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Effects of metal ions on the catalytic degradation of dicofol by cellulase

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ABSTRACT

A new technique whereby cellulase immobilized on aminated silica was applied to catalyze the degradation of dicofol, an organochlorine pesticide. In order to evaluate the performance of free and immobilized cellulase, experiments were carried out to measure the degradation efficiency. The Michaelis constant, K_m, of the reaction catalyzed by immobilized cellulase was 9.16 mg/L, and the maximum reaction rate, V_{max}, was 0.40 mg/L/min, while that of free cellulase was K_m = 8.18 mg/L, and V_{max} = 0.79 mg/L/min, respectively. The kinetic constants of catalytic degradation were calculated to estimate substrate affinity. Considering that metal ions may affect enzyme activity, the effects of different metal ions on the catalytic degradation efficiency were explored. The results showed that the substrate affinity decreased after immobilization. Monovalent metal ions had no effect on the reaction, while divalent metal ions had either positive or inhibitory effects, including activation by Mn^{2+}, reversible competition with Cd^{2+}, and irreversible inhibition by Pb^{2+}. Ca^{2+} promoted the catalytic degradation of dicofol at low concentrations, but inhibited it at high concentrations. Compared with free cellulase, immobilized cellulase was affected less by metal ions. This work provided a basis for further studies on the co-occurrence of endocrine-disrupting chemicals and heavy metal ions in the environment.

Introduction

Dicofol, or 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol, is an insecticide synthesized from dichlorodiphenyltrichloroethane (DDT). It is used widely in farming to control pest mites on cotton, fruit trees, and vegetables (Qiu et al., 2005). Dicofol is an endocrine-disrupting chemical and difficult to degrade (Hoekstra et al., 2006). It not only interferes with the endocrine systems of fish, birds, and rodents, but also has strong estrogenic effects on humans (El-Din et al., 2011; Guo et al., 2012; Skinner et al., 2011). It can even increase the prevalence of human prostate cancer (Settimi et al., 2003). A risk assessment of manufacturing equipment surfaces contaminated with DDTs and dicofol conducted by Luo et al. (2014) showed that the hazard index of dicofol exceeded the acceptable hazard level. Fujii et al. (2011) confirmed that dicofol was detectable in human breast milk, with a geometric mean concentration in the Chinese breast milk samples of 9.6 ng/g lipid. This indicated the presence of extensive emission sources of dicofol in China. Between 1988 and 2002, 54,000 tons of technical DDT were used to produce about 40,000 tons of dicofol in China (Qiu et al., 2005). Therefore, dicofol is a great threat to the environment and human health.

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Biodegradation is regarded as an effective approach to remove dicofol (Osman et al., 2008; Zhao et al., 2010; Zhang et al., 2011). Catalysis with enzymes is one of the biodegradation pathways (Du et al., 2013; Pradub and Wattanachai, 2012). Research has shown that metal ions had impacts on enzyme activity and biological degradation efficiency (Jernejc and Legiša, 2002; Grasso et al., 2012; Madukasi et al., 2010; Gopinath et al., 2011). The co-occurrence of dicofol and heavy metal ions in farmland is common in China (Sun et al., 2012; Zhang and Shan, 2014). However, the effects of metal ions on the degradation of dicofol by cellulase are unknown. Therefore, six metal ions were introduced to test the degradation capability of cellulase.

In this study, an immobilization technique was first applied to cellulase to catalyze the degradation of dicofol. The influence of metal ions on the removal efficiency of dicofol by immobilized and free cellulase was compared. The results will provide support for practical application of this method to treat these environmental contaminants, especially in wastewater and wastewater sludge with high concentrations of toxic metals.

1. Materials and methods

1.1. Materials and equipment

Cellulase (extracted from *Trichoderma longibrachiatum*) was purchased from Ningxia Heshibi Biological Technology Co. (Ningxia, China). Dicofol was purchased from Yangzhou Pesticide Factory (Jiangsu, China). The chemicals glutaraldehyde (50% analytical grade) and 3,5-dinitrosalicylic acid (DNS, pure chemical) were obtained from Beijing Yili Fine Chemical and Sinopharm Chemical Reagent. Carboxymethyl cellulose sodium salt (CMC, pure chemical) were obtained from Beijing Xudong Chemical Works (Beijing, China). Manganous chloride and pyridine were obtained from Shantou Xilong Chemical Works (Guangdong, China). Lead chloride (analytic grade) was purchased from Beijing Hongxing Chemical Works (Beijing, China). Aminated silica was provided by the School of Chemistry and Molecular Engineering of East China University of Science and Technology (Shanghai, China). All other chemicals were purchased from Beijing Chemical Works (Beijing, China).

