Bioresource Technology 101 (2010) 6354–6360

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Degradation of triclosan by an integrated nano-bio redox process

Varima Bokare, Kumarasamy Murugesan, Young-Mo Kim, Jong-Rok Jeon, Eun-Ju Kim, Yoon Seok Chang *

School of Environmental Science and Engineering, Pohang University of Science and Technology (POSTECH), Pohang 790-784, South Korea

ARTICLE INFO

Article history:
Received 14 December 2009
Received in revised form 11 March 2010
Accepted 15 March 2010
Available online 8 April 2010

Keywords:
Triclosan
Pd/nFe
Laccase
2-Phenoxyphenol
Redox process

ABSTRACT

In this study, a sequential reduction–oxidation method was developed for complete degradation of triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether, TCS) in aqueous solution. Rapid reductive dechlorination of TCS was achieved with palladized zero-valent iron nanoparticles (Pd/nFe), under anaerobic conditions, with generation of 2-phenoxyphenol as the sole dechlorination product. Sequentially, 2-phenoxyphenol was transformed into a non-toxic polymer using laccase (EC:1.10.3.2) derived from *Trametes versicolor* in the presence of natural redox mediator syringaldehyde (SYD). High performance liquid chromatography combined with electrospray ionization mass spectroscopy (HPLC–ESI-MS) revealed the formation of dimer and trimer products during the laccase-mediated transformation process. The efficiency of the integrated method is critically dependent on the Fe^{2+} concentration, which was effectively controlled by optimizing the solution pH. To the best of our knowledge, this is the first report of a redox two-step hybrid system for the complete transformation of TCS into non-toxic products.

1. Introduction

Triclosan, 2,4,4′-trichloro-2′-hydroxydiphenyl ether or TCS, is widely used as a broad-spectrum anti-microbial agent in diverse products such as soaps, deodorants, toothpaste, plastic cutting boards and socks (Adolfson-Erici et al., 2002). Due to its high anti-microbial activity, ~350 tons of triclosan are sold every year as the active ingredient of pharmaceutical and personal care products (Singer et al., 2002). This widespread use of triclosan is responsible for its occurrence in numerous municipal wastewater influent samples in the concentration range of 0.5–1.3 μg/L (Lindstrom et al., 2002). Its target-specific antibacterial activity further contributes to acute toxicity towards aquatic algae and protozoa (Wilson et al., 2003) and development of triclosan resistance in bacteria (Heath et al., 1999). It has also been shown to occur in human milk (Adolfson-Erici et al., 2002). The hydrophobic nature of triclosan ($\log K_{OW} \sim 4.86$) allows its easy removal in wastewater treatment plants (94% removal by biodegradation and sorption, Singer et al., 2002), but residual concentrations of ~40–200 and 140 ng/L have still been found in secondary wastewater effluents (Singer et al., 2002) and surface water, respectively (Kolpin et al., 2002).

To control triclosan concentrations in wastewater, chemical oxidation of the phenol moiety using free chlorine, ozonation or UV/TiO_2 photocatalysis has been successfully achieved (Rafqah et al., 2006; Suarez et al., 2007; Yu et al., 2006). However, using free chlorine, the generation of toxic byproducts like chlorinated phenoxy-phenols, chlorinated phenols and trihalomethanes is a huge disadvantage (Rule et al., 2005). On the other hand, ozonation is not cost-effective and suffers the potential generation of carcinogenic byproducts (Zhang et al., 2008). Additionally and more significantly, triclosan under exposure to sunlight or UV irradiation produces highly toxic and persistent chlorinated products like 2,8-dichlorodibenzo-*p*-dioxin (Latch et al., 2003). Triclosan oxidation using alternative chemical oxidants like ferrate (K_2FeO_4), permanganate ($KMnO_4$) or manganese oxides (MnO_2) has also been reported (Lee et al., 2009; Jiang et al., 2009; Zhang and Huang, 2003). But instability of ferrate in water and violet coloration of water due to elevated manganese levels are drawbacks for practical applications. Due to its inhibitory activity against a wide range of bacteria, bacterial degradation of triclosan is also limited. Bacteria known to transform halogenated diphenyl ether compounds which are structurally similar to triclosan, failed to degrade it (McMurry et al., 1998).

