Chemosphere 181 (2017) 320–327

Contents lists available at ScienceDirect

Chemosphere

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

Degradation of sulfadimethoxine catalyzed by laccase with soybean meal extract as natural mediator: Mechanism and reaction pathway

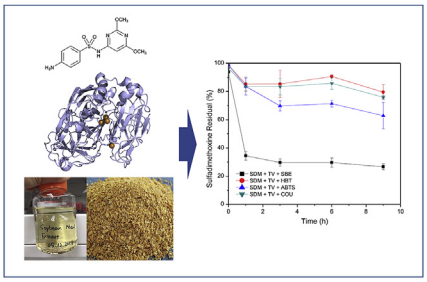
Shangtao Liang, Qi Luo, Qingguo Huang*

College of Agricultural and Environmental Sciences, Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30223, United States

HIGHLIGHTS

- Soybean meal extract mediated efficient sulfadimethoxine degradation by laccase.
- Vanillin, apocynin and daidzein were identified as phenolic mediators in soybean meal extract.
- Sulfadimethoxine degradation pathway was proposed on the basis of HRMS product identification.
- SO_2 excursion happened during sulfadimethoxine degradation, leading to elimination of the antimicrobial functional group.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
12 April 2017
Accepted 21 April 2017
Available online 23 April 2017

Handling Editor: Tamara S. Galloway

Keywords:
Soybean meal
Laccase
Mediator
Sulfadimethoxine
Remediation

ABSTRACT

Natural laccase-mediator systems have been well recognized as an eco-friendly and energy-saving approach in environmental remediation, whose further application is however limited by the high cost of natural mediators and relatively long treatment time span. This study evaluated the water extract of soybean meal, a low-cost compound system, in mediating the laccase catalyzed degradation of a model contaminant of emerging concern, sulfadimethoxine (SDM), and demonstrated it as a promising alternative mediator for soil and water remediation. Removal of 73.3% and 65.6% was achieved in 9 h using soybean meal extract (SBE) as the mediating system for laccase-catalyzed degradation of sulfadimethoxine at the concentration of 1 ppm and 10 ppm, respectively. Further degradation of sulfadimethoxine was observed with multiple SBE additions. Using SBE as mediator increased the 9-h removal of SDM at 1 ppm initial concentration by 52.9%, 49.4%, and 36.3% in comparison to the system mediated by 1-Hydroxybenzotriazole (HBT), *p*-Coumaric acid (COU) and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS), respectively. With the detection of stable coupling products formed with radical scavenger (5,5-Dimethyl-1-pyrroline N-oxide, DMPO), three phenolic compounds (vanillin, apocynin, and daidzein) in SBE were confirmed to serve as mediators for *Trametes versicolor* laccase. Reaction pathways were proposed based on the results of High Resolution Mass Spectrometry. SO_2 excursion happened during SDM transformation, leading to elimination of antimicrobial activity. Therefore, as a natural, phenol rich, and affordable compound system, the future application of SBE in wastewater and soil remediation is worth exploring.

1. Introduction

Sulfadimethoxine (SDM), a sulfonamide antibiotic, is commonly

* Corresponding author.
E-mail addresses: sliang@uga.edu (S. Liang), lisaluo@uga.edu (Q. Luo), qhuang@uga.edu (Q. Huang).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.100
0045-6535/

used to treat infections in livestock and poultry production, and it is approved in some countries to treat human disease. In fresh water fishery industry, sulfadimethoxine is often used in combination with ormetoprim to prevent spread of disease (Guerard et al., 2009). Antimicrobials and their biologically active degradation products may be introduced into terrestrial system through manure application, or in the case of aquafarming, discharged directly into surface waters, thus endangering the aquatic ecology. In environmental systems, sulfonamides were detected at concentrations ranging from 2 mg/kg to 20 mg/kg in liquid manure samples (Haller et al., 2002; Jacobsen and Halling-Sørensen, 2006) and from 0.06 to 15 μg/L in surface water samples (Lindsey et al., 2001). The half-lives of sulfadimethoxine range from 3 to 11 d in manure samples (Wang et al., 2006b), from 10.5 to 49.8 d in natural water samples (Zhang et al., 2012), from 42.0 to 58.2 d in lake water sediments (Zhang et al., 2012), and from 1.36 to 10.2 d in manure amended soils (Wang et al., 2006a).

Widespread use of antibiotics and release of their metabolites into the environment has promoted the evolution of antibiotic resistance genes and impacted microcosm functionality. The prevalence of sulfonamide resistance bacteria and significant enrichment of antibiotic resistance genes (ARGS) was observed in pig slurry samples, river water, sediments, and soils adjacent to livestock production facilities worldwide (Byrne-Bailey et al., 2009; Hsu et al., 2014; Pei et al., 2006; Zhu et al., 2013). In a previous study, the quantity of *sul*(*I*) and *sul*(*II*), two predominant sulfonamide-resistant genes, increased by more than 40% in agricultural soil with periodic additions of manure than in the untreated land (Heuer et al., 2011).

