Journal of Hazardous Materials 321 (2017) 37–46

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Toxicity mechanisms and synergies of silver nanoparticles in 2,4-dichlorophenol degradation by *Phanerochaete chrysosporium*

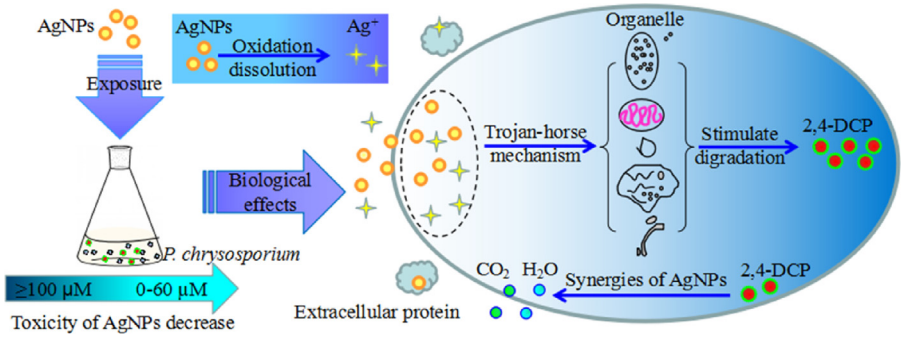
Zhenzhen Huang[a,b], Guiqiu Chen[a,b,*], Guangming Zeng[a,b,*], Zhi Guo[a,b], Kai He[a,b], Liang Hu[a,b], Jing Wu[a,b], Lihua Zhang[a,b], Yuan Zhu[a,b], Zhongxian Song[c]

[a] College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China
[c] Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, PR China

HIGHLIGHTS

- Synergies of AgNPs at low doses (0–60 μM) in 2,4-DCP biodegradation were observed.
- Maximum degradation rates of 2,4-DCP were more than 94% at low-level AgNPs.
- AgNPs-mediated toxicity to *P. chrysosporium* arised from the "Trojan-horse" effects.
- 2,4-DCP was completely degraded into CO_2 and H_2O at optimum conditions.
- Amino, carboxyl, carbonyl and sulfur-containing groups assist in Ag transportation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form 29 August 2016
Accepted 30 August 2016
Available online 31 August 2016

Keywords:
Silver nanoparticles
2,4-Dichlorophenol
Phanerochaete chrysosporium
Synergies
Biodegradation

ABSTRACT

Mechanisms of silver nanoparticles-mediated toxicity to *Phanerochaete chrysosporium* and the influence of silver nanoparticles (AgNPs) on the biodegradation of 2,4-dichlorophenol (2,4-DCP) have been systematically investigated. AgNPs at low doses (0–60 μM) have greatly enhanced the degradation ability of *P. chrysosporium* to 2,4-DCP with the maximum degradation rates of more than 94%, exhibiting excellent synergies between AgNPs and *P. chrysosporium* in the degradation of 2,4-DCP. Meanwhile, removal of total Ag was also at high levels and highly pH dependent. However, significant inhibition was highlighted on 2,4-DCP biodegradation and Ag removal upon treatment with AgNPs at high doses and $AgNO_3$ at low-level exposure. Results also suggested that AgNPs-induced cytotoxicity could arise from the "Trojan-horse" mechanism executing particle effects, ion effects, or both, ruling out extracellularly

* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
E-mail addresses: gqchen@hnu.edu.cn (G. Chen), zgming@hnu.edu.cn (G. Zeng).

http://dx.doi.org/10.1016/j.jhazmat.2016.08.075

released Ag^+. Moreover, under relatively low concentrations of AgNPs exposure, 2,4-DCP was broken into linear chain organics, and eventually turned into CO_2 and H_2O through reductive dechlorination and reaction with hydroxyl radicals. FTIR analysis showed that amino, carboxyl, carbonyl, and sulfur-containing functional groups played crucial roles in Ag transportation and the reduction of Ag^+ to Ag^0.

1. Introduction

2,4-Dichlorophenol (2,4-DCP) is extensively used in many industries such as fungicides, insecticides, pesticides and pharmaceuticals [1], and has been listed as prior pollutants by the U.S. Environmental Protection Agency (EPA) owing to its high toxicity, suspected carcinogenicity, bioaccumulation and mutagenicity to living organisms [2]. 2,4-DCP is abundant in the contaminated groundwater [3], and can cause itch, faint, anemia and comedo [4], provoke disturbances in the structure of cellular bilayer phospholipids [5], pose a serious ecological problem, and seriously affect both public health and environmental quality.