Compared to bacteria, triclosan transformation by white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* has been reported (Hundt et al., 2000) but fungal growth was still markedly inhibited in low triclosan concentrations. To avoid this inhibitory effect, use of isolated fungal enzymes to degrade triclosan is a better alternative. Laccases (EC.1.10.3.2) from white rot fungi can degrade a wide range of organic pollutants (Bollag et al., 2003) by utilizing molecular oxygen as an electron acceptor. Laccase-mediated triclosan oxidation *via* oligomerization has been recently demonstrated using laccases isolated from *T. versicolor* and *Coriolopsis polyzona* (Cabana et al., 2007; Kim and Nicell, 2006). However, the formation of triclosan oligomers does not destroy the structural backbone or generate dechlorinated products, which

* Corresponding author. Tel.: +82 54 279 2281; fax: +82 54 279 8299.
E-mail address: yschang@postech.ac.kr (Y.S. Chang).

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doi:10.1016/j.biortech.2010.03.062

make the decontamination process more difficult. In this regard, the removal of chlorine atoms from the diphenyl ether moiety before laccase-mediated oligomerization is critically essential to yield less toxic or non-toxic products.

In this study, we have attempted to design a hybrid redox process incorporating reductive dechlorination of triclosan by palladized-zero-valent iron nanoparticles (Pd/nFe) under anaerobic conditions and subsequent laccase-mediated oxidation of the dechlorinated products. The Pd/nFe bimetallic system is very effective for the rapid dechlorination of hazardous compounds like polychlorinated biphenyls, dioxins and halogenated organics. We demonstrate that such a sequential reduction–oxidation process not only removes triclosan and its dechlorinated product from aqueous solution, but also reduces the overall toxicity. The effects of solution pH and concentration of nanoparticle-leached-Fe^{2+} ions on triclosan removal were also studied to examine the inhibitory role of ferrous ions on laccase activity.

2. Methods

2.1. Chemicals

The following chemicals were used as received: triclosan (Sigma–Aldrich, USA), acetone (Merck, Germany), ethanol (Merck, Germany), ethyl acetate (Merck, Germany), iron(II) sulfate ($FeSO_4{\cdot}7H_2O$, Sigma–Aldrich, USA), sodium borohydride ($NaBH_4$, Sigma–Aldrich, USA), palladium acetate ($Pd(COOCH_3)_2$, Sigma–Aldrich, USA), laccase (from *T. versicolor* EC:1.10.3.2, Fluka, USA), 1-hydroxybenzotriazole (HBT, Sigma–Aldrich, USA), syringaldehyde (SYD, Sigma–Aldrich, USA), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS, Fluka, USA) and *N*,*O*-bis trimethylsilyl-trifluoroacetamide (BSTFA, Sigma–Aldrich, USA). All solutions were prepared in ultrapure water (18 MΩ cm) prepared by Barnstead purification system. For bacterial toxicity studies, *E. coli* Dα strain was grown in nutrient broth purchased from Merck, Germany.

2.2. Synthesis and characterization of Pd/nFe nanoparticles

Synthesis of iron nanoparticles was carried out using $FeSO_4{\cdot}7H_2O$ (6.2 g) and reducing it with $NaBH_4$ (3.1 g) in 100 mL degassed ultra-pure water (Wang and Zhang, 1997). The solution was mixed vigorously for 15 min, wherein ferrous ion was reduced by borohydride according to the following reaction:

$$4Fe^{2+} + 3BH_4^- + 9H_2O \rightarrow 4Fe^0 \downarrow + 3H_2BO_3^- + 12H^+ + 6H_2 \quad (1)$$

The metal nanoparticles were washed with large volumes (>100 mL/g iron) of degassed ultra-pure water for at least 3–4 times to remove excess borohydride. Palladium deposition on iron nanoparticles was achieved by soaking freshly-prepared particles in an acetone solution of palladium acetate (0.2 wt.%). This caused the reduction and subsequent deposition of Pd on the Fe surface:

$$Fe^0 + Pd^{2+} \rightarrow Fe^{2+} + Pd^0 \quad (2)$$

The palladized iron nanoparticles were washed with ethanol followed by acetone and finally dried in a vacuum oven at 80 °C.

