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Biotransformation of chloramphenicol by white-rot-fungi *Trametes versicolor* under cadmium stress

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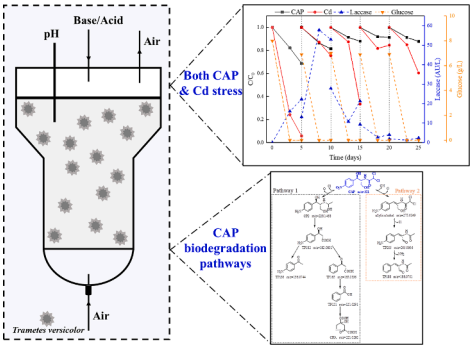
HIGHLIGHTS

- *Trametes versicolor* is capable of simultaneous biodegrading CAP and removing Cd.
- Nine transformation products (TPs) were identified, including six novel TPs.
- Two novel CAP biodegradation pathways were proposed through the TPs.
- Biodegradation and adsorption were the main mechanism of CAP removal by *T. versicolor*.
- Cellular accumulation and surface adsorption were the main mechanism of Cd removal.

GRAPHICAL ABSTRACT



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ABSTRACT

The recalcitrant chloramphenicol (CAP) combined with heavy metals cadmium (Cd) commonly co-existed in the environment, posing threat to environment health. The capacity of *Trametes versicolor* to remove/biodegrade CAP in air-pulse fluidized-bed reactor was evaluated, even under Cd stress. *T. versicolor* could remove 44 % CAP of 5 mg/L in 15 days, even 51 % CAP under 1 mg/L Cd stress. Sustained Cd stress inhibited CAP biodegradation and Cd removal in a 5-batches of a 5-days cycle sequential batch reactor. Nine transformation products and two novel pathways were proposed, with initial multi-step transformation reaction into CP2 and allylic alcohol, respectively. Furthermore, the main mechanism of Cd removal by *T. versicolor* was extracellular surface bioadsorption and intracellular accumulation. This study filled the gap of the mechanism of simultaneous CAP removal/biodegradation and Cd removal by white-rot fungi *T. versicolor*, which offer a theoretical basis for future application of biological removal of CAP containing wastewater.

1. Introduction

Antibiotics has been widely applied in human medical and livestock and aquaculture breeding industry to meet the demand of population growth and human health (Carvalho and Santos, 2016; Wang et al., 2020a,b). However, its over-application might generate environmental risks and human health threats (Kumar et al., 2019; Wang et al., 2016). Chloramphenicol (CAP) is a broad-spectrum antibiotic, which has been

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extensively administrated as a veterinary antibiotic (Hanekamp and Bast, 2015). Due to the incompletely metabolism characteristics by human beings or animals, most un-metabolized CAP (30–90 %) were excreted out into the environment through urine and excrement (Van Boeckel et al., 2015). So, those environmental CAP residues threatened the environment health, for example, decline of soil fertility and microbial diversity (Machowska and Stålsby Lundborg, 2019; Zhang et al., 2020a,b), promotion of antibiotics resistance genes spreading (Lu et al., 2020), even threaten human health through food chain (Pan and Chu, 2017). Due to the stable structure, recalcitrant and anti-bacteria properties, CAP can exist in a long time and accumulated in the environment (Hanekamp and Bast, 2015). So, CAP residues have been detected in many environmental media, such as livestock manure, municipal sludge, aquaculture wastewater, river sediment, farm soil, and even in some food resources, such as radish, rape, coriander, eggs, rabbit, and etc (Chung et al., 2017; Song et al., 2021; Wang et al., 2020a,b). Thus, it is urgent to seek a highly-efficient strategy to reduce or eliminate CAP residues in the environment.

In addition, cadmium (Cd), a common heavy metal and toxic metallic element for all organism, was commonly detected in animal feeds and manure in low concentration (Kirkham, 2006; Shahid et al., 2017). So, Cd commonly entered into the environment with antibiotics residue through livestock manure and wastewater (Adamse et al., 2017; Liang and Hu, 2021; Shao et al., 2019), which means that Cd and CAP often co-exist in livestock waste. Traditional method to remove organic/inorganic pollutants include physical, chemical and biological methods, such as chemical precipitation, ion exchange, adsorption, advanced oxidation processes, electrochemical oxidation, photocatalytic oxidation, microbial biodegradation/immobilization, plant/microbe accumulation, and etc (Teng et al., 2019; Uddin, 2017; Wang et al., 2018; Xu et al., 2020; Li et al., 2022). Compared with chemical and physical methods, microbial biodegradation is a promising, low cost, and environmentally friendly methods. But most microbial biodegradation methods focused on bacterial CAP biodegradation and microbial Cd accumulation (Ma et al., 2020; Ma et al., 2019; Tan et al., 2022). There is scarce information about CAP biodegradation by fungi even under Cd stress, especially by white rot fungi (WRF).

