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Eliminating antibiotics by white-rot-fungi *Trametes versicolor* from manure solids and synthetic wastewater☆

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ABSTRACT

Antibiotics have been abused in livestock as veterinary drug and feed additive. Their incomplete metabolization by animals resulted in heavy accumulation in livestock manure, and therefore they can pose a threat to the environment. In this study, the mechanism of three antibiotics (oxytetracycline (OTC), sulfadiazine (SDZ), enrofloxacin (ENR)) removal/biodegradation by *Trametes versicolor* pellets in air-pulse fluidized-bed reactor was explored, and the effects of wood immobilized *T. versicolor* on four antibiotics (OTC, SDZ, ENR and chloramphenicol (CAP)) removal in solid cow manures were evaluated. *T. versicolor* could remove OTC, SDZ, ENR through adsorption and biodegradation, with the removal efficiency at 92 % and 98 % in 21 h and 98 % after 68 h, respectively. The removal kinetics of those three antibiotics fitted well with the first-order kinetic model, with the removal constant k at −0.238 h^{-1}, -0.102 h^{-1} and -0.023 h^{-1}, respectively. *T. versicolor* could biodegrade those three antibiotics using laccase and cytochrome P450 system with the order SDZ ≈ OTC > ENR. Furthermore, wood immobilized *T. versicolor* promoted SDZ, OTC, ENR and chloramphenicol (CAP) antibiotic biodegradation in cow manure, especially in high inoculation ratio (wood immobilized *T. versicolor*: solid cow manures = 1:2). This study revealed the mechanism of simultaneous SDZ, OTC, ENR and CAP antibiotic removal/biodegradation by white-rot fungi *T. versicolor* even by wood immobilized *T. versicolor* in solid cow manures, which provide a theoretical basis for future application of biological removal of antibiotics present in wastewater and solid manures.

1. Introduction

Antibiotics has been wildly applied in human and animals with the advantages of controlling diseases and promoting growth (Hanekamp and Bast, 2015). Those antibiotics used only in livestock (such as swine, poultry and cattle) production were evaluated to be 118,940 tons in 2013, and are expected to increase by 52 % in 2030 (Oberoi et al., 2019). Antibiotics can not be completely metabolized by animals and thus nearly 50–90 % of the parent compounds are excreted out via feces and urine (Si et al., 2022). Due to the extensive use of antibiotics, incomplete waste management and incomplete remove by waste treatment approaches, antibiotics are continuously released into the environment. Thus, antibiotic residues has been detected in various environmetal media and food sources, such as manure-ammended soils, sewage sludge, aquaculture farm, children's urine, eggs, milk, etc (Fang et al., 2023; Ke et al., 2023; Liu et al., 2021). For example, Shen et al. (2023) found that ciprofloxacin, enrofloxacin, oxytetracycline, tetracycline, neomycin were detected in pig manures, whose concentration were at 87.5 μg/kg, 662.7 μg/kg, 43.3 mg/kg, 2.1 mg/kg and 0.6 mg/kg, respectively. Besides, Salma et al. (2025) detected 26 antibiotics in Buriganga River water in Bangladesh, especially maximum concentration of sulfonamides and fluroquinolones at 790 and 710 ng/L. Furthermore, the accumulation of antibiotics in the environment leads various adverse damages to the environment and human beings, such as inducing the promotion of antibiotic-resistant bacteria (ARBs) and genes (ARG) (Zhang et al., 2022), damaging soil microbial community

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structure (Chow et al., 2021) and reducing soil fertility (Lyu et al., 2020). Therefore, it's imperitive to seek efficiency and eco-friendly approaches to eliminate antibiotics residues in the environment.

In order to meet the increasing global meat production, modern husbandry is experiencing rapid development and expansion, leading to a rise in manure production, which had serious detrimental effects on the environment (Fang et al., 2023; Jia et al., 2018). It was estimated that the amount of manure production reached nearly 551 Mt (dry weight) in 2014, and only 38 % of manure could be directly recycled to apply into the filed soil (Jia et al., 2018). Consequently, a large amount of manure waste remained, causing adverse effect on environment health, such as ammonia evaporatuon, nutrient leaching, ARG and ARB migration (Hong et al., 2023; Zhao et al., 2024). Furthermore, with the abuse of antibiotics in husbandry, manure usually contains high contents of multiple antibiotics, leading to adverse risks to the ecosystem and human health (He et al., 2023). However, due to its complex composition, manure can absorb and accumulate a wide variety of antibiotics, reducing their transformation potential and their migration to the environment (Chi et al., 2022; Ma et al., 2022). Therefore, manure treatment for reducing or eliminating antibiotics residues is imminent before its recycle utilization.

White rot fungi (WRF) refer to a group of fungi that could colonize on wood and are named for the characteristic white, fibrous decay they cause (Chen et al., 2022). They are well-known degraders in nature, capable of decomposing both lignin and cellulose biopolymers in lignocellulosic biomass (Gao et al., 2024). *T. versicolor*, as a WRF, can aerobically degrade diverse oganic matters, including many persistent oganic pollutants, such as diuron, bentazone, chlorpyrifos, quinolone and etc. For example, in our previous study, we proved that *T. versicolor* was capable of biodegrading chloramphenicol (CAP), a wide-spectrum antibitics (Tan et al., 2023). Furthermore, immobilized WRF has been wildly applied in many oganic pollutants removal due to its higher level of enzymatic activity and more resilence to enviornmental perturbations. For example, Toran et al. (2017) found that the immobilized *T. versicolor* on different lignocellulosic materials (pine bark, nutshell, hazelnut shell and wood pallet) could remove ibuprofen, ketoprofen and naproxen in real hospital wastewater. While, there are scarce information of multiple antibiotics biodegradation by WRF at the same time, especially by wood immobilized WRF in non-sterilized realistic system. Therefore, it's necessary to determin the potential of degrading multiple antibiotics by *T. versicolor* in air-pulse fluidized-bed reactor or by wood immobilized *T. versicolor* in non-sterilized cow manures before realistic application.

