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Degradation of Pentachlorophenol by Potato Polyphenol Oxidase

Mei-Fang Hou,[†,§] Xiao-Yan Tang,[§,△] Wei-De Zhang,[*,†] Lin Liao,[§] and Hong-Fu Wan[§]

[†]School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510641, China

[§]Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-environmental and Soil Sciences, Guangzhou 510650, China

[△]Faculty of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, Lin'an 311300, China

ABSTRACT: In this study, polyphenol oxidase (PPO) was extracted from commercial potatoes. Degradation of pentachlorophenol by potato PPO was investigated. The experimental results show that potato PPO is more active in weak acid than in basic condition and that the optimum pH for the reaction is 5.0. The degradation of pentachlorophenol by potato PPO reaches a maximum at 298 K. After reaction for 1 h, the removal of both pentachlorophenol and total organic carbon is >70% with 6.0 units/mL potato PPO at pH 5.0 and 298 K. Pentachlorophenol can be degraded through dechlorination and ring-opening by potato PPO. The work demonstrates that pentachlorophenol can be effectively eliminated by crude potato PPO.

KEYWORDS: pentachlorophenol, polyphenol oxidase, degradation, enzyme

■ INTRODUCTION

Pentachlorophenol (PCP, C_6Cl_5OH), an ionizable hydrophobic organic contaminant, has been used extensively in agricultural, industrial, and domestic applications as a component of fungicides, bactericides, herbicides, insecticides, molluscides, biocides, and wood preservatives.[1−3] PCP not only gives rise to accumulation and biological amplification but also causes immunological and endocrine disorders and infertility problems in humans.[1] On the basis of evidence from animal toxicity studies and human clinical data, the U.S. Environmental Protection Agency (U.S. EPA) has classified PCP as a probable human carcinogenic chemical (B2). There are several techniques for the degradation of chlorophenols, such as zero-valent metal-based reduction,[3,4] advanced oxidation processes (AOPs),[5−10] and bioremediation.[11−19] With the aim of producing highly potent nonspecific oxidants, especially to obtain hydroxyl radical (•OH), AOPs have been developed for the degradation of recalcitrant organic contaminants.[20] Besides AOPs, enzymes can also produce reactive oxygen species (ROS).[21−24] Being powerful biocatalysts, enzyme-catalyzed oxidation as the alternative method for the degradation of PCP has attracted much attention in recent years.[16−19]

Copper-containing enzymes are usually involved in dioxygen binding, activation, and reduction and perform a variety of critical biological functions,[25−27] during which •OH, H_2O_2, and other ROS are produced through the Fenton reaction.[28] Polyphenol oxidases (i. e., laccase and tyrosinase) are a group of copper-containing enzymes that can catalyze the oxidation of phenol derivatives in the presence of O_2.[29,30] In the past three decades, polyphenol oxidase (PPO) has been used for the degradation of organic contaminants.[18,31−33] The substrate specificity and catalytic competence vary greatly for PPOs from different sources.[26] Laccase is often found in fungi, and fungal laccase has been widely studied for degrading various contaminants.[26,34] Compared with the sparse availability and high cost of laccase, plant PPO can be obtained easily and cheaply for potential applications in wastewater and soil treatment.[35] However, there are few reports of the degradation of PCP by plant PPO thus far. Potato is widespread and cheap for extracting PPO,[36] and potato PPO is very effective in the removal of dye pollutants.[37]

The objective of this work is to investigate the degradation of PCP by potato PPO. The degradation mechanism of PCP will be discussed afterward.

■ MATERIALS AND METHODS

Materials. PCP (98%) was obtained from Aldrich. Acetic acid, isopropanol, ascorbic acid, phosphate, catechol, and other chemicals were purchased in analytical grade from Guangzhou Chemical Co., Guangzhou, China. Potatoes were purchased from the local vegetable market, Guangzhou, China. Methanol and hexane were obtained in HPLC grade from Acros. Unless specified, all chemicals were used as received.

Potato Polyphenol Oxidase. The extraction procedure for the PPO is derived from the literature[36−39] and modified as follows: Potatoes (100 g) were homogenized with 100 mL of 0.1 M cold phosphate buffer at pH 7.0 in a blender. The homogenate was filtered through cheesecloth and centrifuged at 4500 rpm for 10 min. The supernatant was collected and added into 200 mL of cold acetone under stirring for 15 min before being sealed for 3 h. All steps above were carried out at 277 K. The sediments in the cold acetone solution were collected, centrifuged, and dried with vacuum freezing, and then the potato PPO was obtained and stored at 261 K.

