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Reaction mechanism of dicofol removal by cellulase

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ABSTRACT

It remains unclear whether dicofol should be defined as a persistent organic pollutant. Its environmental persistence has gained attention. This study focused on its degradation by cellulase. Cellulase was separated using a gel chromatogram, and its degradation activity towards dicofol involved its endoglucanase activity. By analyzing the kinetic parameters of cellulase reacting with mixed substrates, it was shown that cellulase reacted on dicofol and carboxyl methyl cellulose through two different active centers. Thus, the degradation of dicofol was shown to be an oxidative process by cellulase. Next, by comparing the impacts of tert-butyl alcohol (a typical OH free-radical inhibitor) on the removal efficiencies of dicofol under both cellulase and Fenton reagent systems, it was shown that the removal of dicofol was initiated by OH free radicals produced by cellulase. Finally, 4,4′-dichloro-dibenzophenone and chloride were detected using gas chromatography mass spectrometry and ion chromatography analysis, which supported our hypothesis. The reaction mechanism was analyzed and involved an attack by OH free radicals at the orthocarbon of dicofol, resulting in the degradation product 4,4′-dichloro-dibenzophenone.

Introduction

Dicofol, with the chemical name 2,2,2-trichloro-1,1-bis (4-chlorophenyl) ethanol, is a low-toxicity pesticide used globally in many countries for farming and planting, such as apple and cotton (Zahm and Ward, 1998). Dicofol is synthesized by dichlorodiphenyltrichloroethane (DDT), a pesticide that has been restricted by the Stockholm Convention as a persistent organic pollutant (POP). Dicofol is structurally similar to DDT and is an endocrine disturbance that is difficult to degrade in the environment (Hoekstra et al., 2006). It is now believed to exert a negative influence on both animals (Jadaramkunti and Kaliwal, 2002; Kojima et al., 2004; Wiemeyer et al., 2001) and humans (Reynolds et al., 2005; Settimi et al., 2003). The toxicity and environmental persistence of dicofol have drawn attention, and it has been prohibited in most developed countries (Li et al., 2015).

Due to the lack of evidence regarding its environmental persistence, it remains unclear whether dicofol should be listed as a POP and regulated worldwide. Thus, the environmental persistence and degradation process of dicofol are of great interest to both academia and policy makers.

Soil receives various pesticides for agricultural purposes, and the microbial population may be either adapted to these pesticides or capable of degrading them (Barr and Aust, 1994; Bartha et al., 1967; Fogarty and Tuovinen, 1991). Thus, bioremediation is an effective pollution control technology, and many studies have focused on biodegradation of pesticides (Alexander, 1999; Johnston and Camper, 1991; Salama et al., 1999). Many biological approaches such as enzymatic

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remediation have been used to clean up DDT (Kantachote et al., 2004; Purnomo et al., 2008; Zhao et al., 2010). However, limited data are available on dicofol removal (Osman et al., 2008). Thus, we investigated the removal of dicofol from water using cellulose, and found that cellulase can catalyze the degradation of dicofol effectively without other substrates (Zhang et al., 2011).

This work is an extension of our former studies on dicofol removal by cellulase (Zhang et al., 2011), and depicted its degradation process and identified the reaction mechanism. We separated cellulase into various components and tested the features and reaction activities towards dicofol. Bisubstrates of dicofol and carboxyl methyl cellulase (CMC) were applied and reaction mechanisms were tested to identify the active center of dicofol. By contrast experiments of Fenton reagent and product detection using gas chromatography mass spectrometry (GC–MS) and ion chromatography, the reaction process was depicted.

1. Experimental methods

1.1. Materials and equipment

Cellulase (extracted from *Trichoderma longbrachiatum*) was purchased from Ningxia Heshibi Bio-tec. Co. Ltd., China. Dicofol (original drug) was purchased from Yangzhou Pesticide Factory, China. Potassium sodium tartrate, sodium sulfite, citric acid, N-hexane, sodium hydroxide, hydrochloric acid (all analytically pure), and 30% peroxide (excellent purity) were purchased from the Beijing Yili Fine Chemical Co. Ltd., China. Compound 3,5 dinitrosalicylic acid (DNS, chemically pure), ferrous sulfate (analytically pure), and methylene blue (indicator) were produced by Sinopharm Chemical Co. Ltd., China. Carboxyl methyl cellulase (CMC, chemically pure) and crystalline phenol (analytically pure) were produced by Beijing Xudong Chemical Co. Ltd., China. Sodium citrate (analytically pure) was produced by Beijing Chemical Regent Company, China. Pyridine (analytically pure) was purchased from Shantou Xilong Chemical Co. Ltd., China. Sephadex G-75 (with a grain size of 40–120 μm) was purchased from Beijing Ruida Henghui Co., Ltd., China. Tert-butyl alcohol (analytically pure) was purchased from Beijing Yili Fine Chemical Co., Ltd., China.

