Ecotoxicology and Environmental Safety 154 (2018) 45–51

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

# Cadmium-induced stress response of *Phanerochaete chrysosporium* during the biodegradation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)

Mi Feng[a], Hua Yin[a,*], Yajuan Cao[a], Hui Peng[b], Guining Lu[a], Zehua Liu[a], Zhi Dang[a]

[a] Key Laboratory of Ministry of Education on Pollution Control and Ecosystem Restoration in Industry Clusters, Guangdong Provincial Engineering and Technology Research Center for Environmental Risk Prevention and Emergency Disposal, School of Environment and Energy, South China University of Technology, Guangzhou 510006, Guangdong, China

[b] Department of Chemistry, Jinan University, Guangzhou 510632, Guangdong, China

ARTICLE INFO

*Keywords:*
*Phanerochaete chrysosporium*
Cadmium
Stress response
2,2′,4,4′-tetrabromodiphenyl ether

ABSTRACT

Cd-induced stress response of *Phanerochaete chrysosporium* during the biodegradation of BDE-47 was investigated in this study, with the goal of elucidating the tolerance behavior and the detoxification mechanisms of *P. chrysosporium* to resist the Cd stress in the course of BDE-47 biodegradation, which has implications for expanding the application of *P. chrysosporium* in the bioremediation of Cd and BDE-47 combined pollution. The results suggested that single BDE-47 exposure did not induce obvious oxidative stress in *P. chrysosporium*, but coexistent Cd significantly triggered ROS generation, both intracellular ROS level and $H_2O_2$ content showed positive correlation with Cd concentration. The activities of SOD and CAT were enhanced by low level of Cd ($\leq$ 1 mg/L), but Cd of higher doses ( > 1 mg/L) depressed the expression of these two antioxidant enzymes at the later exposure period (3–5 days). The intracellular content of GSH along with GSH/GSSG ratio also exhibited a bell-shaped response with a maximum value at Cd of 1 mg/L. Furthermore, Cd-induced ROS generation resulted in the lipid peroxidation, as indicated by a noticeable increment of MDA content found after 3 days. Moreover, the study also indicated that Cd less than 1 mg/L promoted the production of extracellular protein and quickened the decrease of pH value in the medium, while excessive Cd ( > 1 mg/L) would lead to inhibition. These findings obtained demonstrated that *P. chrysosporium* had a certain degree of tolerance to Cd within a specific concentration range via regulating the antioxidant levels, inducing the synthesis of extracellular protein as well as stimulating the production of organic acids, and 1 mg/L is suggested to be the tolerance threshold of this strains under Cd stress during BDE-47 biodegradation.

## 1. Introduction

Cadmium (Cd) has drawn considerable public attention as one of the most deleterious and ubiquitous heavy metals (Radosavljevic et al., 2012). Many industrial processes involving electronic equipment, battery manufacturing, electroplating, pigments and alloy preparation gave rise to massive discharge of wastes containing high level of cadmium, which has posed a great threat to human health and the environment in that cadmium has been shown to be able to easily translocate from plant roots to aboveground tissues and enter into living organisms and human body through the transfer process of food chain, exerting numerous toxic action at molecular, cellular and physiological levels (Chakraborty et al., 2014; Xu et al., 2012).

As the world's largest market for the import of electronic waste, China has become increasingly concerned that a wide range of polybrominated diphenyl ethers (PBDEs) has been found in different environmental mediums contaminated by Cd in the e-waste dismantling area (Shi et al., 2013). Among PBDEs, 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) is the most frequently detected one, which was reported to tend to persist and accumulate in living organisms owing to its high liposolubility and stability (Malarvannan et al., 2013; Stapleton et al., 2004). Consequently, the hazards of combined pollution of Cd and BDE-47 may be higher than either of these two contaminants. More and more efforts should be paid on removing such co-pollutants from the contaminated environment.

Microbial treatment using microorganisms to transform and degrade the hazardous substance has been considered as an attractive and promising option for the in-situ remediation of the environment co-contaminated with organic pollutants and heavy metals because this technology is highly efficient, low cost and environmentally benign (Basak et al., 2014). Nevertheless, since the toxicity of heavy metals can severely affect the physiological condition, bioactivity and colonization

* Corresponding author.
*E-mail address:* huayin@scut.edu.cn (H. Yin).

https://doi.org/10.1016/j.ecoenv.2018.02.018
Received 16 November 2017; Received in revised form 1 February 2018; Accepted 2 February 2018
Available online 22 February 2018
0147-6513/ 

of microorganism, the degree of organic pollutants biodegradation in co-contaminated system depends largely on both the type and content of co-existed heavy metals and the capability of degrader to survive under heavy metal stress (Chen et al., 2013). As is evidenced by the fact that Cd accumulated in cells could interfere with not only the individual biological reactions but also the complex metabolic processes, and could particularly induce oxidative stress by overproducing reactive oxygen species (ROS) in living cells, thus resulting in the inactivation of enzymes and the disorders of metabolism (Gallego et al., 2012; Sun et al., 2013), such detrimental effects of Cd are bound to have adverse effect on the biotransformation of its organic co-pollutants. But it is worth noting that microorganism, like many other living beings, has developed a series of defense mechanism to counter the toxicity of heavy metal, enhancing the resistance and tolerance to metal stress (Baldrian, 2003). Hence, taking an investigation on the stress response of microorganism to Cd during BDE-47 biodegradation is imperative for obtaining a better understanding of the tolerance limit of degraders and maximizing their potential in bioremediation of co-contaminated Cd and BDE-47 in e-waste recycling regions.