WRF are a group of basidiomycetous fungus, including many species, such as *Trametes versicolor*, *Ganoderma lucidum*, *Pleurotus ostreatus*, *Stropharia rugosoannulata*, *Irpex lacteus* etc. WRF, as is known to all, can aerobically decompose lignin and other organic pollutants using its non-specific intracellular and extracellular enzymes, such as ligninolytic enzymes, cytochrome P450 enzyme system, laccases (Mir-Tutusaus et al., 2018). For example, white rot fungus *P. ostreatus* could biodegrade di(2-ethyl hexyl) phthalate (endocrine-disruptor) through de-esterification, oxidation and oxidation-hydrolysis pathway (Ahuactzin-Pérez et al., 2018). *Phanerochaete chrysosporium* could biodegrade 49 % of insecticide thiamethoxam in 15 days at the initial concentration of 10 mg/L (Chen et al., 2021). And *P. chrysosporium* (MTCC-787) could biodegrade 71.5 % of cyclophosphamide and 98.4 % of etoposide in 6 days at the initial concentration of 0.3 mg/L, respectively (Yadav et al., 2022). *T. versicolor* is a common species of WRF, and it has been verified to biodegrade various of organic pollutants, such as cyclophosphamide, bentazone, etoposide, diuron, chlorpyrifos, dicofol, cypermethrin and fluoroquinolone, etc (García-Vara et al., 2021; Hu et al., 2020a,b; Manasfi et al., 2020; Yadav et al., 2022).

The aim of this study was to evaluate the ability of biodegrading CAP by *T. versicolor* as WRF in an air-pulse fluidized bioreactor, even under Cd stress, and was to reveal the biodegradation pathway of CAP by identifying the transformation products (TPs) formed during the biodegradation process. Furthermore, the enzymatic system related to the biodegradation process was explored. The biodegradation pattern of CAP by *T. versicolor* even under Cd stress provides a new insight of the CAP transformation fate in heavy metal contaminated environments, and also provide a new candidate for both CAP and Cd bioremediation in the future. So, this study is essential before developing a wastewater treatment process of real wastewater containing CAP with *T. versicolor*.

2. Materials and methods

2.1. Strain and media

Trametes versicolor ATCC 42,530 was obtained from American Type Culture Collection. The subcultures, blended mycelial suspension and pellets were maintained following Hu et al. (2020a,b).

Defined medium (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 Hu et al. (2020).

2.2. Chemicals and reagents

The standard of chloramphenicol (CAP, purity $\geq$ 98 %), commercial laccase (20 AU/mg, purified from *T. versicolor*), the laccase mediator 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, purity $\geq$ 98 %) were purchased from Sigma-Aldrich (Barcelona, Spain). HPLC (high performance liquid chromatography) gradient grade acetonitrile was purchased from Carlo Erba Reagents S.A. S (Barcelona, Spain). Methanol was obtained from Merck (Darmstadt, Germany). Stock solution of CAP (1 g/L) was prepared by appropriate dilution of the substances in ethanol and sterilized through 0.22 μm sterilized nylon filter.

2.3. Fungal degradation of CAP under Cd stress

T. versicolor pellets were transferred into air-pulsed fluidized-bed bioreactor, with the initial biomass amount of 2.5 g dry weight. The bioreactor has at the bottom, a ceramic plate as air diffuser, and a cylindrical vertical center body which is connected diametrically wider head. The valid volume of the bioreactor was 1.5 L, and 1 L defined medium was added into the bioreactor. As described in Hu et al. (2021), the medium pH was controlled at a constant value of 4.5 $\pm$ 0.1, aeration rate was maintained at 0.8 L/min (1 s air pulse every 3 s).

Two kinds of experiment were operated using air-pulse fluidized-bed bioreactor: (a) four different experiments were carried out where the defined medium was spiked with 0 mg/L, 5 mg/L, 10 mg/L CAP, and both 5 mg/L CAP and 1 mg/L Cd. At the beginning and end of each batch, samples were collected for laccase activity, glucose concentration, CAP concentration, Cd concentration analyses, and all the pellets were harvested to quantify the biomass at the end of batch; (b) according to the previous result of CAP and Cd removal by *T. versicolor*, 5 batches of a 5-days cycle sequential batch reactor were operated at the same conditions of initial biomass, pH value, aeration rate. The defined medium was spiked with both 5 mg/L CAP and 1 mg/L Cd at the beginning. At the end of each batch, 200 mL supernatant were withdrawn, and same volume of new concentrated defined medium was added into the bioreactor to keep each medium component with the same concentration at the beginning. And at the beginning of each batch, CAP and Cd were added. Samples were collected at the beginning and end of each batch, and laccase activity, glucose concentration, CAP concentration, Cd concentration were tested. At the end of the experiment, all the pellets were harvested to quantify the biomass.

