the experiments slurry (soil to water ratio of 1 g:3 ml) was prepared by using 15 g soil and 45 ml water.

Determination of immobilized laccase activity

Immobilized laccase, 0.1 g, and 1.8 ml of reaction solution containing 200 μl of 500 mM $C_3H_4O_4$–$C_3H_2O_4Na_2$ (pH 4.5), 100 μl of 20 mM ABTS, and 1500 μL of H_2O were used. The reaction solution was mixed for 2 min at 28 °C, then the supernatant was taken into the HPLC system and the reaction was started. The increase in the absorbance at 420 nm was measured for the duration of the experiment, which was 3 min. The measured value was used to calculate the immobilized laccase activity. The immobilized laccase activity was calculated by the following formula:

$$U_i = \frac{1000\Delta AV}{\Delta t \varepsilon_{420} M_0 l} \quad \text{(S-1)}$$

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where ΔA was the increase in the absorbance for the duration of the experiment – 3 min, V was the volume of the reaction solution – 2 ml, Δt was 3 min, M_0 was the mass of the immobilized laccase – 0.1 g, U_i was the activity of immobilized laccase.

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