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Transformation, products, and pathways of chlorophenols via electro-enzymatic catalysis: How to control toxic intermediate products

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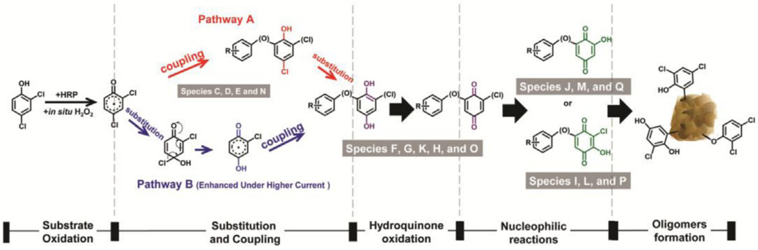
HIGHLIGHTS

- A detailed pathway map for DCP transformation in electro-enzymatic system is proposed.
- Higher current can particularly enhance dechlorination via hydroxylation steps.
- A controllable electro-enzymatic method is developed for DCP detoxification.
- Removal of DCP achieves 99.7% within 10 min of reaction at an optimal condition.
- CO_2 may significant promote the enzymatic pathways by pH buffer effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlorophenols can be easily oxidized into chlorobenzoquinones (CBQs), which are highly toxic and have been linked to bladder cancer risk. Herein, we report the transformation, products, and pathways of 2,4-dichlorophenol (DCP) by horseradish peroxidase (HRP) and electro-generated hydrogen peroxide (H_2O_2) and suggest methods to control the formation of toxic intermediate products. After a 10-min electroenzymatic process, 99.7% DCP removal may be achieved under optimal conditions. A total of 16 reaction products, most of which are subsequently verified as DCP polymers and related quinone derivatives, are identified by using ultra-performance liquid chromatography-time-of-flight mass spectrometry (UPLC-TOF-MS). A five-step reaction pathway for DCP transformation, including HRP-driven substrate oxidation, substitution and radical coupling, quick redox equilibrium, nucleophilic reaction and precipitation from aqueous solution, is proposed. Current variations and the presence of CO_2 could significantly affect these reaction pathways. In particular, higher currents enhance the hydroxylation process by promoting alkaline conditions and abundant H_2O_2 formation. As both OH^- and H_2O_2 are strong nucleophiles, they easily react with CBQ products to form hydroxylated products, which can significantly reduce solution toxicity. An adequate supply of CO_2 can provide favorable pH conditions and facilitate enzymatic steps, such as substrate oxidation and radical coupling, to generate precipitable polymerized products. All of the results

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suggest that toxic intermediate products can be effectively reduced and controlled during the electro-enzymatic process to remove DCP and other phenolic pollutants from wastewaters.

1. Introduction

Chlorophenols have been widely used as intermediates in the preparation of herbicides and other industrial precursors (Ahlborg and Thunberg, 1980; Contreras et al., 2003). Considering their high solubility in water and toxicity to microorganisms (Cooper and Jones, 2008; Angelini et al., 2014), chlorophenols have been designated as priority contaminants in effluents in many countries and regions (Zhang et al., 2010). Chlorophenols can be easily oxidized into chlorobenzoquinones (CBQs), which have recently been confirmed as even more toxic emerging pollutants with links to bladder cancer risk (Bull et al., 2011; Zhao et al., 2012). Considering their potential health risk, the transformation of chlorophenols during water treatment have drawn increasing attention.

Peroxidases and laccases, in the presence of hydrogen peroxide (H_2O_2) or oxygen (O_2), can effectively catalyze the oxidation of phenolic compounds and generate various phenoxy radicals. These active radicals may subsequently couple with each other, forming polymerized products (Veitch, 2004; Riva, 2006). Recent studies have reported that pollutants, including halogenated pesticides (Torres-Duarte et al., 2009), antibiotics (Lu et al., 2009; Gulkowska et al., 2013) and some endocrine disrupting chemicals (Huang and Weber, 2005; Feng et al., 2013; Melo and Dezotti, 2013), could be highly removed from wastewater through enzymatic oxidative processes. Horseradish peroxidase (HRP) is a class of naturally-occurring extracellular enzymes that catalyze oxidative coupling reactions (Veitch, 2004). HRP belongs to a group of heme-containing enzymes, which performs two one-electron reduction steps in one catalytic cycle in the presence of H_2O_2. While HRP effectively promotes the oxidative removal of many phenolic pollutants, the high storage and transportation costs of H_2O_2 greatly limit the development of the HRP method. Interestingly, combined electro-enzymatic technology appears to be able to overcome this problem. Through reduction of dissolved O_2 on a cathode, *in situ* H_2O_2 could be rapidly generated, and the new formed H_2O_2 immediately participate in subsequent reactions. Moon S.H.'s group has worked on the electro-enzymatic technique for several years, and has successfully applied the method to the removal of various contaminants, such as 2,4,6-trinitrotoluene (Lee et al., 2003), azo dye (Kim et al., 2005), and phenols (Cho et al., 2008). The electro-enzymatic method is considered a fast detoxification method and has been developed well as a result of rapid H_2O_2 generation and effective enzymatic catalysis.