2.4. In vitro CAP biodegradation by laccase

According to García-Vara et al. (2021), commercial laccase was added into the laccase reaction solution (250 mM sodium malonate dibasic monohydrate, pH 4.5) with the final enzyme activity at 500 AU/L. Laccase Treatment: CAP was added into the reaction solution with a final concentration at 5 mg/L. Laccase + ABTS Treatment: laccase mediator ABTS was added into Laccase Treatment with a final concentration at 1 mM. All experiment was incubated on dark at 135 rpm orbital shaker and 25 °C. 1 mL sample was collected at 0, 3, 6, 12, 24 h,

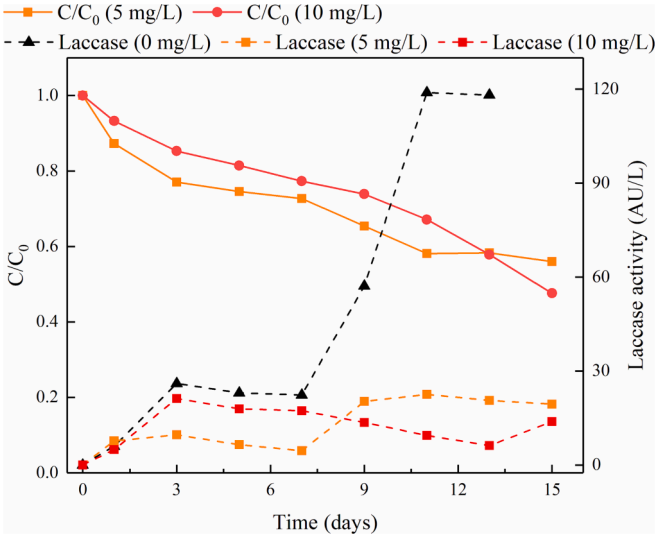


Fig. 1. Time-course of CAP remaining and laccase activity of *T. versicolor* under no CAP and 5/10 mg/L CAP conditions.

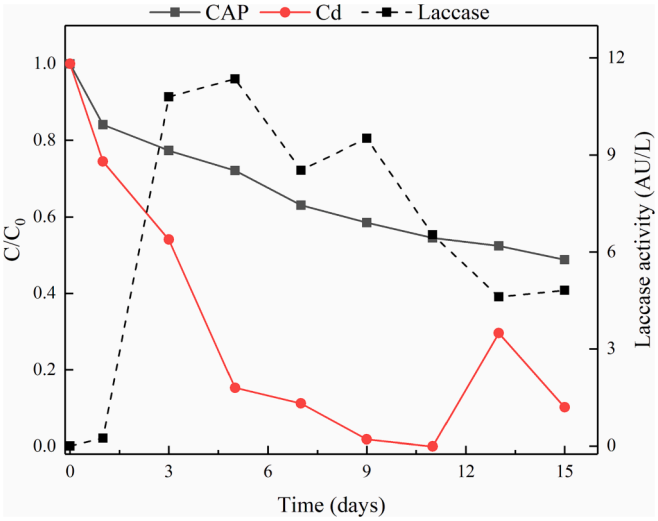


Fig. 2. Time-course of CAP remaining, Cd remaining and laccase activity of *T. versicolor* under both 5 mg/L CAP and 1 mg/L Cd condition.

and then mixed with 0.1 mL of 1 M HCl to stop the reaction. The concentration of CAP was then tested by HPLC.

2.5. Analytical methods

2.5.1. Laccase activity and glucose concentration testing

According to the conventional methods our lab, laccase activity was measured through the oxidation of 2,6-dymetoxyphenol (DMP) by laccase without the presence of a cofactor (Hu et al., 2021). The glucose concentration was tested by biochemistry analyzer (2700 select, Yellow Springs Instrument, USA).

2.5.2. CAP concentration

CAP concentration was detected by HPLC (Ultimate 3000, Dionex, USA), which equipped with a UV detector and with a C18 reversed-phase column (Phenomenex®, Kinetex® EVO C18 100 Å, 4.6 mm × 150 mm, 5 μm). The running conditions were set as follows: injection volume at 2 μL, mobile phase at 60 % methanol and 40 % water, flow rate at 0.8 mL/min, column temperature at 30 °C, detection wavelength at 287 nm.

2.5.3. Cd concentration

The collected liquid samples were filtered through 0.22 μm filter before Cd concentration testing. To quantify the Cd concentration of cellular surface adsorption, the fungi cell pellets were collected and washed with 10 mM EDTA in three times, and the washing solution was combined together. Subsequently the washed pellets were dried at 105 °C to constant weight for cellular accumulated Cd concentration. 1 mL liquid sample and 0.25 g solid sample were digested with HNO_3, and then the Cd concentration was measured by ICP-MS (7900, Agilent, USA). The instrument conditions were set as follows: S/C temperature, 2℃; RF matching, 1.10 w; RF power, 1600 w; 77 carrier gas, 0.82 L/min; Smpl Depth, 10.00 mm; Nebulizer pump, 0.1 rps; Pump rate, 40 r/min.

