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A novel mechanism of bisphenol A removal during electro-enzymatic oxidative process: Chain reactions from self-polymerization to cross-coupling oxidation

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HIGHLIGHTS

- Electro-enzymatic method can effectively improve BPA removal with humic acid (HA).
- Removal of BPA achieved 100% within 2 min reaction at an optimal condition.
- A novel mechanism of BPA removal is proposed during electro-enzymatic process.
- HA provides a pathway of BPA self-polymers incorporated into HA matrix.
- Chain reactions from BPA self-polymerization to cross-coupling oxidation.

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ABSTRACT

The catalyzed removal of bisphenol A (BPA) by a horseradish peroxidase (HRP) cathode in the presence of humic acid (HA) was investigated. At an optimal condition, the removal of BPA achieved 100% within 2 min reaction. In the electro-enzymatic process, products were analyzed by high performance liquid chromatography with diode array detector (HPLC-DAD) and high performance size exclusion chromatography (HPSEC). HPLC-DAD results showed that BPA was oxidized into self-polymers and then self-polymers as important intermediate products decreased and disappeared. HPSEC results showed the order of molecular weight (MW): HA + BPA cross-coupling products > HA self-coupling products > initial HA. According to above results, a novel mechanism of BPA transformation in the presence of HA was proposed in electro-enzymatic process. In summary, under oxidation of *in situ* hydrogen peroxide on HRP electrode, the BPA first are polymerized into self-polymers, and then, the polymers may be incorporated into HA matrix and finally larger MW of BPA_n-HA might be formed. The presence of HA can provide chain reactions from BPA self-polymerization to cross-coupling oxidation. Therefore, in the presence of HA, the electro-enzymatic oxidation is an effective way to improve BPA removal.

1. Introduction

An array of synthetic chemicals are classified as endocrine disrupting chemicals (EDCs), which can modulate sexual development, growth, and reproduction of vertebrates by disrupting the hormone/steroid receptor signaling (Auriol et al., 2007). Many EDCs have been detected in raw and treated sewage, surface waters, ground waters, and drinking waters. Removal of EDCs from water has been becoming an interesting topic in recent years.

Traditional water purification technologies, such as biological method (Zhang et al., 2007), coagulation/flocculation (Choi et al., 2006), chlorination (Choi et al., 2006) and adsorption (Kitaoka and Hayashi, 2002; Choi et al., 2006), have been used for EDCs removal. However, those methods show some of drawbacks such as long retention time, low efficiency, generation of by-products, and high cost for complete removal of trace EDCs. Advanced oxidation processes (AOPs), such as Fenton reaction (Ioan et al., 2007), ozonization (Choi et al., 2006), photocatalytic oxidation (Fukahori et al., 2003), ultrasonic oxidation (Inoue et al., 2008) and combined techniques (Ioan et al., 2007), can efficiently oxidize EDCs due to the production of hydroxyl radicals. However, AOPs hardly mineralize trace EDCs completely due to the presence of natural organic matter (NOM) in surface or ground water, which may lead to the

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competitive inhibition for EDCs removal (He et al., 2009). Furthermore, many intermediate products and by-products of EDCs during AOPs may be as toxic as their parent compounds, or even more harmful (Zhao et al., 2008). Additionally, NOM such as humic acid (HA) has been verified to participate in the formation of harmful disinfection by-products during chlorination process (Weber et al., 2005).

Recently, enzymatic catalysis technology has attracted more attentions in treatment of EDCs at low concentrations due to its high reactivity and high selectivity (Huang and Weber, 2005; Lu et al., 2009; Mao et al., 2010b; Li et al., 2011). In enzymatic catalysis process, peroxidase or polyphenol oxidase can catalyze phenolic hydroxyl for initiating polymerization with the aid of hydrogen peroxide or oxygen. The EDCs, such as natural and synthetic hormones (Auriol et al., 2007; Mao et al., 2010b), acetaminophen (Lu et al., 2009) and bisphenol A (BPA) (Huang and Weber, 2005; Li and Nicell, 2008), can be transformed into the insoluble dimer or multimer. Then, these polymers can be easily separated from liquid phase through precipitation or filtration. HA molecules, with phenolic hydroxyl groups, can also be oxidized for polymerization by enzyme-catalyzed oxidative coupling reaction (Piccolo et al., 2000). The enzyme-catalyzed polymerization can increase the hydrophobicity and molecular size of HA by the formation of aryl and alkyl ethers. Furthermore, it has been proved that phenol (Huang and Weber, 2004), acetaminophen (Lu and Huang, 2009), sulfonamide drugs (Bialk et al., 2005), polychlorinated biphenyl (Colosi et al., 2006), and 17β-estradiol (Mao et al., 2010a) can be oxidized for polymerization into HA structure through enzyme-catalyzed cross-coupling reaction. However, the enzymatic method still shows some drawbacks such as high cost of one time used enzyme and requirement of additional oxidants. Interestingly, an electro-enzymatic technology seems to potentially deal with above problems due to the advantages of reusability of enzyme immobilized on electrode and generation of H_2O_2 *in situ*. The electro-enzymatic method has been used for degradation of various pollutants, such as phenol (Cho et al., 2007), pentachlorophenol (Kim and Moon, 2005), 2,4,6-trinitrotoluene (Lee et al., 2003), chlorpyrifos (Tang et al., 2011), 2-chlorobiphenyl (Khan et al., 2007), haloacetic acids (Li et al., 2007), azo dye (Kim et al., 2005), and BPA (Tang et al., 2010; Xu et al., 2011). Although the electro-enzymatic method has received some researcher attentions, to the best of our knowledge no research focused on the interaction of BPA and HA during enzymatic oxidative coupling process. Furthermore, BPA and HA cross-coupling oxidation with *in situ* hydrogen peroxide on horseradish peroxidase (HRP) electrode has not been investigated to date.

