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Detoxification of U(VI) by *Paecilomyces catenlannulatus* investigated by batch, XANES and EXAFS techniques

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ABSTRACT

Paecilomyces catenlannulatus (*P. catenlannulatus*) as a genus of entomogenous fungus presented a variety of surface reactive groups by batch characterizations. The detoxification of U(VI) by *P. catenlannulatus* was investigated under different water chemistry (pH, incubation time, foreign anions and U(VI) concentration) by batch techniques. Approximately 75% of U(VI) was reduced to U(IV) (i.e., $U^{(IV)}O_2(s)$) by *P. catenlannulatus* at pH 5.5 and 7 days under glovebox conditions, therefore the formation of precipitates decreased the toxicity of U(VI) for *P. catenlannulatus*. In addition, phosphate facilitate the U(VI) reduction, whereas carbonate and sulfate inhibited the U(VI) reduction. The activities of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) level were stimulated exposure to 1-30 mg/L U(VI), indicating that CAT, SOD and GSH were antagonized for the oxidant stress derived from U(VI) at low concentrations. According to XPS and XANES analysis, the occurrence of U(IV) revealed the reduction of adsorbed U(VI) to U(IV) by *P. catenlannulatus*. The results of EXAFS analysis indicated that the fitting of U-O and U-U shell for U-loaded *P. catenlannulatus* was similar to that of $U^{(IV)}O_2(s)$). The formation of U-bearing precipitates decreased the toxicity of U(VI) for *P. catenlannulatus*. These findings indicated that *P. catenlannulatus* is capable to detoxify U(VI) by extracellar/intracellar defense systems. Therefore, *P. catenlannulatus* can be utilized as a promising bioadsorbents for remediation of uranium-contaminated wastewater in environmental cleanup.

1. Introduction

The discharge of uranium into environment during uranium mining and processing leads to the environmental pollution, which has become a serious environmental problem (Sun et al., 2014a). Compared with tetravalent uranium (U(IV)), hexavalent uranium (U(VI)) has the excellent water-solubility and bioavailability, thereby resulting in potential threat to eco-environment and human health (Liu et al., 2017; Sun et al., 2016a). Therefore, it is of great importance to remove U(VI) from aqueous solutions before discharge into sub-environment. Among these methods, bioremediation has been extensively utilized in recent years due to environmentally friendly, low-cost and high efficient performance (Sun et al., 2016b). It is demonstrated that bioremediation, including biosorption, bioaccumulation and biotransformation, presented the high removal performance for U(VI) due to the existence of various functional groups such as oxygen-, sulfur- and nitrogen-bearing functional groups. Considering the sparingly solubility of $U(IV)O_2(s)$, the bio-reduction of U(VI) to U(IV) could be an effective approach for immobilization of uranium-contaminated wastewater under anaerobic conditions in environmental cleanup (Ding et al., 2015).

Several filamentous fungi presented bio-transform for various environmental contaminants. For example, Xu et al. demonstrated that the Cr(VI) can be reduced to Cr(III) by *Paeilomyces lilacinus XLA* (Xu et al., 2017). *Paecilomyces catenlannulatus* (*P. catenlannulatus*) as a genus of entomogenous fungus has been extensively used in a variety of fields due to many functional groups such as oxygen-, sulfur- and nitrogen-bearing functional groups (Yang et al., 2015). In our previous studies, *P. catenlannulatus* was recently used as bio-adsorbents for removal of heavy metals (Li et al., 2014a; Sheng et al., 2015) and radionuclides (Li et al., 2013, 2014b). However, the effect of water chemistry on the detoxification of U(VI) by *P. catenlannulatus* was not available nowadays.

The objectives of this study were (1) to investigate the effect of water chemistry (e.g., pH, incubation time, foreign ions and concentration) on reduction of U(VI) to U(IV) by *P. catenlannulatus* by batch techniques; (2) to determine the role of antioxidant (non)-enzymatic system (e.g., catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH)) in fungal cell response to redox-active U(VI); and

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(3) to demonstrate the reduction mechanism of U(VI) on *P. catenlannulatus* using XPS, XANES and EXAFS analysis. The highlight of this manuscript is to utilize fungi-based biosorbent for in-situ pre-concentration and immobilization of radionuclides under anaerobic conditions.