2.5.4. Identification of transformation products

The transformation products of CAP were identified by UPLC (Agilent, USA)-Q-TOF-MS spectrometer (Acquity, Bruker, Germany). The UPLC was equipped with a DAD detector (1200RR, Agilent) and with a C18 reversed-phase column (Phenomenex®, Luna Omega C18 100 Å, 4.6 mm × 250 mm, 5 μm). And the UPLC separation conditions were set as follows: mobile phase at 0.1 % formic acid in H_2O (solvent A) and 0.1 % formic acid in methanol (solvent B), flow rate at 0.8 mL/min, column temperature at 40 °C, injection volume at 2 μL, detection wavelength at 278 nm. The separation gradient properties followed at: maintained 10 % B in 1 min initially, increased to 95 % over 22 min and maintained at 95 % B for 3 min, decreased to 10 % B over 1 min, and then maintained at 10 % B for 13 min, for a total runtime of 40 min. The tandem HRMS (microTOFQ, Bruker) analysis was performed using an electrospray ionization source in positive and negative polarity with a spray voltage at 4.0 KV (in ESI +) or 3.5KV (in ESI-), dry heater at 210 °C, a dry gas at 8.0 L/min, nebulizer at 4.0 bar. The MS acquisition was performed in full scan mode 50–1000 Da. The molecular structure for CAP and their degradation products were tentatively proposed by the detection of predicted mass and isotope pattern.

3. Results and discussion

3.1. CAP biodegradation at different initial concentration

The chloramphenicol (CAP) removal and biodegradation ability by *Trametes versicolor* in different concentration was evaluated in an air-pulse fluidized-bed bioreactor. As it is shown in Fig. 1, the removal efficiency in 5 mg/L CAP was slightly higher than that in 10 mg/L in 11 days incubation, and the CAP removal efficiency were 42 % and 33 %, respectively. But the CAP removal ability was nearly the same at day 13, and the removal efficiency of CAP were 42 % and 42 % at 5 and 10 mg/L CAP, respectively. At the end of the batch treatment, the removal efficiency of CAP in 10 mg/L CAP was higher than that in 5 mg/L CAP, the efficiency was 53 % and 44 %. Furthermore, the removal kinetics of CAP by *T. versicolor* fitted well with the first-order kinetic model under 5 and 10 mg/L CAP conditions (see supplementary material), with a correlation coefficient R^2 of 0.938 and 0.967 (see supplementary material), respectively. The kinetic of removal constant under 5 mg/L CAP condition was higher than that under 10 mg/L CAP condition, the constant k was 0.039 d^{-1} and 0.037 d^{-1} (see supplementary material), respectively, which mean that high antibiotics concentration inhibit the antibiotic biodegradation by fungi. The inhibition effect of high antibiotic concentration on antibiotic biodegrading strain has been commonly observed. Holanda et al. (2019) found that strains *Aspergillus* sp. BIORG 9 and *Trichoderma* sp. BIORG 7 could biodegrade 29.3 % and 25.2 % of 100 mg/L CAP in 9 days, respectively, and CAP concentration was the main factor with the highest influence over biodegradation. Furthermore, the adsorption of organic pollutants was inevitable, so the adsorption of CAP by *T. versicolor* were determined using killed *T. versicolor*. The adsorption of CAP stabilized in 1 day, and then plateaued between day, so the adsorption efficiency of CAP was nearly 14 % (0.25 mg/g dry weight) (see supplementary material).

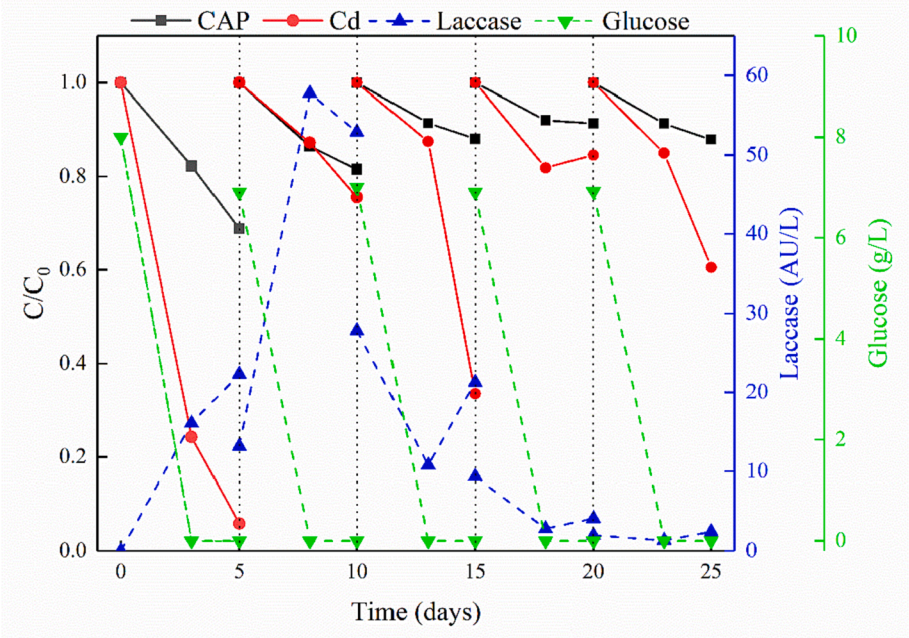


Fig. 3. Remaining of CAP and Cd, and laccase activity, and glucose concentration by *T. versicolor* in sequencing batch bioreactor (SBR) under initial 5 mg/L CAP and 1 mg/L Cd.