Activity Test. Potato PPO activity was assayed with 0.20 M catechol as a substrate by UV−vis spectrophotometry (TU1800-PC, Beijing, China); 0.20 mL of 0.20 M catechol and 2.7 mL of 50 mM phosphate buffer at pH 6.5 were added with 0.10 mL of 4 mg/mL PPO in a 1 cm light path cuvette. The total assay volume was 3.0 mL. The increase in absorbance at 400 nm and 298 K was recorded automatically. Each sample was assayed in triplicate. One unit of PPO activity was defined as the amount of enzyme that caused a change in absorbance of 0.0010 per min.

Table 1. Pseudo-First-Order Kinetic Constants (k_1) of the Degradation of PCP

10 mg/L PCP, 6.0 Units/mL Potato PPO, at 298 K for Initial 30 min						
pH	4.0	4.5	5.0	6.0	7.0	8.0
k_1 (1/min)	0.019	0.025	0.045	0.022	0.025	0.014
R^2	0.986	0.953	0.937	0.996	0.916	0.896

10 mg/L PCP, 6.0 Units/mL Potato PPO, at pH 5.0 for Initial 30 min						
temperature (K)	293	298	303	308	313	318
k_1 (1/min)	0.019	0.045	0.020	0.017	0.012	0.004
R^2	0.993	0.937	0.903	0.914	0.930	0.944

10 mg/L PCP, at pH 5.0 and 298 K for Initial 30 min					
enzyme dose (units/mL)	1.5	3.0	4.5	6.0	7.5
k_1 (1/min)	0.022	0.032	0.038	0.045	0.049
R^2	0.985	0.980	0.959	0.937	0.887

| 6.0 Units/mL Potato PPO, at pH 5.0 and 298 K for Initial 30 min				
PCP C_0 (mg/L)	5	10	15	20
k_1 (1/min)	0.058	0.045	0.019	0.008
R^2	0.911	0.937	0.860	0.952

Enzymatic Degradation of PCP. Unless specified, batch experiments were carried out for the degradation of PCP by PPO in a 250 mL flask exposed to air at 298 K in the dark. To obtain a high concentration of PCP, ethanol was used with the ratio of 1.0% (v/v) to help the dissolution of PCP in the solution. Experiments for determination of the optimum pH were conducted in 50 mM citrate/phosphate buffer in the range of 3.5−8.0 for the degradation of PCP in the reaction solution of 200 mL. The effects of temperature (293−318 K), the dosage of enzyme (1.5−7.5 units/mL), and the initial concentration of PCP (5−20 mg/L) on its degradation were examined at the optimum pH.

It is reported that the enzymatic oxidation of PPO and the degradation of chlorophenols by AOPs can be described by the pseudo-first-order kinetics model (eq 1).[40,41] Thus, the degradation kinetics of PCP by PPO in this work was fitted by the pseudo-first-order kinetic model

$$-\ln\left(\frac{C}{C_0}\right) = k_1 t \qquad (1)$$

where C and C_0 are the concentration of PCP at time t and 0 min, respectively, and k_1 is the pseudo-first-order rate constant for the degradation of PCP by PPO (1/min). The plot of $-\ln(C/C_0)$ versus t should give a linear relationship from which k_1 can be determined from the slope of the plot.

Characterization and Analysis. The pH value during the reaction was monitored by a pH-meter (pHS-3C, Shanghai, China). The concentration of PCP was determined by UV−vis spectrophotometry (TU1800-PC, Beijing, China) and HPLC (Waters 1525/2487). In HPLC, a mobile phase consisting of 1.0% (w/w) acetic acid (20%) and methanol (80%) was used and PCP was detected by a UV detector at 295 nm.[42] The total organic carbon (TOC) measurements were measured with a Shimadzu 5000A (Japan). To detect the TOC removal of PCP, several control experiments were conducted in triplicate, and the clear supernatant was taken for analysis. The intermediates were determined by gas chromatography−mass spectrometry (GC-MS, Thermo Trace-DSQ-2000) on a Finnigan Trace DSO Ultra instrument equipped with an Agilent silicon capillary column (0.25 mm inside diameter by 30 m length). The supernatant samples were extracted by hexane and then acetylated by purified acetic anhydride before GC-MS analysis.[6,8] The efficiency of hexane extraction was no less than 95%.
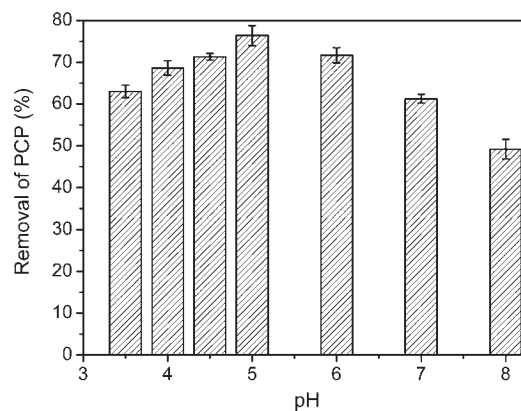
Figure 1. Effect of pH value on the degradation of 10 mg/L PCP by 6.0 units/mL potato PPO at 298 K for 60 min.

