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Degradation of the herbicide isoproturon by laccase-mediator systems

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ABSTRACT

This study investigated the degradation of the herbicide isoproturon using *Trametes versicolor* laccase and its laccase-mediator systems. Isoproturon was poorly degraded with laccase alone, due to the presence of the relatively strong withdrawing electron group (—NH—CO—$N(CH_3)_2$) in the chemical structure of isoproturon. This study showed that laccase-mediator systems can effectively enhance the degradation rate of isoproturon. Within 24 h, isoproturon was completely degraded in the presence of 0.3 U/mL laccase and 1 mM 1-hydroxybenzotriazole (HBT). Compared with natural mediators, synthetic mediators are more effective in the laccase-mediated degradation of isoproturon. However, laccase activity rapidly declined in the presence of the synthetic mediator HBT. Degradation occurred at an acidic pH and optimum temperature was 50 °C. A high concentration (10 mM) of metal ions Cu^{2+}, Zn^{2+} and Cd^{2+} positively enhanced isoproturon degradation with the laccase-HBT system. Polyethylene glycol (PEG) can reduce the HBT dosage in isoproturon degradation with the laccase-HBT system and enhance the degradation rate of isoproturon by increasing laccase stability. Moreover, a higher degradation rate of isoproturon was observed when incubating isoproturon with the laccase-HBT system in real wastewater compared to that in sodium citrate buffer. Finally, transformation products showed much lower ecotoxicity to green algae than the original isoproturon. This study concludes that laccase-mediator systems have great potential to treat industrial wastewater containing the herbicide isoproturon.

1. Introduction

Pesticides and herbicides are commonly used in modern agriculture and support the effective production of a variety of agricultural crops [1]. Isoproturon, a type of phenylurea herbicide, is used mostly to control the growth of weeds such as ryegrass, silky bentgrass, and many broadleaf species in spring and winter wheat [2]. Isoproturon, which produces a carcinogenic metabolite, negatively affects the environment, specifically aquatic invertebrates, algae, and microbes. Its half-life is approximately 15 days in tropical climates and 40 days in moderate climates [3–5]. In 1997, approximately 3300 t of isoproturon were applied to 3.0 million hectares of agricultural land in the United Kingdom (UK). Due to its widespread and extensive usage, isoproturon has been detected as a pollutant in rivers, streams, marine waters, and groundwater [6]. Given its environmental impacts, it is important to remove this compound from wastewater. Degrading organic pollutants using physical or chemical methods such as photocatalytic degradation, flocculation, coagulation, ozonation, and fenton oxidation, is time-consuming, costly, and largely ineffective [7,8]. Low photonic efficiency of the photocatalytic method makes it difficult to treat highly loaded chemical industry wastewater or wastewater less transparent [9,10]; flocculation, coagulation and ozonation have resulted in poor removal of aromatic load in real wastewater [11]; fenton oxidation is an efficient procedure for organic pollutants transformation, however, it necessitates the addition of catalysts that are expensive and may lead to secondary pollution in the treatment of wastewater [12]. More recently, researchers have investigated the use of microorganic approaches to degrade herbicides and other organic contaminants, especially through white-hot fungi [2,13]. However, these methods are also time-consuming and microorganism survival in the target field is difficult [1].

Given these challenges, enzymatic transformation of herbicides or pesticides has gained increasing attention. White-hot fungi produce extracellular oxidoreductase, containing laccase, lignin peroxidase, manganese peroxidase and versatile peroxidase. These enzymes can be used to degrade herbicides or other recalcitrant pollutants [14]. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) belongs to the family of multicopper oxidases and can catalyze the oxidation of various phenolic compounds and aromatic amines with concomitant four-electron reduction of molecular

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oxygen to water [15]. The enzyme laccase is known to be specific, ecologically sustainable, and a proficient catalyst; it has been used in many different fields, such as food-processing industry [16], dye decolorization [17], paper and pulp industry [18], and bioremediation [19]. Moreover, to confront increasing environmental pollution problems, the ability of laccase to remove phenolics, xenobiotics, and other aromatic compounds has elicited increasing interest [20,21].

Due to its low–redox potential, laccase only directly degrades low-redox-potential phenolic compounds. It can not oxidize the most recalcitrant aromatic compounds, including some herbicides [22]. Nonetheless, mediators can serve as an electron shuttle between laccase and target compounds, extending the range of laccase to different substrates. This makes laccase an attractive option to increase the number of targeted pollutants [23]. In the presence of a suitable redox mediator, laccase can therefore also oxidize non-phenolic structures. Laccase-mediator systems, including natural and synthetic mediators, have been used to degrade a wide range of pollutants, such as those found in personal care products, dyes, and herbicides [24–26]. However, no study has investigated the effect of mediators on laccase stability during herbicides or pesticides degradation using laccase-mediator systems. There have also been few reports about the effects of different parameters on the degradation ability of laccase-mediator systems.