In this study, four category antibiotics (tetracyclines: oxytetracycline (OTC); quinolones: enrofloxacin (ENR); sulfonamides: sulfadiazine (SDZ); chloramphenicols: CAP) were selected for further study due to their wide application in livestock cultivation and high detection rate in livestock manures. The aim of this study was to investigate the potential for antibiotic biodegradation by *T. versicolor*, firstly using fungal pellets in an air-pulse fluidized-bed reactor to overcome the bottleneck of Erlenmeyer flask scale studies, and secondly using wood immobilized *T. versicolor* to treat solid cattle manure, thus working in a more realistic system and under non-sterile conditions. The results from this study could elucidate the biodegradtion mechanism of antibiotics by *T. versicolor*, and may provide new insights for developing a treatment process for wastewater/manure containing antibiotics by *T. versicolor*.

2. Materials and methods

2.1. Fungal strain and medium

T. versicolor ATCC 42530 was obtained from American Type Culture Collection previous and stored in our lab in −80 and −20 °C. Blended mycelial suspension and pellets were prepared according to our previous work (Tan et al., 2023). The malt extract meidum (2 %) and defined medium (DM), glucose 8 g/L, NH_4Cl 1.42 g/L, micronutrients 10 mL/L, macronutrients 100 mL/L, pH 4.5) was prepared as described by (Blánquez et al., 2006).

2.2. Chemicals and reagents

The standard of oxytetracycline (OTC, CAS#79-57-2), sulfadiazine (SDZ, CAS#68-35-9), enrofloxacin (ENR, CAS#93106-60-6), chloramphenicol (CAP, CAS#56-75-7), the laccase mediator 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, CAS#30931-67-0), the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT, CAS#1614-12-6) were purchased from Sigma-Aldrich (Barcelona, Spain), with all reagents having a purity ≥98 % (Table 1). Commercial laccase (20 AU/mg, purified from *T. versicolor*, CAS#80498-15-3), were also purchased from Sigma-Aldrich (Barcelona, Spain). Chromatographic grade acetonitrile and methanol were provided by Merck (Darmstadt, Germany).

Antibiotics stock solutions were prepared at a concentration of 1 g/L with ethanol and sterilized through 0.22 μm sterilized nylon filter before use. All the commercial inorganic and organic compounds were analytical grade and chromatographic grade, respectively. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) 0.1 M solution, citric acid anhydrous and disodium hydrogen phosphate salts obtained from Panreac (Barcelona, Spain). McIlvaine buffer at pH 4 was prepared by mixing (for 100 mL) 38.5 ml of a 0.2 M Na_2HPO_4 and 61.5 mL of a 0.1 M citric acid solution. Analytical standards used for quantification were of high purity grade (>90 %). Isotopically labelled standards (IS), used as surrogate standards (enrofloxacin-d5, diclofenac-d5, sulfadiazine-d4, tetracycline-d6), were also purchased from Sigma-Aldrich (Madrid, Spain) and Toronto Research Chemicals (Ontario, Canada). Fresh stock solutions of antibiotics were prepared monthly for the chromatographic analysis.

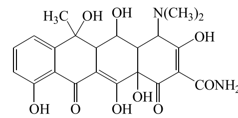
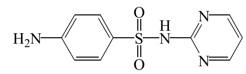
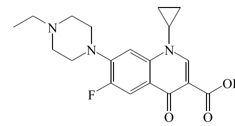
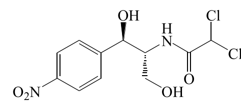
2.3. Degradation experiments in an air-pulsed fluidized-bed bioreactor

The experimental air-pulsed fluidized-bed bioreactor was configured as shown in Fig. S1. The bioreactor has a cylindrical vertical center body connected with a ceramic plate as air diffuser at the bottom and a wider head. The working volume of the bioreactor was approximately 1.5 L. One liter DM medium spiked with 10 mg/L SDZ, OTC, ENR was added into the bioreactor. *T. versicolor* pellets were inoculated into the air-pulsed fluidized-bed bioreactor to reach 2.5 g/L (dry weight). Glucose was initially added and then supplemented to a concentration of 4 g/L when glucose was completely consumed. Antibiotics were supplemented into the medium at the end of each batch. The medium pH was controlled at pH 4.5 ± 0.1 by adding NaOH or HCl (1M), and the aeration rate was maintained at 0.8 L/min with 1 s air pulse in every 3 s. Temperature was set at 25 °C. The bioreactor operated in three batches, the first batch performed in 192 h, the second 143 h, the third 44 h. Samples were collected during the experiment for laccase, glucose and antibiotics detection.

2.4. Hydrolysis, photodegradation and adsorption assay

Before the experiments, killed *T. versicolor* was prepared as follow: *T. versicolor* was autoclaved under 121 °C in 20 min. For the hydrolysis, photolysis and adsorption of the antibiotics, three treatments were conducted: Hydrolysis Treatment (H): antibiotics were added into the DM medium at a final concentration of 10 mg/L under dark conditions; Adsorption Treatment (H + A): killed *T. versicolor* was inoculated into Hydrolysis Treatment at a final concentration of 2.5 g/L (dry weight) under dark conditions; Photolysis Treatment (H + F): Hydrolysis Treatment was performed under light conditions. All the treatments were incubated at 150 rpm, 25 °C. Each experimental treatment was conducted in triplicate. Samples were collected at 0 and 281 h, and the concentration of antibiotics was then detected by HPLC.

Table 1
The characterization of four antibiotics, oxytetracycline, sulfadiazine, enrofloxacin, chloramphenicol.

Compound name	Chemical formula	Measured mass(*m/z*)	Possible structural formula	CAS No.
oxytetracycline	$C_{22}H_{24}N_2O_9$	461.15		CAS#79-57-2
sulfadiazine	$C_{10}H_{10}N_4O_2S$	251.06		CAS#68-35-9
enrofloxacin	$C_{19}H_{22}FN_3O_3$	359.40		CAS#93106-60-6
CAP	$C_{11}H_{12}O_5N_2Cl_2$	323.02		CAS#56-75-7

2.5. Evaluation of the enzymatic system involved in antibiotics degradation

Fungal cytochrome P450 system-mediated antibiotics biodegradation was performed according to Garcia-Vara et al. (2021). Fungal pellets were inoculated into the DM medium containing 10 mg/L antibiotics at a final concentration of 2.5 g/L (dry weight). Two treatments were conducted: EXP: antibiotics were added into the medium at a final concentration of 10 mg/L. EXP + ABT: cytochrome P450 inhibitor ABT was added into EXP Treatment at a final concentration of 5 mM. All the treatments were incubated at 150 rpm, 25 °C. Each experimental treatment was conducted in triplicate. Samples were collected at 0, 1.5, 5, 7, 24, 48 and 96 h, and the concentration of antibiotics was then detected by HPLC.