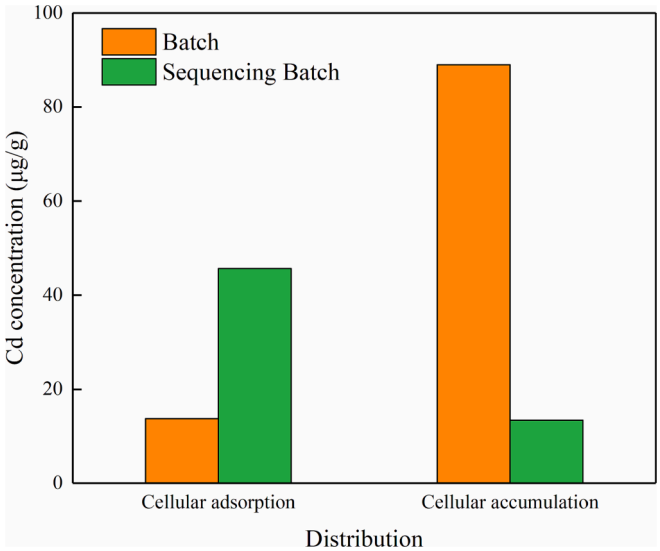


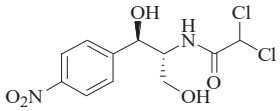
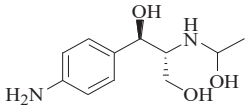
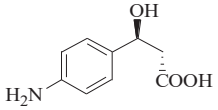
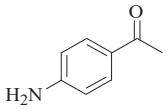
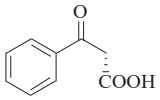
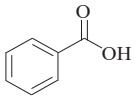
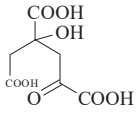
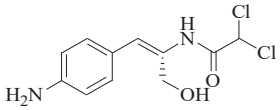
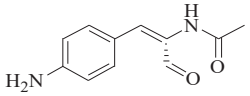
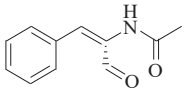
Fig. 4. Cd distribution in *T. versicolor* in bioreactor batch and sequencing batch under initial 5 mg/L CAP and 1 mg/L Cd.

The laccase activity and biomass of *T. versicolor* were also determined under no CAP, and 5/10 mg/L CAP conditions (Fig. 1). With the absence of CAP, the extracellular laccase activity maintained low (24 ± 2 AU/L) until day 7 when it increased sharply to 119 AU/L at day 11 and plateaued between at day 11 to day 13. For the low laccase activity at the beginning, it is may due to the metabolism strategies of many fungi that high concentration of carbon source stimulated the biomass accumulation, but inhibit the laccase production. It is reported that the synthesis of laccase was activated by carbon or nitrogen depletion, and the laccase production was also influenced by some metal ions and xenobiotics (Yang et al., 2017). Interestingly, the trends of laccase activity changes under 5 mg/L CAP condition were the same with that under no CAP condition. But under 10 mg/L CAP condition, the laccase activity increased to 21 AU/L at day 3, and then decreased to 6 AU/L from day 3 to day 13. And with the presence of 5 mg/L CAP, the laccase activity increased from 0 to 10 AU/L in 3 days incubation, and decreased slightly to 5 AU/L at day 7, then increased to 23 AU/L at day 11 and plateaued between at day 11 to day 15. It was reported that if fungal laccase participated in pollutants biodegradation, pollutants concentration corresponded to the time-course laccase activity (Hu et al., 2020a,b; Wang et al., 2022). But, the laccase activity of *T. versicolor* didn't fit with CAP concentration changing, which implied that the laccase of *T. versicolor* may not participate in CAP biodegradation. Furthermore, the biomass of *T. versicolor* with no CAP and with 5/10 mg/L CAP conditions were 7.8, 7.2 and 6.2 g (see supplementary material), respectively. All those results indicated that high CAP concentration (10 mg/L) was more toxic to *T. versicolor*, which represented in inhibiting fungal growth, extracellular enzyme production and CAP biodegradation ability. Same toxic situation was observed in thiamethoxam biodegradation by white-rot fungus *Phanerochaete chrysosporium*, and high concentration of thiamethoxam inhibit its biodegradation rate and catalase and peroxidase activity (Chen et al., 2021).