In this study, the HRP cathode was used for oxidative removal of BPA from water in a membrane cell. Effects of electric current and enzymatic immobilized dosage on BPA removal were investigated. Especially the role of HA in the catalyzed oxidative cross-coupling removal of BPA in electro-enzymatic process was investigated. The products in electro-enzymatic system were analyzed using high performance size exclusion chromatography (HPSEC) and HPLC with diode array detector (HPLC-DAD). Finally, we proposed a possible mechanism of chain reactions during electro-enzymatic oxidation of BPA in the presence of HA.

2. Methods

2.1. Materials

HRP (EC 1.11.1.7) was obtained from Sigma (USA). BPA (97% purity) was obtained from J&K Scientific Ltd. (China). HA was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (China). Graphite felt (GF) was purchased from Hunan Jiuhua Carbon Company Ltd. (China). Nafion 117 proton exchange membrane (PEM) was purchased from Dupont Company (USA). All other chemicals used in this study were analytical reagent grade and were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd (China). All solutions were prepared with Milli-Q water.

2.2. Preparation of enzymatic electrode

To increase the stability, HRP was immobilized on GF as a reported method (Lee et al., 2003). Briefly, the GF was cut into 8 mm × 5 mm × 40 mm pieces and oxidized at 500 °C for 8 h in air. Then, the oxidized GF was fixed onto Ti electrode surface using silver conducting resin, dried at room temperature, and marked as GF/Ti. GF/Ti was immersed in 1 M hydrazine aqueous solution for 3 d, and excess hydrazine was removed by Milli-Q water. HRP was partially oxidized by 0.1 M sodium periodate solution for 30 min in dark. Then, it was added to hydrazine pretreated GF/Ti in 0.05 M acetate buffer (pH 5) containing 0.1 M sodium chloride, which was gently tumbled in a shaker (200 rpm) at room temperature for 30 min and then stored at 4 °C overnight. The HRP loaded graphite felt electrode (HRP-GF/Ti) was washed thoroughly with 0.2 M phosphate buffer (pH 7) prior to use.

2.3. Electro-enzymatic removal of BPA

The electro-enzymatic removal of BPA was carried out in a membrane electrochemical reactor in thermostat water bath at 20 °C. As shown in Fig. 1, the electrochemical reactor was arranged with GF/Ti anode and HRP-GF/Ti cathode with effective volumes of 70 mL and 50 mL, respectively. Nafion 117 proton exchange membrane with diameter of 30 mm was used between two electrochemical cells. Oxygen gas was supplied into the bottom of cathode at 0.8 L min^{-1} for the saturation of the dissolved oxygen. 10 mg L^{-1} of BPA and 0–100 mg L^{-1} HA were dissolved in a 0.1 M phosphate buffer solution (PBS, pH 7.0) as electrolyte. Experiments were conducted under galvanostatic mode using an electrochemical workstation (Metrohm Autolab, Switzerland). All samples were centrifuged for 20 min with acceleration of 10 000*g* before analysis.

2.4. Analytical methods

Enzymatic activity was measured with photometry by using phenol, 4-Amino antipyrine (4-AAP), and hydrogen peroxide (H_2O_2) as color generating reagents (Klibanov et al., 1983). H_2O_2

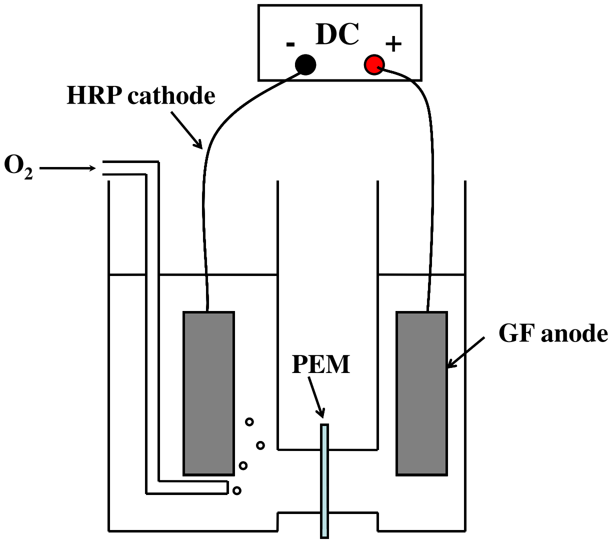

Fig. 1. Schematic diagram of the electro-enzymatic reactor.