Laccase-mediated antibiotics biodegradation was performed according to our previous study (Tan et al., 2023). Commercial laccase was added into 250 mL Erlenmeyer flasks containing 50 mL reaction solution (250 mM sodium malonate dibasic monohydrate, pH 4.5) at a final enzyme activity of 500 AU/L. Laccase mediator ABTS was prepared and added into the reaction solution at a final concentration of 0.5 mM. Three treatments were conducted: Laccase Treatment: antibiotics were added into the reaction solution at a final concentration of 10 mg/L; Laccase + ABTS Treatment: laccase mediator ABTS was supplemented into Laccase Treatment at a final concentration of 0.5 mM; Abiotic control (CK): antibiotics were added into the reaction solution without laccase and laccase mediator ABTS. All treatments were incubated at 150 rpm, 25 °C. Each experimental treatment was conducted in triplicate. Samples were collected and mixed with HCl (1 M) to stop the reaction at 0, 0.5, 1, 4, 24 and 48 h, and the concentration of antibiotics was then detected by HPLC.

2.6. Antibiotics biodegradation by immobilized *T. versicolor* in solid manure

The immobilized *T. versicoclor* was prepared according to (Beltrán-Flores et al., 2020). Firstly, a mycelial suspension of *T. versicolor* was obtained through inoculating its agar subculture (1 cm × 1 cm). Then, the prepared mycelial suspension mixed with the sterilized wood chips (250 mL suspension per kg wood). Finaly, the mixtures were incubated at 25 °C in 30 days before use.

The solid cow manures were spiked with 10 mg/kg each of CAP, SDZ, OTC and ENR. Then, the prepared immobilized *T. versicoclor* woods was mixed with the spiked solid cow manures in different ratio, and five treatments were conducted (Fig. S2): I and II: immobilized wood mixed with solid cow manures with the ratio at 1:0.5 and 1:2 (w/w), respectively; III and IV: sterile wood (previously autoclaved) mixed with solid cow manures with the ratio at 1:0.5 (w/w) and 1:2 (w/w), respectively; V: only solid cow manures. All the treatment incubated at room temperature in dark condition. Cow manures sample were collected after 0, 3 and 6 weeks, and stored at −20 °C before antibiotics detection.

2.7. Analytical methods

2.7.1. Laccase activity and glucose concentration testing

According to the conventional methods in our previous research, the activity of laccase was determined through the reaction of oxidizing 2,6-dymetoxyphenol (DMP) by laccase with the absence of a cofactor (Hu et al., 2022). The concentration of glucose was quantified by a biochemistry analyzer (2700 select, Yellow Springs Instrument, USA).

2.7.2. Antibiotics analysis for liquid samples

Antibiotic concentrations were separated and quantified by HPLC (Ultimate 3000, Dionex, USA), equipped with a UV detector and a C18 reversed-phase column (Phenomenex®, Kinetex® EVO C18 100 Å, 4.6 mm × 150 mm, 5 μm). The mobile phase consisted of 2.5 % methanol (solvent A) and 2.5 % formic acid (solvent B) (v/v). The running conditions were set as follows: mobile phase composition of 80 % A and 20 % B, flow rate at 1 mL/min, injection volume at 5 μL, column temperature at 30 °C, detection wavelength of SDZ, OTC and ENR at 270 nm, 355 nm and 280 nm, respectively. The retention time of SDZ, OTC and ENR were at 3.5 min, 4.9 min and 7.9 min, respectively.

2.7.3. Antibiotics analysis for solid manure samples

Antibiotics from manure samples were extracted with a salt-assisted acetonitrile extraction. Briefly, 1 g of freeze-dried manure was mixed with 5 mL of McIlvaine buffer (pH = 4) and then 6 mL of ACN, vortexing after each step. The tubes were placed in an ultrasonic bath for 15 min (one cycle) and then they were centrifuged for 5 min at 10,000 rpm and 4 °C. The supernatant was collected and diluted with chromatographic grade water to a 1:10 ratio in 1:1 MeOH:H_2O. The analysis was performed with a Waters Acquity Ultra-PerformanceTM liquid chromatography system equipped with two binary pumps systems (Milford, MA, USA) and coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo ion spray source. The positive ionized compounds were analyzed with an Acquity HSS T3 column (50 mm × 2.1 mm i.d., 1.8 μm particle size, Waters Corporation) with acetonitrile and 0.1 % formic acid as mobile phases at 0.5 mL/min, while negative ionized compounds were analyzed with an Acquity BEH C18 column (50 mm × 2.1 mm i.d., 1.7 m particle size), with acetonitrile and an aqueous solution of 5 mM ammonium acetate/ammonia at pH 8 as mobile phases at a 0.6 mL/min flow. Information on the detection parameters is displayed in Table S2.

2.7.4. Identification of transformation products

UPLC (Agilent, USA) coupled with a Q-TOF-MS spectrometer (Acquity, Bruker, Germany) was used to identify the biodegradation products of antibiotics. The UPLC separation conditions were changed as follows: mobile phase consisting of 0.1 % formic acid in water and methanol, flow rate at 1 mL/min, column temperature at 30 °C, injection volume at 10 μL, detection wavelength at 278 nm. The remaining UPLC-TOF-MS parameters was followed by Tan et al. (2023).

3. Results and discussion

3.1. Antibiotics removal by T. versicolor in air-pulsed fluidized-bed bioreactor

The biodegradation abilities of three antibiotics, SDZ, OTC and ENR, by *T. versicolor* pellets were evaluated in an air-pulse fluidized-bed bioreactor (Fig. 1). The removal efficiency of SDZ and OTC by *T. versicolor* increased sharply with the efficiency >92 % in 21 h. While, the removal efficiency of ENR was nearly at 10 % in 21 h, and up to nearly at 98 % after 68 h. Notably, same increasing trend of those three antibiotics (SDZ, OTC, ENR) removal efficiency was observed in the 2nd cycle, but the removal efficiencies were inhibited with the efficiency at 65 %, 20 % and 5 % in 23 h, respectively. Notably, the final removal rate of those three antibiotics was nearly same in the first and second cycle.

