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

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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 hours 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-hour 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. SO₂ 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.

Keywords: Soybean meal; Laccase; Mediator; Sulfadimethoxine; Remediation

1. Introduction

Sulfadimethoxine (SDM), a sulfonamide antibiotic, is commonly 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 – 11 d in manure samples (Wang et al., 2006b), from 10.5 – 49.8 d in natural water samples (Zhang et al., 2012), from 42.0 – 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; Weng et al., 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 $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$. HPLC grade acetonitrile and formic acid were provided by Sigma-Aldrich (St. Louis, MO).

Soybean meal extract (SBE) was prepared by shaking 10g of soybean meal with 300 mL HPLC water at 150 rpm for 5 days. The extract was then passed through $0.45 \mu\text{m}$ 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, $100 \mu\text{l}$ of reaction mixture was taken from the reactor, and the reaction was stopped immediately by adding $900 \mu\text{l}$ 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 mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu phenol reagent and 2 mL of 7.5% (w/v) sodium carbonate. The absorbance was measured at 765 nm after 15 min incubation at 45°C in dark using a Beckman Coulter DU 800 spectrophotometer (Brea, CA). Quantification was done on the basis of a standard curve of gallic

acid, and the total phenolic content was expressed as gallic acid equivalent (GAE, mg L⁻¹).

2.4. Radical scavenging assessment. DMPO was used as a spin trap agent in this study, because it can form stable DMPO adducts with phenoxyl radicals. The reaction sample was prepared by mixing 1 mL of SBE, 50 µl of 100 mg mL⁻¹ freshly made DMPO solution, and 50 µl of 120 U mL⁻¹ laccase solution in a tube and incubated for 5 hours in dark. The final reaction solution was analyzed with an Orbitrap Elite high-resolution mass spectrometer (Thermo Scientific, San Jose, CA) to detect the coupling product formed between DMPO and phenoxyl radicals. Control samples containing SBE but not DMPO was also processed and measured along with the reaction samples.

2.5. UV-Vis absorption spectrum study. The absorption spectra of the soybean meal extract, the initial reaction solution and the reaction solution after 24h treatment were scanned at 1200 nm/min from 200 nm to 500 nm using a Beckman Coulter DU 800 spectrophotometer (Brea, CA).

2.6. Quantification of sulfadimethoxine. Aqueous samples were passed through 0.22 µm cellulose membrane, and 100 µL of sample was reconstituted in 900 µL of acetonitrile/water (50/50, v/v) mixture before further analysis. Sulfadimethoxine was quantified by a Waters ACQUITY UPLC I-class system coupled with a Xevo TQD tandem mass spectrometer (UPLC-MS/MS) (Milford, MA) with a mobile phase consisting of acetonitrile (solvent A) and 0.1% formic acid in water (solvent B) at a flow rate of 0.3 mL/min for a total retention time of 6 minutes. The spectrometer was operated in electrospray positive (ESI+) mode with the following parameters: capillary voltage 2.60 kV, cone voltage 38.34 V, desolvation temperature 500 °C, cone gas flow 50 L/Hr, and desolvation gas flow 500 L/Hr. Multiple reaction monitoring (MRM) mode was employed to detect and quantify sulfadimethoxine based on the precursor ion (m/z

311.00)/daughter ion (m/z 156.10) spectra.

2.7. Identification of products. The sample for product identification was prepared by degradation treatment solution at 1 ppm initial SDT concentration, 0.5 unit/mL laccase, and 1 ppm mediator, which yielded around 50% SDM removal. A positive control that contained only SDM in water, and a negative control that did not contain SDM but SBE were also incubated and processed along with the treatment sample. MS full scan (m/z 50 – 1000, resolution R = 120000) analyses were performed on the treatment and control samples using an Orbitrap Elite high-resolution tandem mass spectrometer (Thermo Scientific, Waltham, MA) operated in electrospray positive mode. Possible products were identified by screening the m/z peaks present in the treatment samples but not in the controls using the mass filtration function in Xcalibur 2.1 software (Thermo Scientific, Waltham, MA). Molecular formulas of the possible products were assigned using Formula Finder with 5 ppm mass error allowed. Tandem MS (MS/MS) was further performed on the possible products (resolution R = 30000), based on which the molecular structure of each product was deduced by general MS fragmentation rules.