3.2. CAP biodegradation under Cd stress

As shown in Fig. 2, the effluence of Cd stress on CAP removal and Cd removal of *T. versicolor* was assessed. At the initial concentration of both 5 mg/L CAP and 1 mg/L Cd condition, the CAP removal pattern of *T. versicolor* was nearly same with that under only 5 mg/L CAP condition at day 0 to day 5. Then the CAP removal efficiency increased and was higher than other conditions at day 7 to day 13, and the highest removal efficiency was 51 % at day 15, which indicated that 1 mg/L Cd promoted CAP removal of *T. versicolor*. As described in previous researches that low concentration of metal had different impact on organic biodegradation by WRF. For example, Liu et al. (2020) reported that low concentration of Cr (VI) (1 mM) greatly promoted reactive black 5 decolorization, from 57 % to 84 %, by white-rot-fungus *T. hirsute*. Furthermore, the removal kinetics of CAP by *T. versicolor* fitted well with the first-order kinetic model under both 5 mg/L CAP and 1 mg/L Cd conditions (see supplementary material), with a correlation coefficient R^2 of 0.957 and the removal constant k of 0.047 d^{-1} (see supplementary material). The removal constant k under 10 mg/L CAP condition was lower than that under 5 mg/L CAP condition and both 5 mg/L CAP and 1 mg/L Cd condition, which indicated that higher CAP concentration

Table 1
The identified CAP biodegradation transformation products by *T. versicolor* using UPLC-QTOF-MS.

Peak number	Elemental composition	Measured mass (*m/z*)	Possible structural formula
CAP	$C_{11}H_{12}O_5N_2Cl_2$	323.02	
CP2	$C_{10}H_9O_3N_2Cl_2$	228.1468	
TP182	$C_9H_{12}O_3N$	182.0801	
TP136	$C_8H_{10}ON$	136.0744	
TP165	$C_9H_9O_3$	165.0536	
TP121	$C_7H_5O_2$	121.0291	
CHA	$C_{11}H_{14}O_3N_2Cl_2$	221.0292	
Allylic alcohol	$C_{11}H_{12}O_2N_2Cl_2$	275.0349	
TP205	$C_{11}H_{13}O_2N_2$	205.0956	
TP188	$C_{11}H_{10}O_2N$	188.0701	

was more toxic than low CAP concentration and even under both Cd condition to *T. versicolor*.

Different WRF species has different strategies in heavy metals, such as adsorption by mycelium, extracellular EPS and other extracellular substrates; immobilization by extracellular/intracellular chelating compounds (such as metallothioneins, oxalate, etc); cell wall adsorption (melanins and phenolic molecules); intracellular accumulation (Baldrian, 2003). For example, Noormohamadi et al. (2019) found that white-rot fungi *Phanerochaete chrysoporium* could remove Cd and Ni through surface biosorption on fungi mycelia in high removal efficiency (96.23 % and 89.48 % respectively). Schlunk et al. (2015) verified that *Tricholoma vaccinum* could encode Mte1 to transport heavy metal into the vacuole, which mediated detoxification. In this research, the Cd concentration decreased sharply, and the removal efficiency were 85 % and 100 % at day 5 and day 11, respectively. But the Cd concentration then increased at day 13, which may due to incident with pH controller and leading Cd de-adsorption from fungi cellular surface at the end of the treatment. The concentration of cellular accumulation and cellular surface adsorption were 89 μg/g and 13.8 μg/g biomass, respectively (Fig. 4), which means Cd removal of *T. versicolor* was mainly attributed to cellular accumulation (61 %).

The laccase activity of *T. versicolor* was also tested, and the changing trend was similar with other conditions at day 0 to day 3, but lower than under other conditions. Then, the laccase activity deceased at day 5 to day 15 (Fig. 2). Furthermore, the biomass of *T. versicolor* was 6.8 g (see supplementary material), which was lower than that under 5 mg/LCAP and higher than that under 10 mg/LCAP. These results indicated that high concentration CAP was more toxic than both low concentration CAP and Cd stress.

3.3. CAP biodegradation assay by laccase

Fungal laccase, a kind of extracellular enzyme, has been reported to have the ability to degrade recalcitrant pollutants, such as olsalazine (Bankole et al., 2021), melanin (Park et al., 2021), bentazone (García-Vara et al., 2021), and etc. So, whether the laccase of *T. versicolor* participated in CAP biodegradation was investigated. The CAP concentration didn't change with or without the presence of laccase mediator ABTS, which indicated that the laccase of *T. versicolor* was not involved in CAP biodegradation (see supplementary material). But, Navada and Kulal (2019) reported that laccase from *T. hirsute* could efficiently degrade 10 mg/L CAP in 48 h with the presence of laccase mediator