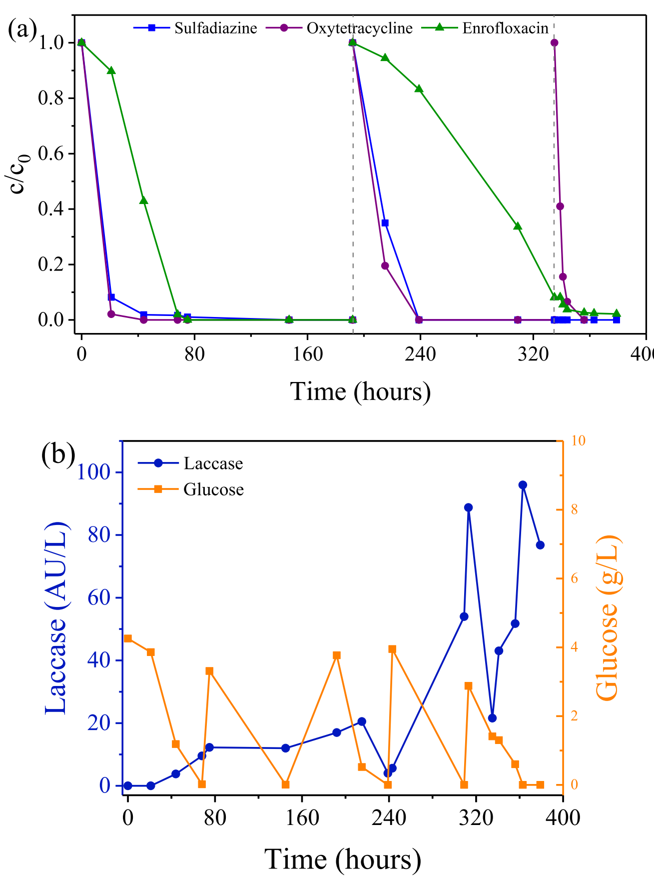


Fig. 1. Time-course of remaining of antibiotics concentration (a), laccase activity, and glucose concentration (b) by *T. versicolor* in air-pulse fluidized-bed reactor with initial antibiotics concentration at 10 mg/L.

However, in the second cycle, the OTC initial concentration was significantly lower (2 mg/L), so a third pulse of OTC to get higher initial concentration in the bioreactor (10 mg/L) while the ENR degradation time was extended. Removal efficiency of OTC reached at 84 % and 100 % after 9 h and 21 h, respectively, which mean the biodegradation of OTC was promoted in the third cycle. It may due to the single addition of OTC in the reactor, whose inhibition effect was lower than those three combined antibiotics. Same situation that sulfanilamide antibiotics were degraded by *T. versicolor* with the efficiency of 96.2 %, which ascribed for MnP and laccase activity (Zhang et al., 2023a,b,c). While, Befenzi et al. (2025) found that WRF *Bjerkandera adusta* could effectively degrade ciprofloxacin and enrofloxacin, with the efficiency at 82 % and 99 % in 7 days, respectively, which were strongly-related with heme peroxidases. Therefore, the comparative low degradation efficiency of ENR may due to the different enzyme system.

The removal kinetics of SDZ, OTC and ENR by *T. versicolor* fitted well with the first-order kinetic model (Fig. 2), with a correlation coefficient R^2 of 0.936, 0.974 and 0.959 (Table S1), respectively.

This observation aligns with previous studies on tetracycline and CAP biodegradation by some bacteria, such as *Sphingobacterium changzhouense* TC931, *Citrobacter* sp. SZW2 and etc (Luo and Tan, 2024; Tan et al., 2021). The kinetic removal constant of OTC by *T. versicolor* was higher than SDZ and ENR, the removal constant k was $-0.238\ h^{-1}$, $-0.102\ h^{-1}$ and $-0.023\ h^{-1}$ (Table S1), respectively, indicating that the removal ability of those three antibiotics by *T. versicolor* were OTC > SDZ > ENR. The difference biodegradation of those three antibiotics by *T. versicolor* may due to the different enzyme catalytic system. For example, fungal laccase can degrade various types of recalcitrant pollutants, such as PAHs, antibiotics and dyes (Dong et al., 2023). Cvancarova et al. (2015) found that the manganese peroxidase was involved in the degradation of norfloxacin (NOR) and ofloxacin (OF) by *Irpex lacteus*.

Furthermore, the laccase activity produced by *T. versicolor* was also determined during the treatment (Fig. 1). Laccase activity increased slightly to 12.2 AU/L at day 75 at the beginning of first cycle, and then plateaued between at hour 75 to hour 215. The lag phase of laccase activity at the beginning may due to the adaptation of *T. versicolor* to the environment, especially under stress. Same observation was reported by our previous study that exogenous 5 mg/L CAP inhibited the laccase activity at the beginning of 7 days, and then the laccase activity increased (Tan et al., 2023). With the supplementation of antibiotics at hour 192, the laccase activity decreased sharply to 4 AU/L at day 239 in

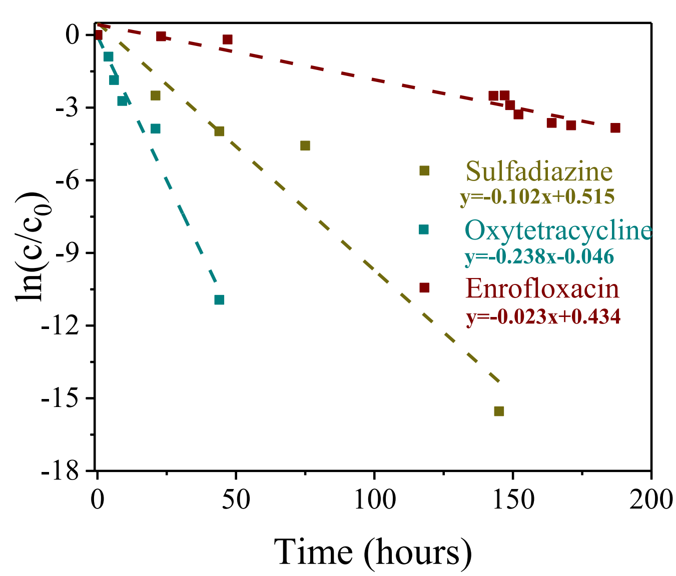


Fig. 2. Pseudo-first-order kinetics model for biodegradation of antibiotics by *T. versicolor*.