3. Results and Discussion

3.1. SDM degradation in LMS. As shown in **Figure 1A** and **1B**, the residual SDM relative to the initial concentration decreased along the degradation duration. After reacting for 9 hours in the systems with SDM initial concentration at 1 mg/L, the removal rates were 73.3%, 37.1%, 23.9%, and 20.4% in reactors containing SBE, ABTS, HBT, and COU respectively. The same trend was observed in the systems with SDM initial concentration at 10 mg/L: the 9-hour removal of SDM in SBE mediated system was 65.6%, which was much higher than those of the systems mediated by ABTS (54.9%), HBT (46.5%) and COU (38.8%), respectively. In previous

studies, ABTS-mediated system was found to achieve the fastest removal rate in laccase-catalyzed sulfadimethoxine oxidations at room temperature (Weng et al., 2012; Weng et al., 2013). Moreover, as the most intensively studied mediators, ABTS, HBT and COU were also reported to be effective in mediating laccase degradation of several other sulfonamide antibiotics (e.g., sulfathiazole, sulfamethoxazole, sulfadiazine, and sulfamethazine) (Rahmani et al., 2015; Shi et al., 2014). The extraordinary performance of soybean meal extract may be attributed to its high contents of nitrogen, phenolic compounds and divalent cations, which will be elucidated in more detail later in this report. Additionally, the concentration of SDM reduced dramatically during the first hour of SBE-mediated degradation in the experiments with 1 mg L⁻¹ initial concentration (**Figure 1A**), with 89.3% of the total SDM removal over the 9-hour reaction achieved within the first hour. This is consistent with the previous finding that the rate-limiting step in LMS is the process of producing free radicals from mediators, which is governed by the laccase-mediator redox status (Li et al., 1999; Xu et al., 2000). The large redox potential difference between phenolic compounds in SBE and *Trametes versicolor* laccase may lead to fast production of phenolic radicals, and thus accelerate the radical-initiated degradation of SDM, especially when the concentration of the substrate is relatively low. Moreover, when the free radical of laccase mediator is present at high concentration, laccase will be inactivated due to the feedback mechanism (Li et al., 1999), which well explains the low enzyme activity observed in SBE mediated systems (**Table S1**).

In the SBE-mediated reaction systems with initial SDM concentrations at both 1 mg L⁻¹ and 10 mg L⁻¹, a plateau phase was observed in the last stage of degradation (**Figure 1**). Also, slightly decreased degradation rate was found in the systems with 10-mg/L initial concentrations (65.6%) compared to those with 1-mg/L initial concentration (73.3%). Both evidences suggested

the depletion of mediators in SBE as the reaction went on, especially in systems with high SDM concentrations. An experiment with multiple mediator additions (2 ml each time) was conducted to test this hypothesis and the result is shown in **Figure S1**. Further removal of SDM was achieved after each SBE addition.

3.2. Identification of laccase mediators in soybean meal extract. Although a great majority of the natural laccase mediators reported so far are of soybean origin phenolic compounds, not all phenolic compounds are laccase substrates and can play the role of mediators. In order to identify those in SBE that can potentially serve as laccase mediators, DMPO was employed as a scavenger to probe the free radicals formed under laccase catalysis. DMPO is ideal for this purpose because it is inactive towards redox reactions, while form relatively stable radical adducts with oxygen-centered free radicals (Guo et al., 2003). Three DMPO adducts (**Figure 2**) were identified using high resolution mass spectrometry and their structures were confirmed based on high resolution MS data (**Table 1**) and MS/MS spectra (**Figure S2**). Vanillin and apocynin (acetovanillone) are known laccase mediators extracted from a variety of plant sources (Martens, 2002; Stuppner et al., 1995; Walton et al., 2003). They significantly promoted the removal of various recalcitrant chemicals (dyes, pentachlorophenol, and polycyclic aromatic hydrocarbons (PAH)) by laccase in previous studies (Camarero et al., 2005; Cañas et al., 2007; Jeon et al., 2008). Daidzein is one of the major isoflavones contained in soybean meal with a concentration as high as 706 µg/g wet weight (Reinli and Block, 1996). To our best knowledge, this is the first report on daidzein as a natural mediator of laccase.

The degradation of sulfadimethoxine in laccase oxidation system mediated by vanillin, apocynin, and daidzein were determined separately. The results (**Figure 3**) confirmed that the addition of each of these three chemicals enhanced the removal of sulfadimethoxine. However,

