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Biotransformation of cadmium-sulfamethazine combined pollutant in aqueous environments: *Phanerochaete chrysosporium* bring cautious optimism

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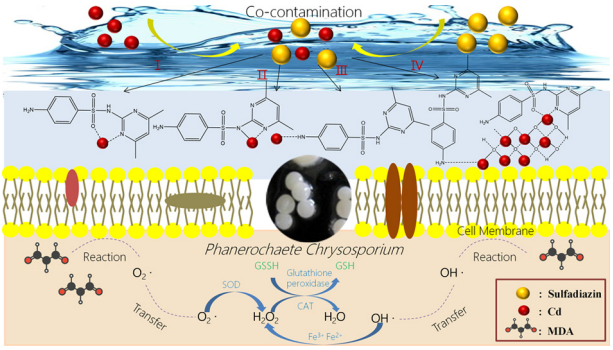
HIGHLIGHTS

- The co-contamination can be efficiently removed by *Phanerochaete chrysosporium*.
- Complex mode of Cd and sulfamethazin was distinctly different at different pH.
- Antioxidant in fungi cell was higher than that induced by individual pollutant.
- Biotransformation efficiency can be enhanced with the concentration ratio changed.

GRAPHICAL ABSTRACT



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ABSTRACT

Microorganism biotransformation of sulfamethazine (SMT) in aqueous environments is a major concern, especially considering their exposure to coexisting SMT and heavy metals. *Phanerochaete chrysosporium* (*P. chrysosporium*) is a more concerned Cadmium (Cd) and SMT hyper accumulation specie. This study, referring to metabolic mechanisms and application, was performed to investigate the single and combined effects of Cd-SMT, including toxicity, resistance, as well as the accumulation and biotransformation by *P. chrysosporium*. The results revealed that Cd-SMT co-contamination caused increasing active oxygen accumulation, and the number of antioxidant enzyme and non-enzymatic antioxidants were higher than that under the stress of their individual pollution. It was found that *P. chrysosporium* accumulated high levels of Cd with the increment of 6.98–23.96% induced by Cd-SMT co-contamination compared to under the stress of Cd individual pollution. What's more, the addition of Cd reduced the toxicity of SMT to *P. chrysosporium*. The decrease of malonaldehyde and the increase of protein also proved that *P. chrysosporium* held enormous potential to fit in the co-contaminated environment, and to remediate the co-contaminated water especially in the long-term treatment. These results undoubtedly

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contribute to the development of fungi-based technologies and the applications of *P. chrysosporium* in realistic environment rather than laboratory simulation environment.

1. Introduction

Sulfonamide antibiotics (SAs) which are widely used by humans and veterinary medicine are of great concerns because of their frequent detections in soils and groundwater. It has been reported that the concentrations of SAs in manure reached 900 mg kg^{-1} [1]. Sulfonamides appear frequently in the effluents of sewage treatment plant with concentrations reported to be 6 μg L^{-1} in Germany, 290 μg L^{-1} in Switzerland, 395–575 μg L^{-1} in Georgia, USA, and 0.06–0.21 μg L^{-1} in Colorado, USA [2–4]. Moreover, evidences showed that the half-life of SAs is 9.6–833 d, therefore the time duration of SAs is too long in the water and sediment of environment and they also pose potential risks to aquatic species, plants and human being [5,6]. Sulfamethazine (SMT), a kind of SAs, is highly hydrophilic (octanol water partition coefficient: SMT log K_{ow} = 0.27) even in neutral form. It shows a good performance in inhibiting the enzymatic reaction in bacteria, via deferring the synthesis of an important coenzyme *para*-aminobenzoic acid and damaging the function of product purines and pyrimidines [7]. Heavy metals (HMs) are another matter of concern for their damage on ecosystem and living organisms [8,9]. Cadmium (Cd) is a highly toxic metal which can easily enrich in food chain and dissolve readily in water by natural processes and anthropogenic activities. It can be easily accumulated in crops, and further cause numerous symptoms and pathologies of animals and humans, such as neurological effects and endocrine dyscrasia [10,11].

Environmental behavior of individual contaminant is a popular topic in laboratory investigation [12–14]. But the research of co-contamination is a more practical issue with the development of industry and the acceleration of urbanization. Therefore, combined pollution has become an important developing direction of environment science. In realistic environment, various pollutants always co-exist and even react with each other to make the environmental pollution diversified and complicated. Therefore, as typical pollutants in the environment, heavy metals-antibiotic combined pollutant, especially Cd-SMT combined pollutant, got popularly attention nowadays.