2nd cycle, and then increased sharply from 5.6 AU/L at hour 243 to 88.7 AU/L at hour 313. The variation of laccase in the medium may due to the antibiotics stress, which was similar with Suboh et al. (2022) that the addition of OTC inhibit the production of laccase nearly 100 %.

3.2. Antibiotics hydrolysis/photodegradation and adsorption assay by *T. versicolor*

Adsorption and biodegradation play an important role in antibiotics removal in waste management in which manures serves as a huge reservoir of antibiotics, especially veterinary antibiotics. Antibiotic compounds usually contain a multitude of functional groups, which induce various biotic and abiotic behaviors, such as adsorption, photodegradation and hydrolysis (Oberoi et al., 2019). As shown in Fig. 3, the concentration of SDZ and ENR remained almost constant after 281h, regardless of light exposure or the presence of killed *T. versicolor* inoculation, indicating that SDZ and ENR were relatively stable in aqueous environment, and neither hydrolysis nor photolysis occur spontaneously, consistent with previous study (Harrabi et al., 2019; Zhang et al., 2023a,b,c; Zhang et al., 2019). However, the concentration of OTC decreased sharply in aqueous condition after 281 days, but there was no significant difference in the decrement of OTC concentration between hydrolysis and photolysis treatment. Furthermore, a small amount of OTC was adsorbed by killed *T. versicolor*. OTC, SDZ and ENR with lower log Kow values (−0.9, −0.09 and 0.702, respectively) showed lower bioadsorption tendencies by *T. versicolor* (Kiki et al., 2020). These results indicated that the chemical structure of OTC was unstable and prone to be hydrolyzed and adsorbed. Same observation was reported by Shi et al. (2021) that hydrolysis is an important part of OTC degradation.

3.3. Antibiotic biodegradation assay by cytochrome P450 enzymatic systems and laccase

Cytochrome P450 system and laccase of WRF have been verified to metabolize various recalcitrant environmental pollutants (Lin et al., 2022; Singh et al., 2023). Cytochrome P450 system was an intracellular enzymatic system, it can convert recalcitrant pollutants into hydrophilic compounds or less toxic forms as a primary catalyst through inserting electrophilic groups (Lin et al., 2022). For example, Garcia-Vara et al. (2021) found that *T. versicolor* could biodegrade bentazone through cytochrome P450 system with the efficiency of 80 % within 42 h. Fungal laccase, an extracellularly diverse enzyme/biocatalyst, was considered as a promising biocatalyst for recalcitrant pollutants due to its low substrate specificity and monoelectronic oxidation of substrates (Dong et al., 2023). Thus, in this study, whether the cytochrome P450 system and laccase of *T. versicolor* was involved in SDZ, OTC and ENR biodegradation were explored.

In Fig. 4, the concentration of SDZ and OTC decreased with the degradation efficiency of 15 % and 90 % within 96 h, respectively. While, with the presence of cytochrome P450 inhibitor 1-aminobenzotriazole (ABT), the concentration of SDZ and OTC remained unaffected. However, the ENR concentration remained unaltered regardless of the ABT presence. It was proved that ENR can be demethylated by the P450 system to ciprofloxacin (CIP) in sea bass, which eventually inactivate the cytochrome P450 enzymes (Vaccaro et al., 2003). But, Wang et al. (2023) found that cytochrome P450 enzymes were responsible for fluoroquinolone degrading of microalgae through molecular analysis. While, in this study, all those results suggested that cytochrome P450 system of *T. versicolor* was responsible for SDZ and OTC biodegradation, but is not involved in ENR decomposition, which may due to the different molecular structure of cytochrome P450 enzymes in different organism. While, Befenzi et al. (2025) found that the biodegradation of ENR (the degradation efficiency at 99 % in 7 days) by WRF *B. adusta* was ascribed by versatile peroxidase (e.g. heme peroxidase).

In Fig. 5, the biodegradation ability of SDZ, OTC and ENR by laccase was determined. The concentration of SDZ and ENR remained stable with or without laccase in 48 h treatment. However, OTC concentration decreased slightly, with reductions of 29 % and 46 % with or without laccase, respectively, which was attributed to direct degradation by laccase and hydrolysis. The laccase-mediator system (LMS) enhances the ability of laccase to decompose various organic compounds other than phenolic ones, including natural and synthetic laccase mediators (e.g., ferulic acid, acetovanillone, 1-hydroxybenzotriazole and ABTS) (Spina et al., 2020; Dong et al., 2023). In the presence of mediator ABTS on laccase degradation system, the concentration of SDZ, OTC and ENR decreased sharply, with efficiency of 95 %, 91 % and 50 % in 0.5, 0.5 and 48 h, respectively. These results indicated that laccase could biodegrade the antibiotics with the order SDZ ≈ OTC > ENR, which was similar with the results in SBR.

3.4. Antibiotics biodegradation products

The biodegradation products of those three antibiotics by *T. versicolor* were identified through HPLC-TOF-MS, and two biodegradation products were identified, compound 2 (m/z = 334, $C_{17}H_{20}N_3FO_3$) and compound 3 (m/z = 332, $C_{17}H_{18}N_3FO_3$). Compound 3 was identified as CIP due to the exactly similar m/z, with the deethylation of the ethyl on the piperazin ring, which was the most metabolite species and active metabolite of ENR (Trouchon and Lefebvre, 2016). This phenomenon has been observed in various studies where CIP residues were detected after ENR administration to animals such as goats and dairy cows (Idowu et al., 2010; Rao et al., 2002). During the degradation of ENR by *T. versicolor*, the formation of compound 3 exhibited a lag-phase after ENR supplementation, and its concentration increased at the beginning and then decreased (Fig. 6a). During the degradation process of ENR by *T. versicolor*, ENR was