■ RESULTS AND DISCUSSION

Effect of pH on the Degradation of PCP. It is well-known that changes in the pH value of the reaction solution may not only affect the shape of an enzyme protein but may also change the charge properties of the substrate. The pH value of the reaction solution might have a significant effect on the degradation of PCP by potato PPO. Experiments were carried out for the degradation of 10 mg/L PCP by 6.0 units/mL potato PPO at 298 K. The results are shown in Figure 1, and the pseudo-first-order kinetics constants are listed in Table 1. It can be seen that the optimum pH value is 5.0.

The optimum pH for PPO catalyzing various substrates is different, which might be ascribed to the nature of the sources of enzyme, the properties of substrates or additives, and the purity of enzyme.[43,44] The transformation of chlorinated hydroxyphenylureas by laccase achieved a maximum between pH 4.0 and 5.0.[45] Mushroom PPO catalyzed the transformation of phenol very effectively at pH values ranging from 5.0 to 8.0 with an optimum at pH 7.0.[44] The optimum pH values for PPO from *Ferula* leaves and stems were found to be 7.0 with catechol, 6.5 with (+)-catechin, and 6.0 with 4-methylcatechol, chlorogenic acid, and (−)-epicatechin.[44] The common pH range for optimal grape PPO activity, as well as other fruits, is known to be pH 5.0−7.0, such as for Victoria grape PPO, with the maximum activity at pH 5.0.[30] It can be seen that the optimal pH for the most PPO ranged from 5.0 to 7.0 and that PPO is more active in weak acid conditions than in basic conditions. It is reported that the range between pH 4.0 and 5.0 is better than the lower or higher pH of the reaction solution for the degradation of PCP by horseradish peroxidase in the presence of hydrogen peroxide.[15] The experimental results in this study show that the degradation of PCP by potato PPO achieves a maximum activity at pH 5.0. Thus, a pH value of 5.0 was used in the following experiments.

Effect of Temperature on the Degradation of PCP. There are different optimum temperatures for PPOs catalyzing different substrates such as PPO from *Ferula* leaves at 285 K with catechol, at 286 K with (−)-epicatechin, at 293 K with chlorogenic acid, at 295 K with 4-methylcatechol, and at 303 K with (+)-catechin.[46]

The effect of temperature on the degradation of 10 mg/L PCP by 6.0 units/mL potato PPO was examined at pH 5.0 for 60 min. The results show that the peak of degradation appears at 298 K (Figure 2 and Table 1). When the temperature is below 303 K,

the degradation of PCP by potato PPO can be >60% and up to 76% at 298 K. It can be concluded that the effective degradation of PCP can be achieved with cheap potato PPO at room temperature.

Initial Concentration of PCP and PPO Dosage. When the dosage of potato PPO is 6.0 units/mL, the degradation kinetics constants of PCP decrease from 0.058 to 0.008 1/min with the increase of initial concentration of PCP from 5 to 20 mg/L (Table 1). To discover the effect of PPO dosage on the degradation of PCP, experiments were conducted at pH 5.0 and 298 K. The degradation kinetics constants of 10 mg/L PCP rise from 0.022 to 0.049 1/min upon the increase of PPO dosage from 1.5 to 7.5 units/mL (Table 1). It can be seen that the highly
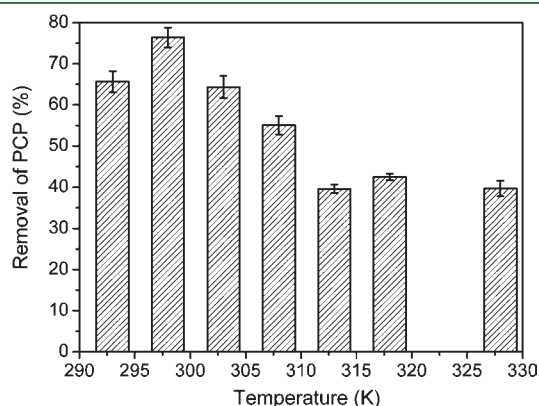