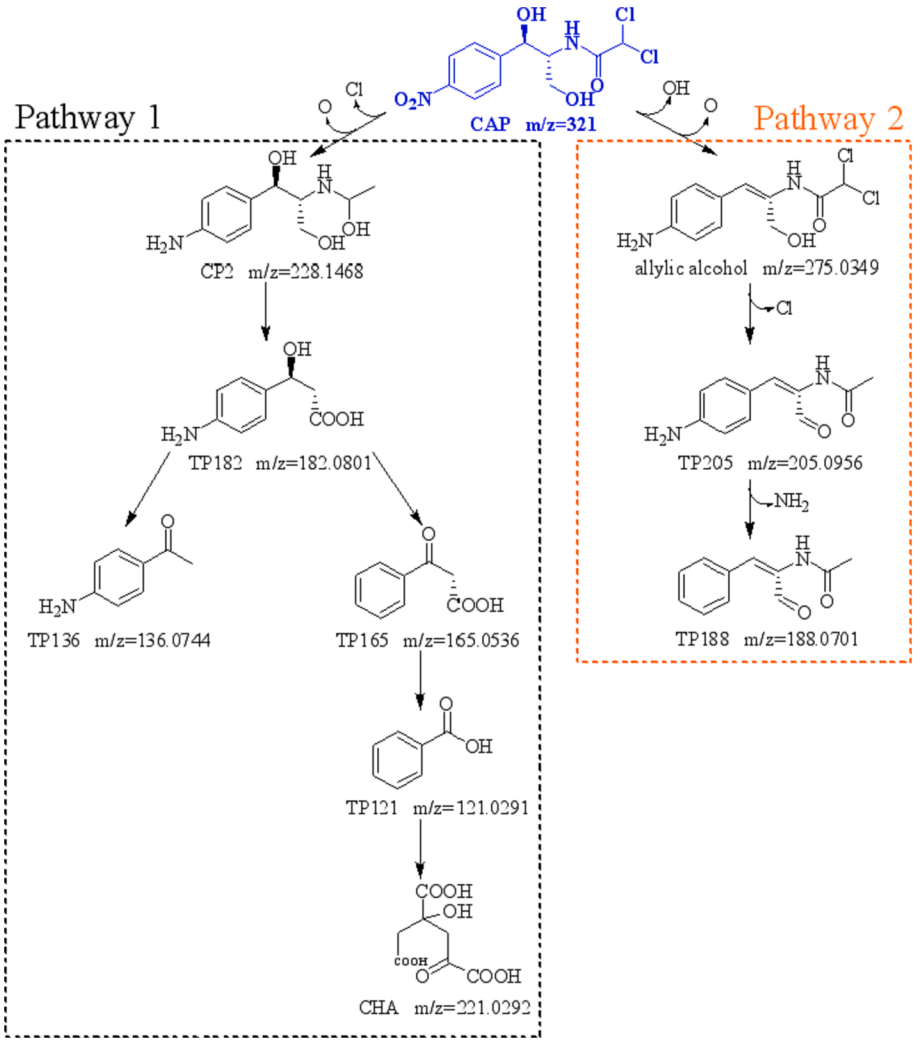


Fig. 5. The proposed CAP biodegradation pathways by *T. versicolor*.

system, such as vanillin and ABTS. The reason for different activity of laccase from different fungi on CAP biodegradation is that fungal laccase has several gene families (5–17 family members) and the function between them are vary, for example, Lcc3/5, Lac4, LacA, LccA and etc (Iimura et al., 2018; Yang et al., 2017). So, in conclusion, there was no relationship between CAP biodegradation and laccase activity among *T. versicolor*.

3.4. CAP biodegradation and Cd removal in sequencing batch bioreactor

To investigate the sustained Cd stress on CAP removal/biodegradation by *T. versicolor*, 5 batches of 5-days cycle were operated (Fig. 3), and CAP and Cd in fresh medium were supplemented at the end of each batch according with the previous results (Fig. 2). In the first batch, the CAP removal efficiency and Cd removal efficiency were nearly the same with previous results in Fig. 2, 31 % and 94 %, respectively (Fig. 3). And with the following batches (batches 2 to 5), the inhibition of CAP removal efficiency increased with the increasing batches by the sustained Cd stress. It was reported that low heavy metal concentration could promote the growth of fungi, but high concentration of heavy metal (especially in sustained Cd stress) could inhibit their growth, such as Sharma et al. (2020) reported that low Cd and Pb concentration (1–2 μM) promoted the growth and its removal efficiency of *Phlebia brevispora*, but high concentration of Cd (100 μM) was toxic to its growth and inhibited their removal efficiency. Interestingly, the final Cd removal efficiency in each batch were fluctuated between batches, but the Cd removal efficiency was lower than that in batch 1, and the Cd removal efficiency in each batch were 94, 24, 67, 15 and 40 %, respectively. It was reported that heavy metal stress could induce reactive oxygen species (ROS), low concentration of ROS could promote heavy metal removal, but high concentration of ROS could result in membrane damage and enzyme inactivation (Sharma et al., 2020; Zhang et al., 2015). The irregular removal rate of Cd in each batch may due to the lagging production or different production amount of ROS.