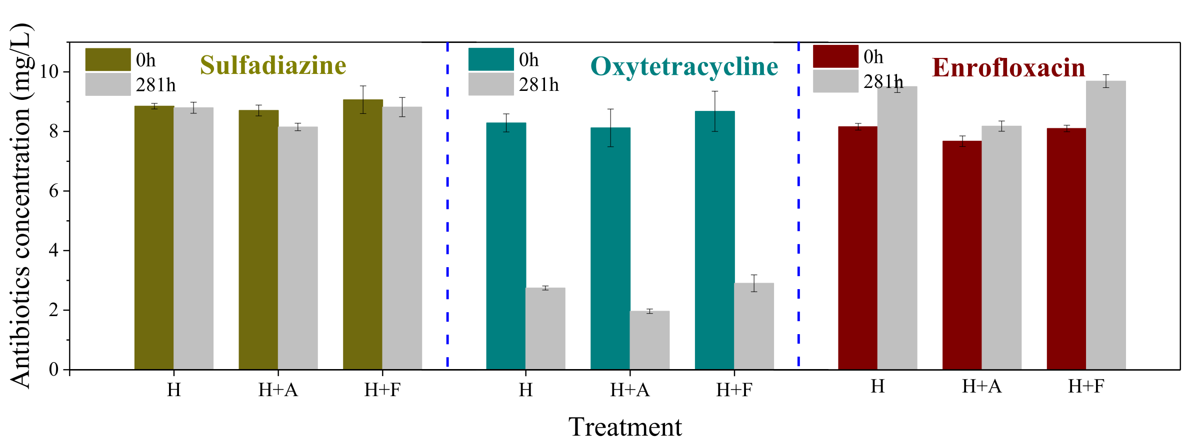


Fig. 3. Hydrolysis (H), adsorption(A) and photodegradation (F) assay of SDZ, OTC and ENR by killed *T. versicolor*.

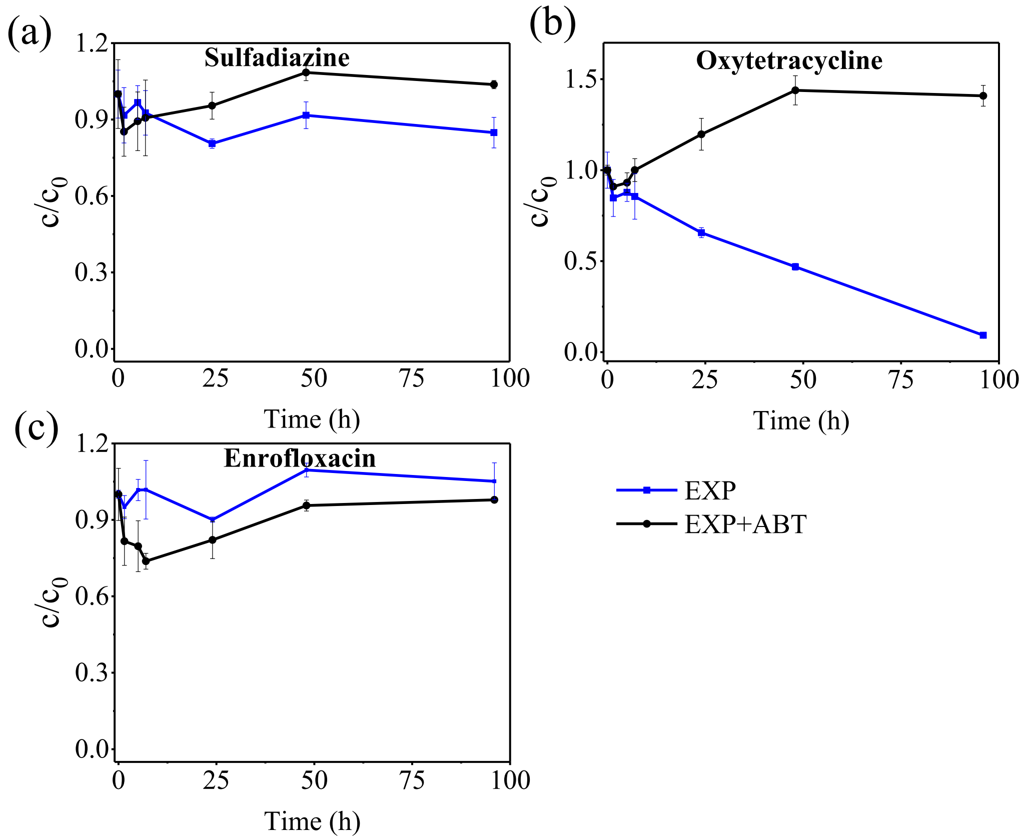


Fig. 4. The biodegradation assay of SDZ (a), OTC (b) and ENR (c) by CP450 system of *T. versicolor*.

deethylated and CIP (compound 3) formed subsequently. Then CIP was oxidized with the production of compound 2 (Fig. 6b). Furthermore, the toxicity of ENR parent compound and those tow biotransformation products (compound 2 and CIP) were evaluated using ECOSAR Program (Table 2). The predicted lethal concentration of compound 2 and CIP were higher than ENR to Fish, Daphnid, Green Algae, indicating the toxicity of the original parent compound ENR exceeded that of the final biotransformation products. Thus, ENR was difficult to degrade, and its biodegradation process was more toxic than other two antibiotics to *T. versicolor* due to the formation of the active metabolite CIP.