The size and morphology of the nanoparticles was imaged on a transmission electron microscope (JEM 2200FS with Cs Corrector, Japan) operated at 200 kV. The distribution of palladium and iron within the nanoparticle was also obtained by elemental mapping. Brunauer, Emmett and Teller (BET) surface area was determined using N_2 adsorption at 77 K using a Micromeritics ASAP 2000 instrument. X-ray diffraction (XRD) spectrum for Fe–Pd nanoparticles was obtained using Cu Kα radiation (MAC Science Co., M18XHF).

2.3. Dechlorination experiments and analyses

Batch experiments were performed in 50 mL glass reactors under anaerobic conditions (continuous N_2 purging). The TCS concentration used in all experiments was fixed at 5 mg/L (or 17.3 μM). Although TCS concentration in wastewater and drinking water is generally <200 ng/L, recent reports have indicated that bioaccumulation in lipophilic matrices and partitioning in organic matter can result in higher TCS concentrations (from μg/L to mg/L) (Xu et al., 2009; Savage, 2009). In this study, however, we have used high TCS concentration to allow easy estimation of the degradation products and establish an accurate reaction mechanism. Simultaneously, the efficiency of the integrated system when dealing with concentrated TCS solutions can be established. Initial dechlorination experiments were carried out under neutral pH conditions using Pd/nFe nanoparticles (1 g/L) under continuous N_2 purging and shaking (350 rpm). Samples (1 mL) were withdrawn at regular time intervals from the reactor and filtered through a 0.45 μm hydrophillic PTFE filters (Millipore) prior to analysis. All experiments were carried out in triplicates.

Quantitative analysis of TCS degradation was done using a HPLC (Agilent 1100) equipped with a C-18 column (Agilent Zorbax 300SB) and a diode-array detector. The eluent composition was 0.1% phosphoric acid aqueous solution and acetonitrile (40:60 v/v). Chloride ions were quantified using an ion chromatograph (IC, Dionex DX-120) equipped with Dionex IonPac AS-14 column and a conductivity detector. The eluent composition was 3.5 mM Na_2CO_3 + 1 mM $NaHCO_3$ at a flow rate of 1.2 mL/min. Concentration of total dissolved Fe^{2+} and Pd^{2+} in treated samples was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Jarrell Ash Corp. IRIS/AP). To identify the dechlorination products, the reaction mixture was extracted thrice with equal volume of ethyl acetate. The extract was dehydrated with anhydrous sodium sulfate, dried under N_2 gas, and redissolved in acetone. It was then derivitized using BSTFA [*N*,*O*-bis(trimethylsilyl)-trifluoroacetamide] by incubating at 60 °C for 1 h. The derivitized samples were analyzed by gas chromatography mass spectrometry (GC–MS) on a Trace GC system coupled with Polaris Q Iontrap Mass Spectrometer (ThermoQuest, Jan Jose, CA) equipped with DB-5 column (30 m, 0.25 mm ID and 0.5 μm film thickness). The column temperature was started at 60 °C, then held for 3 min, ramped up to 230 °C at a rate of 10 °C/min and finally held for 10 min. About 1 μL samples were injected each time.

2.4. Transformation of dechlorinated product by laccase

After complete TCS dechlorination, nanoparticles were allowed to settle down with help of a strong magnet. The solution was then decanted and filtered samples (0.45 μm hydrophilic PTFE filter) were subjected to enzymatic treatment under open reactor conditions. Preliminary experiments were performed with filtered samples to optimize the concentrations of laccase (0.25–5 U/mL) and redox mediator (25–500 μM). About 1 mL solution of Pd/nFe-treated TCS aqueous solution and laccase was added to glass vials (2 mL) and incubated at 30 °C in dark for 3 h. After 3 h, the enzymatic reaction was stopped by acidifying the reaction mixture to pH 2 with concentrated HCl, and the solution was analyzed by HPLC. To check the effect of redox mediators on laccase-mediated product removal, 1-hydroxybenzotriazole (HBT) and syringaldehyde (SYD) were tested individually along with laccase. For kinetics of product transformation from both unfiltered and filtered samples, reactions were conducted with optimum laccase (2 U/mL) and redox mediator (100 μM) concentrations. To check the effect of Fe^{2+} on the enzymatic removal of the dechlorinated products in the presence of nanoparticles, laccase (2, 10 and 50 U/mL) and redox mediator (100 μM) were added into the reactor immedi-