Nanomaterials of noble metals have drawn considerable attention due to their specific mechanical, electrical, optical and catalytic properties compared to their bulk materials [6]. Silver nanoparticles (AgNPs) have been used for many applications in consumer products including textiles, disinfecting medical devices and home appliances, as well as their potential capability of water treatment [7,8]. Considerable interest has arisen in the use of AgNPs for point-of-use systems for emergency response following disasters, and for water systems that are not connected to a central network, owing to the potent and broad-spectrum antimicrobial properties of AgNPs [9,10]. However, with the significant increase in production, application, and market request of AgNPs, it is inevitable for the release of AgNPs into the environment (with the predicted environmental concentration of tens and perhaps hundreds ng/L in water) [11], affecting the normal wastewater processing. According to EPA and World Health Organization, the maximum admissible concentration of silver in drinking water is regulated at 0.1 mg/L.

Furthermore, AgNPs have been reported to be toxic both to humans and the environment [12–15]; and their fate, transport and toxicity can be influenced by water chemistry properties. In turn, the special characteristics of AgNPs will affect the removal of toxic organic pollutants and impact on microbial growth during the process of sewage treatment in the presence of AgNPs in the wastewater.

Researchers have shown that AgNPs can be implicated as efficient catalysts for removal of environmental contaminants, including 4-nitrophenol [16], pentachlorophenol [17], dyes [18], and aromatic nitro compounds [19]. The current reports are mostly concerned with the removal of 2,4-DCP using Ni/Fe [20], Pb/Fe [3], Fe_3O_4 [21], Cu@TiO_2 [22], and other functionalized nanoparticles [23,24], as well as the detection of 2,4-DCP with the nanocomposite acting as a sensor [4]. However, few studies have been reported on the influence of AgNPs on the biodegradation of 2,4-DCP in the wastewater. Among various microorganisms screened, *Phanerochaete chrysosporium* (*P. chrysosporium*), as the model species of white-rot fungi, has been reported to degrade and transform a wide range of organic substrates containing 2,4-DCP in wastewater treatment [25], and, hence, was selected as the testing microorganism in this research.

In order to evaluate the toxicity of AgNPs to *P. chrysosporium* and the effects of AgNPs on the performance of 2,4-DCP biodegradation, dose-response assays of AgNPs, $AgNO_3$ and 2,4-DCP were investigated. Additionally, other factors involved in 2,4-DCP degradation, such as dissolved Ag^+, total Ag (including Ag^+ and AgNPs), pH values, and extracellular proteins content, were also addressed. Based on the analyses of scanning electron microscopy (SEM) equipped with an energy dispersive X-ray (EDX) attachment, Fourier transform infrared spectrometry (FTIR) and gas chromatography–mass spectrometry (GC–MS), toxicity mechanism of AgNPs and the biodegradation pathway of 2,4-DCP were proposed.

2. Materials and methods

2.1. Microorganism and chemicals

P. chrysosporium strain BKMF-1767 (CCTCC AF96007) was obtained from the China Center for Type Culture Collection (Wuhan, China) and maintained on potato dextrose agar slants at 4 °C. Spores suspensions were prepared by scraping the spore into ultrapure water (18.25 MΩ cm), the concentration of which was adjusted to 2.0×10^6 CFU/mL. The cultivation of *P. chrysosporium* was performed in an incubator at 37 °C and 150 rpm for 3 days by inoculating spores suspensions into the culture medium in 500 mL conical flasks. Then, the mycelia were harvested and rinsed several times with 2 mM Na_2HCO_3 buffer solution, which was selected as the exposure medium because it had no effect on silver bioavailability and avoided ligands that might bind with AgNPs/Ag^+ and facilitate precipitation or other confounding effects [26,27].

2,4-DCP and $AgNO_3$ were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analytical reagent grade and obtained from Shanghai First Reagent Co., China.

2.2. Characterization of AgNPs

AgNPs were synthesized via reduction of $AgNO_3$ and utilization of $NaBH_4$ and trisodium citrate as reductants and stabilizing agents, respectively, following a protocol adapted from a previous study with minor modifications [28]. Detailed descriptions on preparation and characterization of AgNPs are available in the Supporting information.

2.3. Dose-response assays of AgNPs and $AgNO_3$

The AgNPs stock solution was diluted with 2 mM Na_2HCO_3 buffer solution to obtain the desired concentrations (0, 1, 10, 30, 60, and 100 μM) used in the test solutions and mixed; the initial 2,4-DCP concentration was maintained at 20 mg/L in the flasks. Equivalent fungal mycelia (0.6 g/L) were added to the solutions and incubated in an incubator at 37 °C with 150 rpm. Dose-response assays of $AgNO_3$ were performed similarly.

2.4. Dose-response assay of 2,4-DCP

In order to determine the effect of 2,4-DCP concentration on the adsorption and degradation performance, 0.6 g/L *P. chrysosporium* pellets were exposed to aqueous solutions containing 0, 5, 10, 20, 40, and 80 mg/L 2,4-DCP, respectively, and maintaining an initial AgNPs concentration of 10 μM. 2,4-DCP concentration was determined by high performance liquid chromatography (HPLC, Agilent 1100) with a previously reported method [29]. The intermediates