3.5. Antibiotics biodegradation by immobilized T. versicolor in solid manure

Antibiotics are wildly used in livestock, and a significant portion of these excreted out antibiotics accumulates in the solid fraction, potentially altering their chemical properties and inhibiting their migration and transformation. For example, Zahedi et al. (2022) found that 4 fluoroquinolones, 3 tetracyclines, 2 sulfonamides and 1 lincosamides were detected in solid cattle manures, ranging from 0 to 8.9 mg/kg. Wang et al. (2024) found that the detection rate of 6 kinds of antibiotics, including sulfamethoxazole (SMX), sulfamerazine (SMR), tetracycline, OTC, NOR, and ENR, were 100 % in 8 manure samples from six farms, with the mean concentration at 26.51 mg/kg, and the pollution level of OTC was the highest. Notably, using immobilized antibiotic-degrading microorganism to reduce or eliminate antibiotics residues in solid manures is considered a promising approach. In this study, the wood immobilized *T. versicolor* was inoculated into the antibiotic-contaminated cow manures at different ratio (1:0.5 and 1:2, w/w) (Table 3). CAP, SDZ and OTC were rapidly removed under natural conditions (treatment 3W_V) after three weeks with the removal efficiency of 99 %, 90 % and 69 %, respectively. However, the removal of ENR was comparatively slow, with an efficiency of 1.3 %. This finding suggests that the indigenous microbial community in cow manure could effectively biodegrade CAP, SDZ and OTC. Same situation was observed in many manures (Zhang et al., 2023a,b,c; Gaballah et al., 2024). Furthermore, some pure antibiotic-degrading bacteria or fungi were isolated from manures or soils after their application. For example, Tan et al. (2021) isolated a TC biodegrading bacterium, *Sphingobacterium changzhouense* TC931, from manure applied soils. In contrast, with the inoculation of killed wood (treatment 3W_III and 3W_IV), the concentration of CAP, SDZ, ENR decreased and was lower than that under natural condition (treatment 3W_V) with the exception of OTC, indicating that the wood can adsorb a small amount of CAP, SDZ and ENR. This might be due to their lower log Kow values, which showed lower bioadsorption by organic matrices. Same observation was reported by Kiki et al. (2020), where fluoroquinolones with log Kow values at −0.3–1.6 showed lower bioadsorption tendencies by microalgae.

Notably, with the inoculation of wood immobilized *T. versicolor* after three weeks (treatment 3W_I and 3W_II), the concentration of ENR decreased sharply with a biodegradation efficiency of 42.5 % and 30 % in the inoculation ratio of 1:0.5 and 1:2, respectively. Therefore, lower inoculation ratio of wood mobilized *T. versicolo* in cow manures means higher manure dosage background and higher concentration of organic matters. Moreover, *T. versicolo* could utilize more organic matters for rapid proliferation and produce high content of laccase. Furthermore, laccase has been proved for SDZ, OTC and ENR biodegradation (Fig. 5). So, a lower inoculation ratio of wood mobilized *T. versicolo* in cow manures resulted in a higher degradation efficiency of antibiotics (e.g., for ENR) in a short term (three weeks). Besides, the concentration of SDZ decreased slightly from 379 mg/kg (only solid manure treatment) to 88 mg/kg and 173 mg/kg in the inoculation ratio of 1:0.5 and 1:2, respectively. On the other hand, CAP and OTC concentrations in the wood immobilized *T. versicolor* treatment increased compared to that

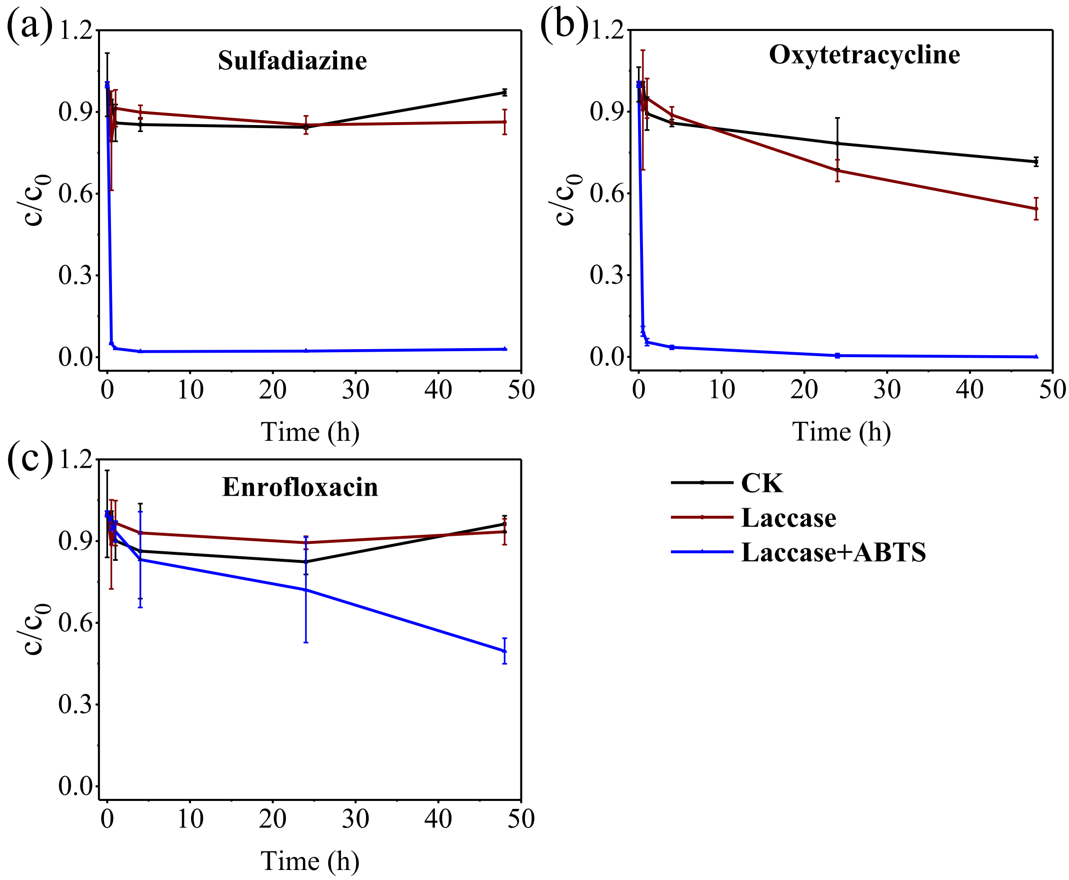


Fig. 5. The biodegradation assay of SDZ (a), OTC (b) and ENR (c) by laccase of *T. versicolor*.