the 20-h removal rate (14.7% for DA, 28.2% for VA, 31.9% for AP) was not as high as that achieved by soybean meal extract in 9 hours (65.6%). The particularly effective laccase mediation effect of soybean meal extract may arise from: 1) soybean meal is a protein-based organic fertilizer rich in nitrogen content that can decay into nitroso compound (Hedler et al., 1979), while it is known that nitroso compound, such as 1-hydroxybenzotriazole (HBT), N-hydroxyphthalimide (HPI) and violuric acid (VLA), is a major class of laccase mediators that has been reported having the best efficiency for laccase oxidation of recalcitrant compounds (Cañas and Camarero, 2010; Morozova et al., 2007); 2) the total phenolic content of SBE was determined to be 91.0 mg L⁻¹ gallic acid equivalent (GAE), which is much higher than those of the other mediators tested in present study (**Table S2**). The total phenolic content may be positively correlated to oxidation efficiency as most of the natural mediators are phenolic compounds; 3) soybean meal extract is especially high in total organic carbon, magnesium, and calcium content (**Table S3**), while it has been found in previous studies that transition metal ions (e.g. Mn²⁺) and metallic complexes can act as mediators when they were oxidized by enzyme (Höfer and Schlosser, 1999; Schlosser and Höfer, 2002), and the organic content in SBE may provide natural chelating agents to complex with metals, thus facilitating its oxidation by enzyme (Schlosser and Höfer, 2002); and 4) co-existence of multiple mediators may lead to synergic effect in laccase-mediator systems. Enhanced reaction rate was observed in previous laccase oxidation study when ABTS and natural mediators were present in combination (Jeon et al., 2008). This is because not only the primary radical species but also the secondary species generated through chain reactions among primary radicals can attack the organic pollutants, leading to more efficient oxidation.

The degradation of SDM was also studied in a series of SBE water diluents at various total

phenolic contents (**Figure 4**). The result revealed a positive relationship between the degradation rate of SDM and the dilution rate of SBE. The treatments achieved 64.2% and 43.4% of SDM removal when SBE (originally ~90 mg GAE/L) was diluted to 50 and 25 mg GAE/L, allowing remediation application by dosing SBE into contaminated water at a specific dilution rate. Further reduction of the amount of SBE addition is achievable as the total phenolic content in SBE can be increased by raising the soybean meal to water ratio during extraction or extending the extraction time. Besides, considering that soybean meal is commonly amended to soil as a nutrient booster, its application in soil remediation is especially promising. The feasibility of applying soybean meal extract in soil remediation is being examined in our ongoing projects.

3.3. Reaction Pathways. Possible reaction products were identified by screening the peaks present in HRMS spectra of the treatment samples while not in those of the controls. The high mass accuracy (5 ppm at m/z 400) allows for an unambiguous assignment of the elemental composition of a possible product, as indicated in **Table 2**. The molecular structure was then deduced based on the daughter ions detected in MS/MS analysis according to common fragmentation rules (McLafferty and František, 1993), as depicted in **Figure S3**. Based on the product identification, reaction pathways were proposed for SDM degradation as illustrated in **Figure 5**. The product (m/z 232.1080) assigned with the elemental composition of $C_{12}H_{13}O_2N_3$ (Table 2) corresponds to the loss of SO_2 and an amino group accompanied by rearrangement of a hydrogen. The relative MS peak intensity of this molecule to SDM was 55.59%. A similar pathway was reported in a study on the reaction of several sulfonamides catalyzed by *Trametes versicolor* laccase, including sulfadimethoxine (García-Galán et al., 2011; Schwarz et al., 2010). Moreover, loss of sulfonate group, also known as SO_2 excursion, is a common transformation pathway found in enzymatic degradation, microbial metabolism, anodic Fenton treatment, and

chlorinolysis of sulfonamides (Gao et al., 2012; García-Galán et al., 2011; Mohatt et al., 2011; Neafsey et al., 2010; Schwarz et al., 2010). This process is critical in detoxification treatments because it destroys sulfonyl and amide functional groups, which are responsible for the antimicrobial activity of sulfonamide antibiotics (Henry, 1943). In the UV-Vis spectrum shown in **Figure 6**, the featured peak of sulfadimethoxine at 265 nm diminished significantly after 24 hour enzymatic treatment, providing strong evidence to the removal of sulfonamide group from the parent chemical. Subsequent loss of $-OCH_2$ or $-CH_2$ group led to the formation of $C_{11}H_{11}ON_3$ (m/z 202.0996) or $C_{11}H_{11}O_2N_3$ (m/z 218.0948), respectively, as shown in **Figure 5**. This is the first report on the detection of these two intermediates in enzymatic oxidation of sulfodimethoxine. These two products may further convert to aniline C_6H_7N (m/z 94.0647), through hydrolysis. Aniline was also detected in a previous study as a metabolite of SDM by fungal laccase (Schwarz et al., 2010). Alternatively, SO_2 and aniline can be eliminated directly from SDM to form a pyrimidine product at m/z 156.0763 ($C_6H_9O_2N_3$). In the presence of ligninolytic enzyme and its mediators, aniline and other intermediates may undergo further degradation/mineralization to form smaller molecules, CO_2 , and water (Mahendra et al., 2007; Xu, 1996; Yang et al., 2016).