Over the past few years, white rot fungi has been gaining mounting attention in the field of microbial remediation due to its abundant metabolic versatility, extensive adaptability to environment, and desired ability to degrade xenobiotics and absorb heavy metals (Feng et al., 2017a). Although the feasibility of applying white rot fungi to remove Cd pollution has been validated (Chen et al., 2011), little attention was paid to the effects of Cd stress on adaptive responses of white rot fungi in the course of BDE-47 biodegradation.

In view of this, *Phanerochaete chrysosporium*, a typical species of white rot fungi proven to be available for simultaneous Cd removal and BDE-47 biodegradation in our previous study (Cao et al., 2017) was chosen as a model strain in current study. The aim of this work was first to assess Cd-induced oxidative stress in *P. chrysosporium* during BDE-47 biodegradation, with particular attention given to the production of reactive oxygen species (ROS), the variation of antioxidative enzyme activity, superoxide dismutase (SOD) and catalase (CAT), together with the intracellular content of glutathione (GSH) and malondialdehyde (MDA), as the changes of these physiological index reflected microbial fitness and survival under Cd stress. Moreover, extracellular proteins and pH changes of the reaction system were also evaluated to understand more about the influence of Cd stress on cellular metabolism and the role of extracellular secretions in detoxification of Cd.

## 2. Materials and methods

### 2.1. Chemicals

BDE-47 (99% purity) used in our study was purchased from Chem Service (Inc., USA), $Cd(NO_3)_2{\cdot}4H_2O$ (analytical pure) was obtained from Guangzhou Chemical Reagent Company(Guangzhou, China). All other chemicals were of or above analytical grade and were provided by Tianjin Damao Chemical Reagent Factory (Tianjin, China). The stock solution of 100 mg/L BDE-47 and 1000 mg/L Cd was prepared by dissolving certain amount of BDE-47and $Cd(NO_3)_2{\cdot}4H_2O$ in methanol and distilled water separately.

### 2.2. Strain and cultivation

The experimental strain *P. chrysosporium* BKM-F-1767 (GIM3.383) was purchased from Guangdong Microbiology Culture Center. The spore suspension of *P. chrysosporium* was prepared in sterile saline solution and adjusted to approximately $3.0 \times 10^6$ CFU/mL, then, 1 mL of spore suspension was incubated into 19 mL of Tien and Kirk's liquid medium (Kirk et al., 1978). For the toxicity experiments, 100 μL of stock solution of 100 mg/L BDE-47 in methanol was added into a sterile vacant flask and allowed to evaporate in a clean bench at room temperature. Following 3 days of pre-incubation at 30 °C in an incubator under 160 rpm, the mycelial pellets formed along with the aqueous medium were transferred into the flask containing BDE-47 at 0.5 mg/L, and then various amounts of Cd stock solution was rationed into the culture to reach the designated concentrations. The initial concentration of Cd in the medium was regulated to 0, 0.5, 1, 5 and 10 mg/L separately. The mycelial pellets treated without BDE-47 and Cd were set as control.

### 2.3. Determination of ROS and hydrogen peroxide ($H_2O_2$)

The content of intracellular ROS was measured using a specific fluorescence indicator 2,7-dichlorodihydro -fluoresceindiacetate ($H_2$DCF-DA). Generally, $H_2$DCF-DA can be hydrolyzed to 2,7-dichlorodi-hydrofluorescein ($H_2$DCF) by intracellular ester enzymes, if intracellular ROS produce, non-fluorescent $H_2$DCF will be further oxidized to 2,7-dichlorofluorescein (DCF) and emit green fluorescence (Kováčik et al., 2015). The fluorescence intensity of DCF is proportional to extent of ROS production. The pellets harvested were washed twice with PBS (0.05 M, pH 7.4) and stained with10 μM work solution of $H_2$DCF-DA at ambient temperature for 40 min in darkness. The stained pellets were further rinsed with PBS again followed by observation using confocal laser scanning microscope (Pascal 2, Zeiss. Ex at 488 nm, Em at 520–600 nm). The determination of $H_2O_2$ content was performed strictly according to the instruction of hydrogen peroxide detection kit produced by Nanjing Jiancheng Bioengineering Institute, China.

### 2.4. Analysis of antioxidant components

SOD, CAT activities and glutathione content were evaluated using total superoxide dismutase assay kit, catalase assay kit and GSH and GSSG Assay Kit, respectively. All the kits were provided by Beyotime Institute of Biotechnology, China.

### 2.5. Measurement of malondialdehyde (MDA) content

MDA content was detected by means of thiobarbituric acid method (Zhou et al., 2016). The pellets collected were washed with PBS and then homogenized in 10% trichloroacetic acid solution. After centrifuging at 10,000 g for 15 min, the supernatant was harvested and further incubated with 0.6% thiobarbituric acid in boiling water bath for 20 min. The cooled supernatant was recentrifuged at 4000 g for 5 min, and the absorbance was recorded at 532 nm, 600 nm and 450 nm, respectively.

### 2.6. Determination of extracellular protein and pH of the medium

The quantification of protein concentration in the extracellular medium was fulfilled by Bradford protein assay kit according to the manufacturer's instructions, this kit was purchased from Beyotime Institute of Biotechnology, China. And the pH value of medium was measured with a pH meter (Sartorius AG 37070, Goettingen, Germany).

### 2.7. Statistical analysis

All the experiments were run in triplicate, and the results are presented as the means ± standard deviations of three replicates. Statistical analysis was conducted using GraphPad Prism 7.0 software. All the data has been subjected to two-way ANOVA to show any concentration × time effects. Tukey test was performed to determine the significant differences among treatments at $p < 0.05$ significance level.

## 3. Results and discussion

### 3.1. Cd-induced ROS generation during BDE-47 degradation

ROS are a kind of highly reactive molecules or molecular fragments