Journal of Hazardous Materials 331 (2017) 182–188

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Transformation and toxicity evaluation of tetracycline in humic acid solution by laccase coupled with 1-hydroxybenzotriazole

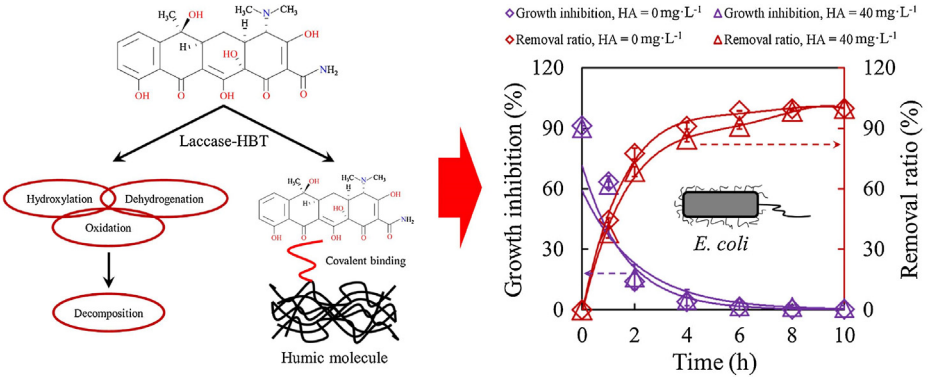
Kai Sun[a,*], Qingguo Huang[b], Shunyao Li[c]

[a] School of Resources and Environment, Anhui Agricultural University, Hefei 230036, China
[b] Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30223, USA
[c] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

HIGHLIGHTS

- Laccase from *Pleurotus ostreatus* could effectively transform TC with HBT present.
- HA inhibited transformation of TC, and the *k* values for TC decreased as HA concentration increased.
- A possible transformation mechanism of TC in HA solution was proposed.
- The antimicrobial activity of TC was significantly reduced with an increasing reaction time.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form 24 February 2017
Accepted 27 February 2017
Available online 1 March 2017

Keywords:
Tetracycline
Laccase
Decomposition
Covalent binding
Antimicrobial activity

ABSTRACT

Enzyme-based catalyzed oxidative coupling reactions (E-COCRs) are considered as viable technologies to transform a variety of pharmaceutical antibiotics. This study indicated that the extracellular fungal laccase from *Pleurotus ostreatus* was effective in transforming tetracycline (TC) with 1-hydroxybenzotriazole (HBT) present at varying conditions during E-COCRs. The presence of humic acid (HA) showed suppressive effect on the transformation rate constants (*k*) of TC, and the *k* values for TC decreased as HA concentration increased. It was ascribed primarily to the covalent binding between TC and HA, which reduced the apparent concentration and availability of TC in water. It is noted that TC molecules from the cross-coupling products were likely re-released under extreme conditions (pH < 2.0). The intermediate products were identified regardless of HA presence by high-resolution mass spectrometry (HRMS). A possible reaction pathway of TC in HA solution including electron transfer, hydroxylation, dehydrogenation, oxidation, radical reaction, decomposition, and covalent binding was proposed. The growth inhibition assays of *Escherichia coli* (*E. coli*) confirmed that the antimicrobial activity of TC was remarkably reduced with an increasing reaction time. These findings provide novel insights into the decomposition and cross-coupling of TC in a multi-solute natural aquatic environment by laccase-based catalyzed oxidative processes.

1. Introduction

Pharmaceutical antibiotics have been produced in large quantities and frequently used for controlling the outbreak of human

* Corresponding author.
E-mail address: sunkai0920@gmail.com (K. Sun).

http://dx.doi.org/10.1016/j.jhazmat.2017.02.058
0304-3894/

and animal diseases and promoting livestock production worldwide [1–3]. In China, approximately 160,000 t of antibiotics is sold annually for use in agriculture and medicine, equivalent to 75% of antibiotics produced [4]. Currently, antibiotics as a category of emerging contaminants have been extensively found in pharmaceutical manufacturing wastewater, household waste, and livestock farm, *etc.*, and a considerable fraction is therefore discharged into natural waters, while only a restricted transformation and an extremely low mineralization rate have been reported [5–7]. It has also been proved that the residues of antibiotics were detected in surface water, groundwater, and even drinking water, which posed a potential risk to the eco-environment [8–10]. For instance, survey studies indicated that antibiotics along with their partial metabolites could damage the structure and function of microbial communities, and induce the expression of antibiotic resistance genes in microorganisms causing a significant threat to human health [11,12]. Hence, how to eliminate the contamination of pharmaceutical antibiotics in natural aquatic environments has attracted increasing concern.

The conventional wastewater treatment technologies (CWTT) such as adsorption, photocatalysis, biodegradation, and other techniques have been widely used for treating antibiotics in water matrices [13–16]. However, these methods displayed high energy consumption and costs and might form the byproducts of higher toxicity [17]. Additionally, CWTT could not effectively transform antibiotics at low concentrations in complex environmental matrices [18]. In particular, natural organic matter (NOM) is ubiquitous in natural waters, which owns multiple functional groups (*e.g.*, —OH, —COOH, and —NH_2) [19,20]. It is well-recognized that NOM can significantly impact the distribution, transformation, and bioavailability of antibiotics by cation bridging, complexation, electron-acceptor, electrostatic interactions, and covalent binding between antibiotics and NOM [21–26]. It has also been indicated that clarithromycin could be bound to humic acid (HA) by the electrostatic interaction of antibiotic-Ca complexes with humic molecules [22]. Nevertheless, microscopic information is available in the literature on the covalent binding mechanisms between antibiotics and NOM, and the binding degree and stability may be related to the type and affinity of HA-reactive intermediates during the natural enzymatic reactions [20,21,27].