Despite the many benefits of electro-enzymatic oxidation, however, many CBQs and benzoquinone derivatives are formed as intermediate products during this reaction. These intermediate products can undergo spontaneous transformation, including redox or nucleophilic reactions, which could largely affect health risks. Thus, identifying reaction products and proposing clear reaction pathways is necessary. As well, determining how reaction parameters affect the relevant reaction pathways during the electro-enzymatic process requires further investigation.

The present work focuses on the transformation and products of DCP during HRP-driven oxidation in a membrane electrochemical reactor. To identify intermediates and products during the electro-enzymatic process, the ultra-performance liquid chromatography (UPLC) coupled with time-of-flight (TOF) mass spectrometry is applied. The effects of electric current and CO_2 supply on reaction products and pathways are also evaluated, and the mechanism of DCP transformation in the electro-enzymatic system is systematically demonstrated.

2. Experimental

2.1. Materials

HRP (EC 1.11.1.7, type VI-A) was obtained from Sigma (USA). DCP (97% purity) was obtained from J&K Scientific Ltd. (China), and the Nafion 117 proton exchange membrane (PEM) was purchased from Dupont Company (USA). All other chemicals used in this study were of analytical-reagent grade and obtained from Sinopharm Chemical Reagent Beijing Co., Ltd (China). All solutions were prepared with Milli-Q water, and HRP activity was measured by the 4-amino antipyrine method, as reported in earlier studies (Klibanov et al., 1983; Li et al., 2013).

2.2. Assessment of DCP removal in electro-enzymatic system

DCP removal efficiency was measured in a membrane electrochemical reactor at 30 °C. The electrochemical reactor was constructed with a pair of Ti electrodes. A Nafion 117 proton exchange membrane with a diameter of 30 mm was used to separate the two electrochemical cells. The membrane would block the interference of anodic reactions, which may generate trace oxidants such as ozone and hydroxyl radical inactivating the enzyme (Cho et al., 2008). The effective volumes of the two cells were both 150 mL. O_2 was supplied to the bottom of the cathode cell at a rate of 0.5 L min^{-1} for the saturation of the dissolved O_2. DCP (1 mg L^{-1}) was dissolved in a solution containing 0.1 M sodium sulfate and 0.01 M sodium bicarbonate as the electrolyte. Sulfuric acid was used to adjust the pH to 7.0 and stop the reaction immediately. CO_2 was also provided as a buffer reagent at a rate of 0.1 L min^{-1} to maintain a neutral or weak acid pH in the solution during the reaction. Different enzyme doses were added to the cathode cell to catalyze the reaction, and the current was examined. The concentration of H_2O_2 was measured by using potassium titanium (IV) oxalate at 400 nm in UV–visible spectrophotometer (Labtech, China) (Sellers, 1980).

Aliquots of 1.0 mL of the reaction mixture were periodically collected in high performance liquid chromatography (HPLC) vials, to which 10 μL of 0.1 M sulfuric acid solution have been injected in advance to immediately cease the reaction. All samples were filtrated by using 0.22 μm polytetrafluoroethylene (PTFE) membrane filters (Millipore, Billerica, MA) before HPLC analysis. The concentrations of DCP residues were determined by using an Agilent 1100 HPLC (Agilent, USA) equipped with a UV detector and a C18 reversed phase (RP) column (150 mm × 2.1 mm, 3.5 μm particle, Agilent). Isocratic elution with 50% Milli-Q water and 50% methanol at a flow rate of 0.25 mL min^{-1} was used as the mobile phase. DCP depletion was calculated by the ratio values between C and C_0. C means the concentration of residual DCP and C_0 means the concentration of original DCP in solution, respectively. C and C_0 were detected by HPLC and calculated based on the prepared standard curve.