Laccase, a blue multicopper oxidase, is capable of transferring four electrons from four reducing substrate molecules to one molecule oxygen with no requirement of cofactors, producing water as the only byproduct. In this process, substrates become radicals, and then may undergo cross-coupling reactions, decarboxylations, demethylations and/or dehalogenations. The unique characteristic of laccase to degrade phenolic aromatic compounds, which are normally toxic or recalcitrant in environmental matrixes, has raised considerable interest as an eco-friendly bioremediation approach for contaminated wastewater and soil. Previous studies have demonstrated successful application of laccase in treating a broad range of substrates, including phenols, methoxy phenols, aromatic amines, polyphenols, and polyamines (Cañas et al., 2007; d'Acunzo et al., 2002).

However, the application of laccase is limited because of its incapability to oxidize pollutants with high redox potentials. Redox mediators are low-molecular-weight phenols that were found to produce active free radicals that can further react with inert chemicals, thus expanding the substrate spectrum. Synthetic mediators, such as 2, 2′-azine-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2,6,6-tetramethyl-1-piperidinyloxyl (TMPO), and 1-Hydroxybenzotriazole (HBT), have been reported to promote laccase-catalyzed oxidation of numerous toxic compounds, including antibiotics (Cañas and Camarero, 2010; Morozova et al., 2007; Weng et al., 2012, 2013). Plant-, especially legume-derived natural phenolic compounds (vanillin, *p*-coumaric acid, sinapic acid, acetovanillone, ferulic acid, salicylic acid, syringaldehyde) can also act as mediators, but are less efficient than synthetic mediators (Camarero et al., 2005; Cañas and Camarero, 2010; Johannes and Majcherczyk, 2000). A recent study compared the effects of both synthetic and natural mediators on sulfadimethoxine degradation and indicated that ABTS-mediated treatment achieved the highest removal rate of 90% at 20 °C (Weng et al., 2013). Similar result was found by another study, suggesting ABTS can lead to faster degradation of sulfadimethoxine than other tested mediators (Weng et al., 2012). Although the laccase-mediator system (LMS) has great potential as an environmentally benign remediation technology, its feasibility is hindered because of the high cost and the potential toxic effects of mediators.

Soybean meal is produced as the residue of oil extraction process. Its superior amino-acid profile, high digestibility, and low cost make it an ideal vegetable-protein meal for animal production. Soybean meal is also commonly used as soil amendment in organic farming to provide nitrogen source for crops. The heating process during soybean meal production can transform aromatic amino acids in soybean into phenolic compounds (Kato et al., 1971), in addition to the phenolic contents (up to 1.13 mg gallic acid equivalent (GAE)/g) originally in soybean (Lee et al., 2011). Its high phenolic content makes soybean meal a potential cheaper and nontoxic alternative mediator for laccase oxidation reaction. The objective of this study is to investigate the feasibility of using the water extract of soybean meal to mediate laccase-catalyzed degradation of sulfadimethoxine. The reaction products were identified using high resolution mass spectrometry, based on which the reaction mechanism is elucidated. The phenolic compounds in SBE acting as mediators were studied, and their reactions with laccase to form radicals were verified via a radical scavenging probe.

2. Materials and methods

2.1. Chemicals and materials

Sulfadimethoxine (SDM), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1-hydroxybenzotriazole (HBT), *p*-coumaric acid (COU), gallic acid (GA), vanillin (VA), apocynin (AP), daidzein (DA), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), Folin-Ciocalteu's phenol reagent, sodium carbonate and laccase from *Trametes versicolor* were obtained from Sigma-Aldrich (St. Louis, MO). Soybean meal (organic soil amendment) was purchased from Eureka Springs Organics (Eureka Springs, AR). Aqueous solutions used in this work were prepared from Barnstead Nanopure system with a resistivity of $\geq$18 MΩ cm^{-1}. HPLC grade acetonitrile and formic acid were provided by Sigma-Aldrich (St. Louis, MO).

Soybean meal extract (SBE) was prepared by shaking 10 g of soybean meal with 300 mL HPLC water at 150 rpm for 5 days. The extract was then passed through 0.45 μm cellulose acetate membrane and stored at 4 °C for subsequent use.

2.2. Degradation experiments

The experiments were conducted in 20 mL polyethylene vials containing 1 mg L^{-1} of sulfadimethoxine, 0.5 U mL^{-1} laccase (one unit of laccase was previously defined (Luo et al., 2015)), and 1 mg L^{-1} mediators (ABTS, HBT, COU, VA, AP, and DA) or various doses of soybean meal extract in a total reaction volume of 10 mL. For reactors containing 10 mg L^{-1} of sulfadimethoxine, the concentrations of laccase and mediators were 5 U mL^{-1} and 10 mg L^{-1} respectively. Reactors were covered by Parafilm to allow passive aeration, and continuously shaken at 150 rpm in dark under room temperature. At each pre-determined sampling point, 100 μl of reaction mixture was taken from the reactor, and the reaction was stopped immediately by adding 900 μl acetonitrile to the sample. All samples were tested in triplicate.

2.3. Folin-Ciocalteu assay for total phenolic content

The total phenolic content of soybean meal extract was examined colorimetrically as modified (Singleton et al., 1999). Briefly, 0.5 mL of soybean meal extract or gallic acid standard solution was