Figure 2. Effect of temperature on the degradation of 10 mg/L PCP by 6.0 units/mL potato PPO at pH 5.0 for 60 min.
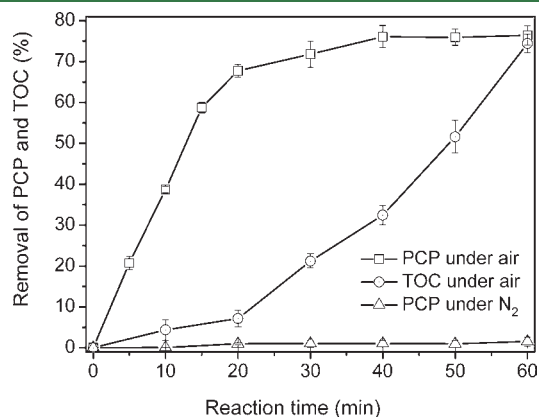

Figure 3. TOC removal and degradation of 10 mg/L PCP by 6.0 units/ mL potato PPO at pH 5.0 and 298 K under air or N_2.

effective degradation of PCP can be achieved with the lower ratio of PPO to PCP.

Mechanism of PCP Degradation. It is reported that the oxidation of phenols by PPO was favored by ensuring an adequate supply O_2 but greatly inhibited by bubbling with N_2.[47] Thus, the effect of N_2 on the degradation of PCP by potato PPO was examined. The result shows that there is little degradation of PCP by potato PPO under N_2 (Figure 3), which suggests that O_2 is a key to degrade PCP and there is little adsorption of PCP onto potato PPO. In the experiments, there is also little polymeric substance detected because the dose of PPO and the concentration of PCP are very low.

Figure 3 shows the TOC removal during the degradation of PCP by potato PPO under air. It can be seen that the TOC removal is below 10% within 20 min and increases obviously to 74% after 60 min under air, which suggests the ring-opening degradation of PCP. The PCP removal is 68% within 20 min and increases slowly to 76% under air. It can be deduced that the dechlorination of PCP by potato PPO happened first and rapidly and was then followed by the ring-opening degradation under air.

The reactivity of the copper site to O_2 in the copper-containing enzymes has been discussed in detail.[27,48−50] The reduction of O_2 by copper-containing enzymes might facilitate the production of ROS through the Fenton reaction.[28] The prepared potato PPO as a copper-containing enzyme can be a catalyst to activate O_2 and then produce ROS for degradation of PCP. Isopropanol is an effective scavenger for •OH.[51,52] When isopropanol was introduced into the reaction solution, the degradation of PCP decreased to near 29%. Thus, •OH may be the important ROS to degrade PCP by potato PPO. Compared with other enzyme-mediated degradation and AOPs (Table 2), potato PPO is a
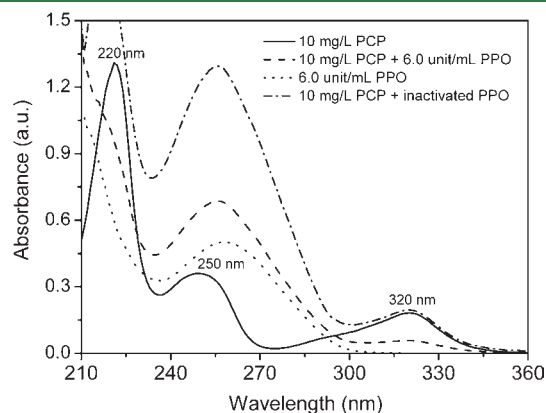

Figure 4. Temporal UV−vis spectra of 10 mg/L PCP by 6.0 units/mL potato PPO at pH 5.0 and 298 K for 30 min.