Instruments included a UV757CRT UV–Vis spectrophotometer (Shanghai Precision Scientific Instrument, Shanghai, China), WMNK-404 temperature controller (Shanghai Medical Instruments, Shanghai, China), and PHS-3A digital pH Meter (Hangzhou Wanda Instruments, Zhejiang, China).

1.2. Preparation of immobilized enzyme

First, 0.15 g of aminated silica carrier was weighed and 5 mL of glutaraldehyde solution was added to it. After the cross-linking reaction and washing away the excess glutaraldehyde, the pale yellow glutaraldehyde cross-linked carrier was obtained. Next, cellulase solution was added to the carrier at the temperature of 40°C, stirred magnetically to mix the cellulase thoroughly, and then washed and filtered to remove excess solution. The product was cellulase immobilized on yellow aminated silica.

1.3. Measurement of cellulase activity

The reaction of the glucose produced by the cellulase hydrolysis of sodium carboxymethyl cellulose (CMC) and 3,5-dinitrosalicylic acid (DNS) generated a brown–red substance with maximum absorption at a wavelength of 540 nm. Then, 0.02 mL cellulase (0.02 mL deionized water as reference) was added to the CMC solution (3.0 mL, 0.51%). Ten minutes later, 1.0 mL of DNS solution was added to 1.0 mL of supernatant, and both were heated in a water bath at the temperature of 100°C for 5 min. After cooling and diluting with water to 10 mL, the absorbance was determined at 540 nm spectrophotometrically. The corresponding cellulase activity was calculated from a standard glucose curve.

The amount of cellulase in 1 mL of enzyme solution completing the hydrolysis of CMC substrate at room temperature generating 1 μmol glucose per min is defined as one enzyme activity unit (U), as calculated by Eq. (1):

$$\text{Free enzyme activity per unit (U/g)} = \frac{\text{Glucose content (mg)} \times 1000}{\text{Enzyme content (mL)} \times 10\ \text{(min)} \times 180}. \quad (1)$$

For the measurement of immobilized cellulose activity, the steps are as follows.

First, 0.15 g of immobilized cellulase was reacted with 3.0 mL of CMC solution for 10 min, and 1.0 mL of supernatant was removed. Then 1.0 mL DNS solution was added. The mixture was heated in a boiling water bath for 5 min and then diluted to 10 mL with water. The absorbance at 540 nm was determined and the activity of immobilized cellulase was calculated from a glucose standard curve.

The unit activity of the immobilized enzyme was defined as the amount of immobilized cellulase completing the hydrolysis of CMC substrate at room temperature generating 1 μmol glucose per min, as calculated by Eq. (2):

$$\text{Immobilized enzyme activity per unit (U/g)} = \frac{\text{Glucose content (mg)} \times 1000}{\text{Enzyme content (g)} \times 10\ \text{(min)} \times 180} \quad (2)$$

1.4. Preparation of dicofol

First, 0.1 g of dicofol powder was extracted and dissolved in 10 mL pyridine. A 5 mL sample containing dicofol was extracted, concentrated with n-hexane, put in a 10 mL centrifuge tube, and evaporated to dryness in a water bath at a constant temperature of 60°C. Subsequently, 0.2 mL of deionized water was added to the centrifuge tube, and then 0.1 mL of 45% NaOH and 2.0 mL pyridine were mixed in and heated in a 100°C water bath for 3 min. After centrifugation for 2 min at 4000 r/min, the absorbance was determined at 530 nm.

1.5. Determination of dicofol concentration

The degradation reaction between 10 mg/L dicofol and 64.98 U/L cellulase was conducted in a 50 mL reaction system at room temperature. Aliquots of the reaction liquid were extracted at specified times and the dicofol concentration was determined to calculate the dicofol removal efficiency. Similarly, 0.2 g of