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Oxidation mechanism and overall removal rates of endocrine disrupting chemicals by aquatic plants

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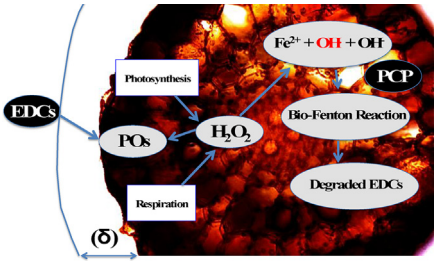
HIGHLIGHTS

- EDCs were stably removed by aquatic plants during long-term experiments.
- Peroxidases were the primal enzymes on EDCs degradation.
- H_2O_2 produced by aquatic plants was a key substance for oxidation of EDCs.
- Overall removal rates of EDCs were governed by mass transfer in liquid films.

GRAPHICAL ABSTRACT

A proposed mechanism for oxidations of endocrine disrupting chemicals and persistent pollutants such as pentachlorophenol in aquatic plants.



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ABSTRACT

The purpose of this study was to evaluate experimentally and theoretically the oxidation mechanisms and overall removal rates of phenolic endocrine disrupting chemicals (EDCs) by aquatic plants. EDCs used in this study were bisphenol-A (BPA), 2,4-dichlorophenol (2,4-DCP), 4-tert-octylphenol (4-t-OP), and pentachlorophenol (PCP). Referring to reported detection levels in aquatic environments and contaminated sites, the feed concentration of each EDC was set from 1 to 100 μg/L. Experimental results showed that, except for PCP, phenolic EDCs were stably and concurrently removed by different types of aquatic plants over 70 days in long-term continuous treatments. Primal enzymes responsible for oxidation of BPA, 2,4-DCP, and 4-t-OP were peroxidases (POs). Moreover, enzymatic removal rates of BPA, 2,4-DCP, and 4-t-OP by POs were more than 2 orders of magnitude larger than those by aquatic plants. Assuming that overall removal rates of EDCs are controlled by mass transfer rates onto liquid films on the surface of aquatic plants, an electrochemical method based on the limiting current theory was developed to measure the mass transfer rates of EDCs. Because of extremely large removal rates of EDCs by POs, observed removal rates by aquatic plants were in reasonably good agreement with calculated results by a mathematical model developed based on an assumption that mass transfer limitation is a rate-limiting step.

1. Introduction

Environmental pollutants such as EDCs have been detected in aquatic environment, and their effects on human health and aquatic life forms have received considerable attention [1]. Especially, the estrogenic effects of BPA, 4-t-OP, and estrogens such as estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) were identified for aquatic lives [1,2]. In addition, there are suspected EDCs such as PCP and 2,4-DCP with potential estrogenic activities. Concentration ranges of these EDCs were reported as 0.5–21 μg/L for BPA in stream/river [3], 5–4 μg/L for 4-t-OP in rivers [4], 26–20 μg/L for 2,4-DCP in rivers [5], and 2–82 ng/L for natural and synthetic estrogens in effluents from sewage treatment plants [6]. Higher levels

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of EDCs up to 7 and 17 mg/L for phenols and BPA were found in leachates of waste landfills, respectively [7,8]. Because of identified and suspected endocrine disrupting effects, effective methods for removal of these EDCs from contaminated sites are required.

Former studies on EDCs removal demonstrated the possibility of EDCs treatments by plants, plant tissues, and plant enzymes. Table 1 shows an example of these studies, demonstrating that EDCs such as BPA, 2,4-DCP, 4-t-OP, NP, E1, E2, and EE2 could be removed by enzymes such as peroxidases (POs) [9–11,19], laccase (LAC) [12,13], polyphenol oxidase (PPO) [10,14], and glutathione S-transferase (GST) [15] as well as plant cells or tissues containing enzymes [16–18]. In addition, some researchers [9,18] confirmed that treatment of phenolic EDCs by plant enzymes or plant tissues was effective to eliminate estrogenic activity from contaminated sites. As shown in Table 1, most studies on EDCs treatment have been conducted based on batch experiments with relatively high concentrations of EDCs (i.e., mg/L level) when compared with observed levels in aquatic environments. Batch experiments could indicate the possibility of removing EDCs, however, continuous performance and/or stability have not been well known. Furthermore, studies focusing on mechanisms and kinetics of EDCs removals have not been conducted extensively; and therefore, rational operation and design conditions with information on appropriate plants have not been available.

This study focused on oxidation mechanisms and removal rates of trace EDCs by aquatic plants. Continuous treatments of BPA, 2,4-DCP, 4-t-OP and PCP were conducted using different aquatic plants, and the stability and removal rates were investigated. In our previous study [19], EDCs concentration was set continuously at 100 μg/L. However, in this study, the feed concentration of BPA, 2,4-DCP, 4-t-OP and PCP was set in the range of 1–100 μg/L to investigate the removal ability of aquatic plants at different influent concentration. In addition, enzymatic removal rates of EDCs were measured to elucidate primal enzymes and reactions responsible for the EDCs removal in phytoremediation process. The same measurements and comparison were made for removals of estrogens such as E1, E2, and EE2. To better understand the overall removal rates by aquatic plants, an electrochemical method based on the limiting current theory was developed to measure the mass transfer rates of EDCs. A mathematical model was developed based on an assumption that overall removal rates of EDCs are controlled by the mass transfer rates in liquid film onto the surface of aquatic plants, and comparisons of calculated and observed results were made to evaluate the net removal rates of EDCs by aquatic plants.