Table 2. Comparison among AOPs, Biotechnique, Potato PPO, and Other Enzyme-Mediated Degradations of PCP

treatment process	$C_0{}^a$ (mg/L)	$pH_0{}^b$	reaction duration	PCP removal (%)	TOC removal (%)	ref
photo-Fenton	13.3	5.0	5 h	90		8
sonication	16	7.3	4 h	100		9
photodegradation	10		25 h	100		10
biodegradation by WRF^c	2.7		6 weeks		42.4	13
HRP^d + H_2O_2	13.3	4−5	3 h	95		15
this work	10	5.0	20 min	68	7.2	
	10	5.0	1 h	76	74	

^{a} C_0 = initial concentration of PCP (mg/L). ^{b} pH_0 = initial pH value of the reaction solution. ^{c} WRF, white rot fungi. ^{d} HRP, horseradish peroxidase.
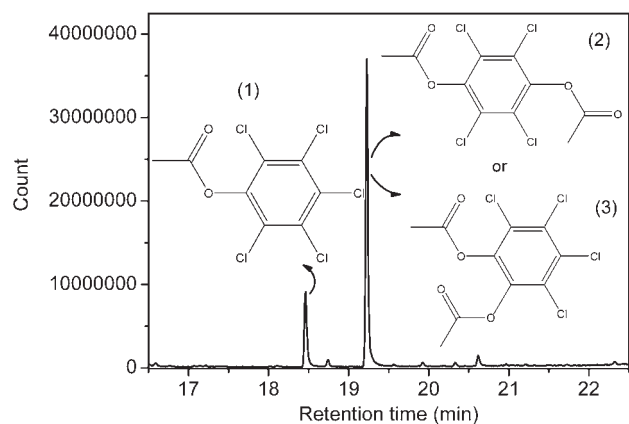
Figure 5. Dechlorinated intermediates detected by GC-MS during the degradation of 10 mg/L PCP by 6.0 units/mL potato PPO at pH 5.0 and 298 K within 10 min.

more effective, economical, and environmentally friendly catalyst for degradation of PCP.

The temporal UV−vis spectra of PCP by potato PPO under air are shown in Figure 4. The strong absorbance band at 220 nm can be ascribed to the substituted aromatic ring of PCP. The bands at 250 and 320 nm can be assigned to conjugated system and free electron of O or Cl atoms of PCP. There is an absorption band around 256 nm but no absorption band at 320 nm in the UV−vis absorption of potato PPO solution. During the degradation of PCP by potato PPO, the absorption of PCP at 320 nm decreases sharply within 30 min and the band around 250 nm also decreases, which suggest the dechlorination of PCP by potato PPO. Meanwhile, the adsorption of PCP at 220 nm decreases and has a blue shift, which suggest the ring-opening degradation of PCP by potato PPO.

The degradation of 10 mg/L PCP by 6.0 units/mL potato PPO was conducted at pH 5.0 and 298 K. The intermediates were detected by GC-MS. As shown in Figure 5, the acetylated products of PCP and its dechlorinated intermediates were detected. According to the GC-MS result, tetrachlorohydroquinone (TeCHQ) and tetrachlorocatechol (TeCC) are the primary dechlorinated intermediates during the initial degradation of PCP by potato PPO. High-chlorinated phenols are expected to be more difficult to degrade than low-chlorinated phenols or unsubstituted phenols.[53,54] Generally, the ortho-Cl and para-Cl of chlorophenols are easily attacked by ·OH or other active species in most AOPs[9,55] and microbe- or enzyme-catalyzed oxidation reactions.[17,56] The dechlorination and the production of more hydroxyl substituent compounds synchronized before the aromatic ring-opening. Thus, PCP can be degraded through dechlorination and ring-opening by potato PPO. On the basis of the results from GC-MS, HPLC, and TOC analysis, it can be seen that the dechlorination is the main step during the initial degradation of PCP. The ring-opening degradation of PCP and the TOC decrease rapidly after the initial dechlorination of PCP by enzyme. As a result, few intermediates were detected by GC-MS analysis.[33,57,58] Further studies are needed to elucidate the degradation mechanism of PCP by PPO.

In summary, potato polyphenol oxidase was extracted from potatoes and its catalytic activity for the degradation of pentachlorophenol was investigated. The potato polyphenol oxidase shows superb catalytic activity for the degradation of pentachlorophenol in the presence of O_2 at room temperature. This finding paves the way for efficiently eliminating the highly toxic pentachlorophenol in the environment. The work also provides a new insight for the potential application of potato polyphenol oxidase.

■ AUTHOR INFORMATION

Corresponding Author
*Phone/fax: 86-20-8711 4099. E-mail: zhangwd@scut.edu.cn.

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