Data from the literature indicate a greater tolerance of white rot fungi (WRF), which are capable of not only degrading SAs but also immobilizing of HMs via unique extracellular oxidative enzyme systems, cell wall cation exchange, extracellular chelation with organic acids and intracellular bioaccumulation [15,16]. Kim et al. [17] discovered that O-methyltransferases in WRF can accelerate SAs degradation by converting the major inhibitors OH-SAs into non-toxic methylated phenolic ones. Rodríguez et al. [18] made a point that WRF-laccase system can promote the degrading efficiency of SAs to 75%. Li et al. [19] proved that the addition of WRF can increase the Cd removal rate from 44.85 to 80.36%. These findings also signaled that WRF possessed potential ability to biotransform HMs-SAs co-contamination. Their high tolerance protects them from the high-level concentrations of organic pollutants and HMs so that their enzyme system could function normally in harsh environments. *Phanerochaete chrysosporium* (*P. chrysosporium*) is a more concerned Cd-SMT combined pollutant hyper accumulation species of WRF [20,21]. The main removal mechanisms were (i) biosynthesis of phytochelatin (PCs) and metallothioneins (MTs) in cells, (ii) phosphate and polyphosphate metabolisms, (iii) chelate with malate and oxalate, (iv) the functional group added to the reactants by CYP450 monooxygenases, such as hydroxyl, carboxyl, or an amine group, and (v) metabolism product conjugated by non-specific extracellular oxidizing agents such as sulfates, glucuronides, glucosides, and glutathione (GSH) [22].

Toxicity and resistance effects, which produced in the interaction process, are closely related to the biotransformation efficiency of pollutant. Most attention has been paid toward the extracellular enzymes, the generation of reactive oxygen species (ROS) and free radical scavenging capacity. These indicators are more beneficial to assess practicability of one biotechnology and reveal the deep mechanisms in transformation process of xenobiotics. In this study, we not only investigated the biotransformation performance of Cd-SMT combined pollutant by *P. chrysosporium*, but also detected the toxicity and resistance indicator, such as ROS, malonaldehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and polyphenol oxidase (PPO), to uncover the underlying mechanisms induced by HMs and antibiotics. Therefore, the objectives of this study were to provide insight into the biotransformation pathway of combined pollutant and to enhance the application and practical value of biotechnology in real environment.

2. Materials and methods

2.1. Microorganism, chemicals, and media

The fungus *P. chrysosporium* strain ATCC-24725 was obtained from China Center for type Culture Collection (Wuhan, China). Fungal cultures were maintained on potato dextrose agar (PDA) slants at 4 °C, and then transferred to PDA plates at 37 °C for 3 days. The spores on the agar surface were gently scraped and blended in the sterile distilled water as spore suspension. The spore concentration was measured by a microscope with a blood cell counting chamber and adjusted to 2.0×10^6 CFU per mL.

The analytical standard of sulfamethazine (99%, w/w) was purchased from Sigma-Aldrich. Analytical reagent grade $FeSO_4$, $K_3Fe(CN)_6$, Na_2HPO_4 and NaH_2PO_4 were obtained from Sinopharm Chemical Reagent Co., Ltd. China. Tert-butanol (TBA), 2,7-dichlorodihydrofluoresceindiacetate (DCFH-DA), hydroxylammonium chloride, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), pyrogallic acid (PAPG), 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), trichloroaceti-cacid (TCA), bovine serum albumin (BSA) and sulfanilic acid were purchased from Aladdin Chemistry Co., Ltd. China. Acetonitrile and methanol were of HPLC grade and obtained from Merck KgaA. Ultrapure water (resistivity of 18.2 MΩ cm) was used throughout the experiments.

2.2. Experimental design

Liquid-state conditions were selected to simulate the real aqueous environment. 5.0 mL of aqueous spore suspension of *P. chrysosporium* was inoculated into 250 mL Erlenmeyer flasks containing 100 mL Kirk's liquid culture medium [23] for 3 days. Controlled the final SMT level at 0, 10, 30 and 50 mg L^{-1} (G1), the final Cd level at 0, 10, 50 and 100 mg L^{-1} (G2) and the final Cd-SMT combined pollutant level at 0, 10–30, 50–30, 100–30, 50–10 and 50–50 mg L^{-1} (G3), respectively. 1.0 g *P. chrysosporium* wet biomass was mixed with 50 mL aqueous solution at various initial Cd and SMT concentrations. Finally, the mixture was incubated in a constant temperature incubator with a constant speed of 120 rpm at 30 °C for 72 h. Sampling was performed after 6, 12, 16, 24, 36, 48, 60 and 72 h. The preparation method of cell free extract was shown in Supplementary information (SI) in detail.

2.3. Quantitative analysis by HPLC

The SMT concentration in samples was monitored by an Agilent high-performance liquid chromatography (HPLC) Series 1100 (Agilent,