Instrumentation included a UV757CRT UV–vis spectrophotometer (Shanghai Lengguang Tech. Corp., China), a WMNK-404 temperature controller (Shanghai Huachen Medical Instrument Corp., China); a PHS-3A Digital pH meter (Hangzhou Huada Instruments Co., Ltd., China), and a chromatography column of 16 × 750 mm (Beijing Ruida Henghui Science & technology development Co., Ltd., China).

1.2. Extracting and determining dicofol in water

Dicofol in water was extracted and concentrated using *n*-hexane and determined using the colorimetric determination method; our former study provided the optimal working conditions (Yang et al., 2009). In this experiment, we conducted recovery tests before applying to the dicofol: the dicofol concentrations used were 35.6, 16.9 and 0 μg/L, with recovery rates in the range of 91.6% to 113.4%, 85.2% to 108.3%, and 87.9% to 108.9% and RSDs of 8.1%, 8.8% and 8.6%, respectively.

1.3. Separating cellulase and determining their features and activities

Cellulase is a complex enzyme containing three components. We separated cellulase components using gel chromatography and tested the active component that catalyzed the degradation of dicofol. The process was as follows: 10 g of Sephadex G-75 was added to 200 mL of deionized water, after which it was heated in a boiling water bath for 2 hr. Flocculent deposit was then added to the tube and kept in solution until reaching 40 cm. After stabilization for 15–20 min, buffer solution was added to neutralize the tube. Subsequently, the valve was closed after the solution was 1–2 mm higher than the gel. Next, 5 mL of enzyme and washing solution was added and washed at a rate of 12 mL/hr; 2 mL of elution was collected per tube. Finally, the absorbance of eluents in each tube was tested using an ultraviolet spectrophotometer with a 280-nm wavelength.

We tested the enzymatic activities of components in these three peak positions and reacted them with dicofol, respectively. We then compared their degradation efficiency and determined the active components during degradation.

1.4. Determining the active center of cellulase

1.4.1. Testing reaction kinetics of cellulase with CMC

Various concentrations of carboxyl methyl cellulase (CMC) were used to react with cellulase (at a concentration of 64.98 U/L) for 10 min at room temperature. The amount of glucose generated in the reaction system represented the hydrolytic reaction rate of cellulase. We then determined the Michaelis constant (K_m, mg/L) and maximum reaction rate (V_m, mg/(L·min)) based on the Lineweaver–Burk method.

1.4.2. Testing reaction kinetics of cellulase with dicofol (DCF)

Various concentrations of dicofol (DCF) were reacted with cellulase (at a concentration of 64.98 U/L) at room temperature in a 50-mL reaction system. Reaction solution was removed at various intervals, the concentrations of DCF were determined, and the DCF removal efficiency was calculated. We then applied the Lineweaver–Burk method to calculate K_m and V_m.

1.4.3. Testing reaction kinetics of cellulase with bisubstrates of DCF and CMC

The initial reaction rates of cellulase degrading bisubstrates of CMC and DCF were measured, and the concentrations of the various substrates were set as the equation below, where K can be expressed as (Keleti et al., 1987):

$$K_{m,CMC} \times C_{DCF} + K_{m,DCF} \times C_{CMC} = K \quad (1)$$

where, K is an arbitrary value close to K_m when concentrations of DCF and CMC are in an applicable range, $K_{m,CMC}$ (mg/L) is the Michaelis constant of enzyme hydrolyzing CMC, C_{DCF} (mg/L) is the concentration of DCF, $K_{m,DCF}$ (mg/L) is the Michaelis constant of enzyme degrading DCF, and C_{CMC} (mg/L) is the concentration of CMC. The concentrations of DCF and CMC are shown in Table 1.