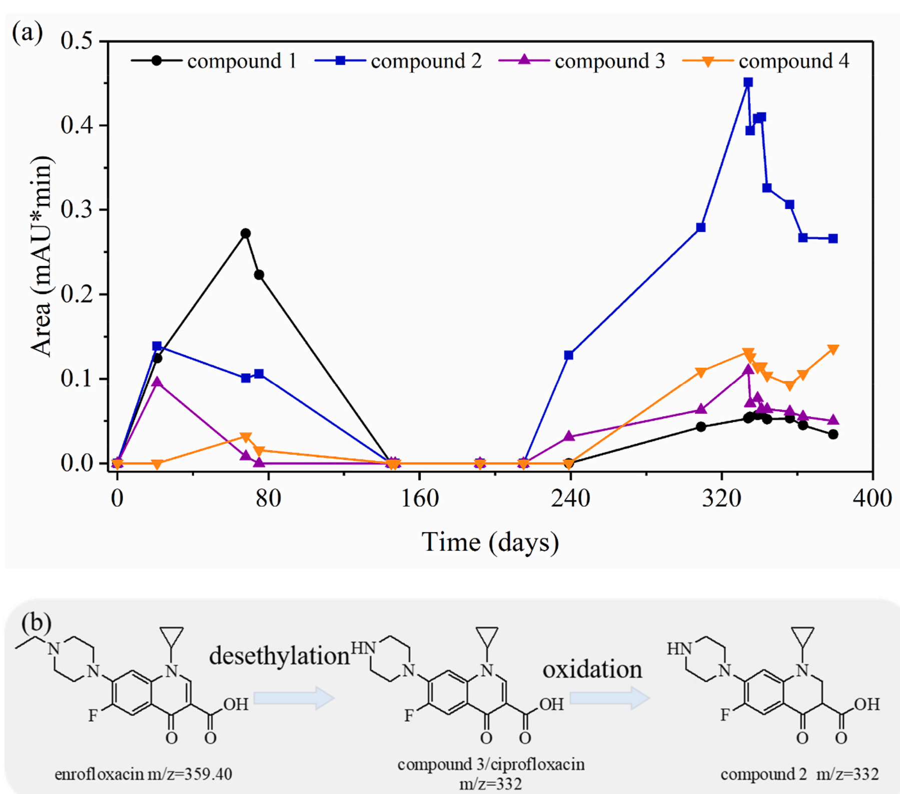


Fig. 6. Degradation characteristics of the potential identified metabolites. (a) the area changes of the detected biotransformation products during the incubation, (b) the proposed biodegradation pathway of ENR.

Table 2
Toxic values of ENR and its biotransformation products evaluated by ECOSAR.

Compounds	Organism	Duration	End Pt	Predicted mg/L (ppm)
ENR	Fish	96h	LC50	4920
	Daphnid	48h	LC50	505
	Green Algae	96h	EC50	561
	Fish		Chv	454
	Daphnid		Chv	35.8
	Green Algae		Chv	167
Compound2	Fish	96h	LC50	18100
	Daphnid	48h	LC50	14000
	Green Algae	96h	EC50	27300
	Fish		Chv	39600
	Daphnid		Chv	755
	Green Algae		Chv	6620
Compound3	Fish	96h	LC50	1310
	Daphnid	48h	LC50	1240
	Green Algae	96h	EC50	1620
	Fish		Chv	1550
	Daphnid		Chv	81.3
	Green Algae		Chv	455

Table 3
The concentration of antibiotics in cow manure treated with wood immobilized *T. versicolor* in different ratio.

Sample	Concentration (ng/g)			
	CAP	SDZ	OTC	ENR
Initial	4755	4025	1187	4905
3W_I	136	88	712	1314
3W_II	62	173	884	1934
3W_V	212	379	369	4849
3W_III	77	151	557	3423
3W_IV	14	204	389	3448
6W_I	4.2	169	425	2162
6W_II	7.9	97	492	1451
6W_V	9.2	287	387	3275
Recovery ± SD (%)	91 ± 4.6	83 ± 11	38 ± 6	57 ± 11
MDL (ng/g)	0.16	0.33	1.35	0.26
MQL (ng/g)	0.56	1.04	4.56	0.86

Note: I and II means mixed solid manures with wood immobilized *T. versicolor* with the ratio of 1:0.5 and 1:2, respectively. III, IV means mixed solid manures with sterilized wood with the ratio of 1:0.5 and 1:2, respectively. V mean only solid manure addition. 3W and 6W means incubation in 3 and 6 weeks, respectively. MDL means minimum detection level. MQL means minimum quantified level.

under natural conditions and killed wood treatment, which might be due to OTC and CAP being desorbed from the wood and the acidic condition formed by *T. versicolor* inhibit the bacterial degradation. Yu et al. (2023) reported that acidic conditions significantly reduced the bacterial diversity and community abundance, thereby reducing many potential antibiotic-resistant bacteria. Furthermore, after 6 weeks, the concentration of those four antibiotics decreased after wood immobilized *T. versicolor* inoculation, except for SDZ and ENR concentration in 1:0.5 wood immobilized *T. versicolor* treatment (treatment 6W_I). All those results suggest that wood immobilized *T. versicolor* promoted antibiotics biodegradation in cow manure, especially in a low inoculation ratio in a short term, but in a high inoculation ratio in a long term.

4. Conclusions

T. versicolor removed OTC, SDZ, ENR through adsorption and biodegradation processes, with the removal efficiency of 92 % and 98 % in 21 h and 98 % in 68 h, respectively. The removal of these antibiotics followed a first-order kinetic model. Moreover, *T. versicolor* biodegraded these three antibiotics using laccase and cytochrome P450 system, with the biodegradation efficiency following the order SDZ ≈ OTC > ENR. Four possible biodegradation products were identified (compound 1–4), with ENR being transformed into CIP through desethylation of the ethyl on the piperazin ring. Furthermore, wood immobilized *T. versicolor* promoted SDZ, OTC, ENR and CAP biodegradation in solid cow manure, particularly at a high mixture ratio of 1:2 (wood immobilized *T. versicolor*: solid cow manures, w/w). Therefore, this study provides support to the use of *T. versicolor* or wood immobilized *T. versicolor* for the removal of antibiotics contaminants from wastewater and manure.

CRediT authorship contribution statement

Zewen Tan: Writing – original draft, Investigation, Data curation. **Eduardo Beltrán-Flores:** Writing – review & editing, Investigation, Data curation. **Gisselle D. Ramos-Meza:** Investigation, Data curation. **Lucas L. Alonso:** Investigation, Data curation. **Montserrat Sarrà:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126504.

Data availability

Data will be made available on request.

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