Currently, enzyme-based catalyzed oxidative coupling reactions (E-COCRs) have become an innovative technology for transforming various persistent organic contaminants in natural waters because of its high-efficiency, mild condition, and eco-friendly process [20,28]. Laccase is a multi-copper oxidase catalyzing elimination of plentiful persistent organic contaminants using molecular oxygen as an electron acceptor via a radical-mediated reaction mechanism [20,29,30], and the laccase-producing white-rot fungi and *Trametes versicolor* have been well-documented for decomposition of antibiotics such as tetracyclines and sulfonamides [31,32]. For example, Llorca et al. demonstrated that the naturally occurring laccase from *Trametes versicolor* could degrade 78% tetracycline (TC) for an 18-h incubation during E-COCRs [33]. Additionally, we previously indicated that laccase could mediate radical–radical coupling between antibacterial agent and humic molecules via covalent bonds [26]. It is noteworthy that the substrate range of laccase could be extended with 1-hydroxybenzotriazole (HBT, as a redox mediator) present by a hydrogen atom transfer mechanism [31,34,35]. However, studies on the decomposition and cross-coupling mechanisms of TC in the presence of NOM are limited during laccase-HBT mediated reactions and have mainly involved endocrine disrupting chemicals and sulfonamides [20,27,36].

In this study, the batch experiments were performed to investigate the removal ratios of TC in aqueous solution at varying conditions by an extracellular fungal laccase from *Pleurotus ostreatus* coupled with HBT, and the enzymatic kinetic parameters (k) and half-lives ($T_{1/2}$) were also calculated in the presence of HA. A potential reaction pathway of TC in HA solution was proposed using high-resolution mass spectrometry (HRMS). Additionally, the antimicrobial activity of TC was also evaluated using *Escherichia coli* (*E. coli*) during laccase-HBT mediated reactions. TC is extensively used for agriculture and medicine in China and other countries and poses a grave threat to eco-environment and human health. These finding provide novel information for understanding the fate and transformation of TC in natural environmental matrices by laccase combined with HBT.

2. Materials and methods

2.1. Chemicals and materials

All reagents and chemicals were purchased in the highest purity available. Tetracycline (TC, CAS 60-54-8), 1-hydroxybenzotriazole (HBT, CAS 123333-53-9), 2,6-dimethoxyphenol (2,6-DMP, CAS 91-10-1), humic acid (HA, from an unknown source, CAS 1415-93-6), and extracellular fungal laccase (from *Pleurotus ostreatus*, CAS 80498-15-3) were purchased from Sigma-Aldrich Chemical Company. Chemical formula and selected physicochemical properties of TC are shown in Fig. S1 (Supplemental material). The elemental compositions (43.38% C, 0.70% N, and 3.02% H) of HA were determined using a Vario MICRO Elemental Analyzer. Additionally, the Supplemental Material also provided the details about HA and laccase stock solution prepared in this study. High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were obtained from Fisher Scientific. All other chemicals and solvents were reagent grade.

2.2. Enzymatic transformation of TC in aqueous solution

Batch experiments were performed in 25-mL autoclave-sterilized tubes to investigate the removal of TC with a 24-h static incubation at 22 °C in darkness by laccase-HBT mediated systems. For each flask, 5 mL of 50 mM phosphate buffer solution (PBS, pH 7.0), consisting of 50 mg L^{-1} TC, 0–80 mg L^{-1} HA, 1.0 U mL^{-1} laccase and 100 mg L^{-1} HBT. At preselected time intervals, the enzymatic reaction was terminated immediately with the same volume of methanol, and then 1 mL of the mixed solution was stored in a vial for determination of TC concentration by HPLC. Our preliminary experiments have confirmed that laccase-mediated reactions could be immediately quenched by adding the same volume of methanol [20]. Similar experiments were also conducted to examine the impact of different conditions on the transformation of TC, such as the initial TC concentration (1–50 mg L^{-1}), laccase dosage (1.0–10.0 U mL^{-1}), pH (3.0–10.0), and HBT concentration (0–400 mg L^{-1}). For comparison, experimental laccase-free solutions were used as controls. All experiments were performed in triplicate.

The residual ratio (R) of TC in laccase-HBT mediated reactions was calculated using the following equation: $R = C_t/C_0$, where C_0 corresponds to the initial TC concentration that was measured prior to the addition of laccase (mg L^{-1}), and C_t is the residual TC concentration measured at reaction time t (mg L^{-1}). The recoveries of TC in the standard samples with HA present (0–80 mg L^{-1}) that were investigated averaged 99.1–100.3% (n = 5) after the entire procedure.

2.3. Determination of laccase activity

Laccase activity was monitored immediately prior to each experiment by ultraviolet (UV) spectrophotometer (Beckman Du 640-B) at 468 nm as described in our previous study [20]. One unit of laccase activity equals the amount of enzyme causes an