2. Materials and methods

2.1. Aquatic plants

Aquatic plants used in this study were floating plants, Amazon frogbit (*Limnobium laevigatum*), Giant Duckweed (*Spirodela polyrhiza*), Duckweed (*Lemna aoukikusa*), Liver wort (*Ricciocarpos natans*), and Crystalwort (*Riccia fluitans*); submerged plants, Willow Moss (*Fontinalis antipyretica*), Hydrilla (*Hydrilla verticillata*), Pondweed (*Potamogeton oxyphyllus*), Hornwort (*Ceratophyllum demersum*); and an emergent plant, Watercress (*Nasturtium officinale*).

Enzyme activities in aquatic plant tissues were measured according to previously studies [13,33,34] and were listed in Table 2 with parameters such as specific surface area (*a*), endogenous H_2O_2 level, and water content. Enzymatic activities were converted to number of units (U) based on extinction coefficient such as 26.6 mM/cm for POs, 1.42 mM/cm for PPOs, 3.6 mM/cm for LAC, and 9.6 mM/cm for GST. One unit of enzyme activity (U) represents the amount of enzyme catalyzing 1 μmoL of a reference substrate per 1 min in the corresponding enzymatic reaction.

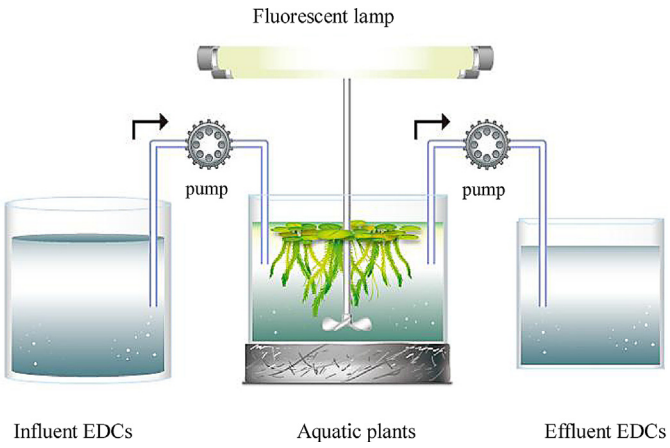

Fig. 1. Scheme of experimental apparatus.

2.2. Continuous treatments of EDCs by aquatic plants

Referring to reported detection levels in contaminated sites, the feed concentration of BPA, 2,4-DCP, 4-t-OP and PCP was set in the range of 1–100 μg/L. The stock solutions of each EDC were prepared and dissolved in a diluted Hoagland solution.

Continuous treatments of BPA, 2,4-DCP, 4-t-OP and PCP were conducted using an experimental apparatus illustrated in Fig. 1, where each plant was cultivated in glass vessels (effective liquid volume was 5 L). All vessels were maintained under intermittent illumination, which consisted of light and dark periods of 16 h and 8 h, respectively. The illumination during the light period was controlled at 3000 lx and was provided by white fluorescent lamps. The flow rate of the feed solution was set at 1.92 L/day (corresponding to HRT = 2.6 days) using peristaltic pumps.

Every experiment and analysis was conducted at a room temperature (25 °C). In continuous experiments, the amount of aquatic plant was set at 5 g-FW (fresh weight)/L and the feed solution was prepared every 5 days. The fresh biomass of aquatic plants was monitored and the excess over than 5 g-FW/L was removed. In parallel to the continuous experiments, a blank test without any aquatic plant was conducted as a reference. After the end of the continuous treatment, plants were taken out from the vessels and the amount of EDCs accumulated in their tissues was analyzed.

To investigate the possible effect of microorganisms on EDCs degradation, part of aquatic plants were collected from the vessels after 35 days of operation, and were disinfected with sodium hypochlorite (5%) for 10 min as described by Toyama et al. [13], and afterwards, washed with distilled water several times to avoid physiological damage. After that, batch treatments of BPA, 2,4-DCP, 4-t-OP and PCP were conducted and compared with those by undisinfected aquatic plants.

2.3. Removal rates of EDCs by extracted crude enzymes

In order to determine the primal enzymes in the EDC treatments, different fractions of crude POs were extracted from homogenized aquatic plants so as to conduct further *in vitro* batch treatments of phenolic EDCs. The following extraction steps were carried out at 4 °C. For each enzyme, the aquatic plant tissues were homogenized in a buffer solution (buffer volume (mL):fresh weight (g) = 1:1) using a mortar with a pestle. The PO fractions, soluble PO (SPO), ionically cell wall-bound PO (IPO), and covalently cell wall-bound PO (CPO) were extracted at pH 6.0 in 50 mM Tris–maleate, 0.2 M $CaCl_2$, and 40 mM Tris–maleate buffer as described in a previous study [34]. The homogenate was centrifuged at 10,000 × *g* for 30 min, and the supernatant was used for the measurement of enzyme