The laccase activity increased in batch 1 and 2, and reached a maximum value of 58 AU/L during the second batch of day 3, but it decreased to nearly 0 at batch 3 to 5. The reason for the laccase activity trends during the experiment maybe that Cd and CAP supply promoted *T. versicolor* laccase secretion at the beginning of batch 2, but the accumulated Cd and CAP in the medium and *T. versicolor* cell poisons cell growth and metabolism in bath 2 to bath 5. As Liu et al. (2020) reported that the decolorization ratio of azo dye (Reactive Black 5) by *Trametes hirsuta* promoted by the increased hexavalent chromium (Cr (VI)) concentration (0–1 mM), then inhibited with the increased Cr(VI) concentration (0–5 mM); and the laccase activity also promoted with the presence of Cr(VI), which was responsible for azo dye decolorization. The glucose concentration was nearly 0 at day3 of each batch (Fig. 3), and the fungal biomass after 5 batches was 5 g (see supplementary material), which was lower than all other treatments. It means that *T. versicolor* could metabolize glucose to grow and synthesize other

substances, but the growth rate was lower than other treatments and the cell was still active. Furthermore, the cellular surface adsorbed and cellular accumulated Cd was 45.6 and 13.4 μg/g (Fig. 4), respectively, which were totally different with that in Section 3.2. So, sustained Cd and CAP addition may change the strategies of *T. versicolor* to remove Cd, which mean *T. versicolor* may pump out more Cd from cell and synthesis more extracellular polymeric substances and cellular surface substances to adsorb Cd. These results indicated that sustained Cd stress in 5 batches made *T. versicolor* adsorbing more Cd on its surface, those adsorbed and accumulated Cd by *T. versicolor* are more toxic and inhibited CAP removal/biodegradation and Cd removal.

3.5. CAP transformation products and pathway

The CAP transformation products by *T. versicolor* were identified through HPLC-QTOF-MS, and nine transformation products were determined, including 6 novel products (Table 1 and see supplementary material). And two novel CAP bio transformation pathways by *T. versicolor* were proposed through the transformation products in Fig. 5, which haven't been reported before. To our acknowledge, four initial CAP biodegradation steps were reported, including amide bond hydrolysis, acetylation, reduction of nitro group, and oxidation of hydroxyl group; and all of them were focused on bacterial biodegradation (Ma et al., 2020; Tan et al., 2022; Zhang et al., 2020a,b). Navada and Kulal (2019) reported that fungi could biodegrade CAP, and only one biodegradation product was identified, chloramphenicol aldehyde, which formed after dehalogenation. In pathway 1, CAP was transformed into CP2 through multi-step reaction, including reduction of nitro group at C4 and dechlorination reaction of chloride at *C2′*. Same multiple reaction step of nitro group reduction was observed in bacterial CAP biodegradation by *Aeromonas media* SZW3 (Tan et al., 2022). And then the C—N bond at the side chain of *C2′* of CP2 was disrupted with the formation of TP182. On the one hand, TP136 was formed after carbon–carbon bond breaking between *C2′* and C3′ of TP182. On the other hand, deamidation reaction was occurred at C4 location of TP182 with the production of TP165, and then TP182 was transformed into TP121 after de-carboxyl reaction at the side chain of *C2′* location. Then further oxidation reaction of ring opening between C3 and C4 caused the production of CHA. In pathway 2, allylic alcohol was formed after nitro group reduction and further dehydroxylation at the side chain of C1′ location of CAP. Allylic alcohol was also detected in some bacterial CAP biodegradation pathway, such as *Haemophilus influenzae* (Smith et al., 2007) and *Aeromonas media* SZW3 (Tan et al., 2022). Then allylic alcohol was transformed into TP205 dechlorination reaction, and then TP205 transformed into TP188 after deamidation reaction.

4. Conclusion

T. versicolor could remove 44 % CAP of 5 mg/L in 15 days, even 51 % CAP under 1 mg/L Cd stress. Sustained Cd stress inhibited CAP biodegradation and Cd removal in 5 batches of a 5-days cycle in FBR. 9 transformation products and two novel pathways were proposed. Extracellular surface bioadsorption and intracellular accumulation were the main mechanism of Cd removal of *T. versicolor*. The findings reveal that *T. versicolor* could biodegrade CAP through two novel pathways and simultaneously remove Cd through cellular adsorption and accumulation. This study is essential before developing a wastewater treatment process of real wastewater containing CAP.

CRediT authorship contribution statement

Zewen Tan: Data curation, Investigation, Writing – original draft. **Diana Losantos:** Investigation. **Yongtao Li:** Supervision, Project administration, Funding acquisition. **Montserrat Sarrà:** Supervision, Writing – review & editing, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.128508.

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