The main objective of this work was to test the ability of the laccase-mediator systems to degrade and detoxify the herbicide isoproturon to help in the reduction of its environmental impact. The effects of natural and synthetic mediators on the stability of laccase during the process of isoproturon degradation using the laccase-mediator systems were also studied. In addition, the influence of the operational parameters (pH, temperature, metal ions, and PEG) on isoproturon degradation using the laccase-HBT system were investigated. Moreover, identification and ecotoxicity evaluation of the degraded products was carried out. Based on these experiments, the potential of the laccase-mediator systems for the bioremediation of wasterwater containing organic pollutants was assessed.

2. Materials and methods

2.1. Chemicals

Isoproturon, violuric acid (VA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1-hydroxybenzotriazole (HBT), vanillin, syringaldehyde, acetosyringone and PEG 4000 were purchased from Sigma-Aldrich. All other chemicals were of analytical grade.

2.2. Fungal strain, media and cultural conditions

Trametes versicolor was obtained from the New Zealand Timber Anticorrosion Research Center. The strain was maintained on potato dextrose agar slants at 4 °C and subcultured every 3 months. One-week-old fully grown slants at 30 °C were used to prepare the inoculum. Four 5-mm disks of fungal mycelia were excised from the agar slants and then transferred to 250 mL Erlenmeyer flasks containing 50 mL of the following inoculum media (g/L): glucose, 10.0; yeast extract, 1.0; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; urea, 0.3; and $MgSO_4{\cdot}7H_2O$, 0.1. The media were adjusted to pH 4.8. The culture was allowed to grow in the media for 5 days and used as inoculums.

Laccase was produced using solid-state fermentation (SSF) by *T.versicolor*. Up to 10 g of solid substrates, containing 6 g of corn bran and 4 g of wheat bran, were added to 250 mL cotton-plugged Erlenmeyer flasks. A basal mineral salt solution was used to adjust the moisture content to 65% with the following composition (g/L): $(NH_4)_2SO_4$, 2.5; $CaCl_2$, 2.4; KH_2PO_4, 2.5; $MgSO_4{\cdot}7H_2O$, 2.5; and $CuSO_4$, 0.1. The initial pH value of the medium was adjusted to 5.0. After being autoclaved at 121 °C for 60 min, each flask was inoculated with 1 mL of inoculum and incubated at 30 °C.

Laccase produced by SSF was extracted with distilled water (10 mL water/g substrate), by shaking the sample at 180 rpm for 1 h at 30 °C. The mixture was centrifuged at 4000 rpm for 20 min at 4 °C to remove the mycelium. The supernatant was collected and used to conduct the enzyme activity assay.

2.3. Enzymatic activity

Laccase activity was determined using ABTS as a substrate [27]. The reaction mixture included 50 μL of culture supernatant, 950 μL of citrate-phosphate buffer (pH 4.5), and 1 mL of ABTS solution (2 mM). The temperature was adjusted to 30 °C. Oxidation was followed by increasing absorbance at 420 nm ($\varepsilon = 3.6 \times 10^4\ cm^{-1}\ M^{-1}$). One activity unit was defined as the amount of enzyme needed to oxidize 1 μM of ABTS per minute.

2.4. Degradation tests

Isoproturon degradation assays were conducted in a 50 mL Erlenmeyer flask, each containing 10 mL of the reaction mixture. The reaction mixture contained sodium citrate buffer (100 mM and pH 5.0), 100 mg/L isoproturon, and laccase with or without mediators. The isoproturon concentration (100 mg/L) was set according to the previous detection of the wastewater of four pesticide plants producing the herbicide isoproturon in our lab, which showed the isoproturon concentration of 50–100 mg/L. A control test with the same amount of heated-denatured laccases and isoproturon concentrations in a 100 mM sodium citrate buffer (pH 5.0) was also conducted. Both degradation and control tests were kept at 30 °C under aerobic conditions, which were maintained by stirring at 120 rpm for 24 h.

Before and after enzymatic treatment, the isoproturon concentrations of all samples were measured using an HPLC system (Agilent 1200, USA) fitted with an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm × 5 μm) and eluted with 65% methanol in water at a flow rate of 1.0 mL/min at 25 °C. The isoproturon concentration was determined using a UV detector at 240 nm. The chemical nature of the degradated products was identified by gas chromatography coupled to a mass detector, GC–MS (Aligent 6890 N Gas chromatograph with 5973 Mass spectrometer). The oven temperature was programmed to rise from 50 °C to 280 °C at 10 °C per min. Ionization was carried out by electron impact (EI) at 70 eV, and the mass range (40–1000 m/z) was scanned in 0.4 s.

The degree of degradation was calculated using the equation $\frac{(C_0-C)}{C_0} \times 100\%$, where C_0 is the initial concentration, and C is the final concentration.

2.5. Effect of laccase mediators on isoproturon degradation

The effect of laccase mediators on isoproturon degradation was tested using three synthetic mediators (ABTS, HBT, and VA) and three natural mediators (vanillin, syringaldehyde, and acetosyringone). The reaction mixtures contained 100 mM sodium citrate buffer (pH 5.0), 100 mg/L isoproturon, 0.3 U/mL laccase, and different mediators at different concentrations (0.2 mM, 0.5 mM, 1 mM, and 2 mM). The reaction was conducted at 30 °C for 24 h; aerobic conditions were maintained by stirring the sample at 120 rpm.