4. Conclusions

The addition of soybean meal extract with laccase effectively transformed sulfadimethoxine at environmentally relevant condition, while the degradation rates were faster than those tested using HBT, ABTS and *p*-Coumaric acid as mediators. Loss of sulfonate group was the major transformation pathway detected, which resulted in the removal of antimicrobial activity. This study is the first to report the degradation process, mediator identification and reaction pathways

in LMS system mediated by the extract of naturally origin soybean meal. It is worthwhile in future studies to understand the relative contributions of phenolic compounds, metal ions, and the interactions between these chemicals and organic pollutants.

ASSOCIATED CONTENT

Supporting Information Available

Results of multi-dosing SBE test; Enzyme activity; HRMS data and MS/MS spectrum of DMPO and mediator coupling products; HRMS data and MS/MS spectrum of SDM degradation products; Total phenolic content, total organic carbon and metal ion contents of soybean meal extract and other mediator solutions.

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Figure captions

Figure 1. Degradation of sulfadimethoxine (SDM) in laccase-mediator systems at 25 °C. (A) 1 mg L⁻¹ initial SDM, 0.5 unit mL⁻¹ enzyme activity, 1 mg L⁻¹ initial mediator concentration; (B) 10 mg L⁻¹ initial SDM, 5 unit mL⁻¹ enzyme activity, 10 mg L⁻¹ initial mediator concentration; TV, *trametes versicolor* laccase; SBE, soybean meal extract; HBT, 1-Hydroxybenzotriazole; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); COU, *p*-coumaric acid.

Figure 2. Proposed coupling reaction between 5, 5-Dimethyl-1-Pyrroline-N-Oxide (DMPO) and phenolic mediators.

Figure 3. Degradation of sulfadimethoxine (SDM) in laccase-mediator systems at 25 °C. TV, *trametes versicolor* laccase; VA, vanillin; AP, apocynin; DA, daidzein. Reaction conditions were the same as Figure 1B.

Figure 4. The removal of sulfadimethoxine (SDM) in SBE diluents at various total phenolic content expressed as gallic acid equivalent (GAE). The initial SDM concentration was 1 ppm, and the initial enzyme activity was 0.5 unit mL⁻¹.

Figure 5. Proposed transformation pathways of sulfadimethoxine in laccase-SBE treatment system.

Figure 6. UV-Vis scan spectrum of 10 mg L⁻¹ SDM before and after laccase-SBE treatments. Reaction conditions were the same as Figure 1B.

Table 1. High resolution mass spectrometry data of DMPO-mediator coupling products.

Notation	Elemental Composition	[M+H] ⁺		Error (ppm)
		Measured Mass	Theoretical Mass	
DMPO + Vanillin	C ₁₄ H ₁₈ O ₄ N	265.1304	265.1309	-1.73
DMPO + Apocynin	C ₁₅ H ₂₀ O ₅ N	295.1410	295.1414	-1.59
DMPO + Daidzein	C ₂₁ H ₂₁ O ₅ N	368.1496	368.1492	0.91

Table 2. High resolution mass spectrometry data of SDM degradation products.

Notation	Elemental Composition	[M+H] ⁺		Error (ppm)	Relative Intensity (%)
		Measured Mass	Theoretical Mass		
Aniline	C ₆ H ₇ N	94.0647	94.0651	-4.53	1.33
SDM-SO ₂ -Aniline	C ₆ H ₉ O ₂ N ₃	156.0763	156.0768	-2.75	0.27
SDM-SO ₂ -NH-OCH ₂	C ₁₁ H ₁₁ ON ₃	202.0975	202.0975	-0.07	11.08
SDM-SO ₂ -NH-CH ₂	C ₁₁ H ₁₁ O ₂ N ₃	218.0923	218.0924	-0.53	9.61
SDM-SO ₂ -NH	C ₁₂ H ₁₃ O ₂ N ₃	232.1080	232.1081	-0.41	55.59
SDM	C ₁₂ H ₁₄ O ₄ N ₄ S	311.0802	311.0809	-2.14	100.00

Figure 1. Degradation of sulfadimethoxine (SDM) in laccase-mediator systems at 25 °C. (A) 1 mg L⁻¹ initial SDM, 0.5 unit mL⁻¹ enzyme activity, 1 mg L⁻¹ initial mediator concentration; (B) 10 mg L⁻¹ initial SDM, 5 unit mL⁻¹ enzyme activity, 10 mg L⁻¹ initial mediator concentration; TV, *trametes versicolor* laccase; SBE, soybean meal extract; HBT, 1-Hydroxybenzotriazole; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); COU, *p*-coumaric acid.

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Figure 5. Proposed transformation pathways of sulfadimethoxine in laccase-SBE treatment system. $r\text{H}$ stands for proton rearrangement.

Figure 6. UV-Vis scan spectrum of 10 mg L⁻¹ SDM before and after laccase-SBE treatments.

Reaction conditions were the same as Figure 1B.