2. Materials and methods

2.1. Materials

U(VI) stock solution (0.1 mol/L) was prepared by dissolving uranyl nitrate ($UO_2(NO_3)_2.6H_2O$, 99.9% purity, Sigma-Aldrich) into DI water. Other chemicals (i.e., Na_3PO_4, Na_2CO_3, Na_2SO_4 and $NaNO_3$) were purchased as analytical grade from Sinopharm Chemical Reagent Co. Ltd.

2.2. Preparation and characterization of P. catenlannulatus

The U(VI)-tolerant fungus *P. catenlannulatus* was isolated from uranium-contaminated soil. Fungus strain *P. catenlannulatus* (CCTCC: M2012136) was cultured using potato dextrose agar (PDA) and then incubated at 303 K for 7 days. Briefly, 1 mL *P. catenlannulatus* suspension ($\sim 10^6$ spores/mL) was incubated at 150 rpm and 30 °C for 3 days. Fungal mycelia were collected by washing it in sterile deionized water for several times and then centrifuging it at 6000 rpm for 10 min. The bacterial growth was inhibited by adding streptomycin ($\sim$20 mg/L) into PDA. The activities of SOD and CAT were assayed by Total SOD Assay Kit with WST-1(S0107) and (S0051), respectively. The levels of GSH were examined with the GSH Assay Kit (S0053, Beyotime Institute of Biotechnology, Beijing, China).

The change in surface groups of *P. catenlannulatus* before and after U (VI) addition was characterized by FT-IR (Nicolet 8700 FT-IR spectrometer) and XPS (Thermo ESCALAB 250 electron spectrometer) with a multidetection analyzer using an Al Kα X-ray source (1486.6 eV) at 10 kV and 5 mA under 10^{-7} Pa techniques.

2.3. Batch experiments

The effect of pH, incubation time, foreign ions (phosphate, carbonate and sulfate) and concentrations on the reduction of U(VI) by *P. catenlannulatus* was investigated in glovebox conditions. The obtained fungal mycelia was served as inoculum an re-inoculated into each fresh medium with 60 mg/L of U(VI). The pH values were adjusted to 2.0–11.0 by 0.1–1.0 mol/L NaOH or HCl solutions. All the cultures were continuously incubated for 20 days at 200 rpm and 30 °C. The reduction of U(VI) on different incubation time and U(VI) concentration was conducted the same following experimental conditions. The effect of foreign ions on U(VI) reduction was conducted by adding 50 mg/L of PO_4^{3-} and CO_3^{2-} and SO_4^{2-} was separately added into each culture with 60 mg/L U(VI) under glovebox conditions. After reaction equilibrium, the culture biomass was collected by centrifuging at 6000 rpm for 15 min and then freeze-dried 12 h. The reduction of U(VI) can be distinguished from the sorption of U(VI) by the desorption of adsorbed U (VI) using 0.2 mol/L Na_2CO_3 for 30 min under stirring conditions (Ding et al., 2015). Briefly, 3 mL supernate was removed after removal experiments by centrifuging it 6000 rpm for 15 min, and then 3 mL of 0.2 mol/L Na_2CO_3 was added into wet pastes. The suspensions were reacted for 30 min under vigorous stirring conditions. Therefore, the adsorbed U(VI) was extracted by Na_2CO_3 solution, the reduction amount of U(VI) can be calculated by the difference of total removal amount and extracted amount by Na_2CO_3 solution. The concentration of U(VI) in supernatant was detected using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, ThermoFisher, XSERIES 2.0). All the experimental data were conducted in triplicate sets. All experimental data were obtained in triplicate sets. The statistical analysis of all data was conducted with SPSS 13.0. All data were plotted by Origin 8.5 pro. The significant level was set at $p < 0.05$.

2.4. XANES and EXAFS analysis

The samples for XANES and EXAFS analysis of U(VI)-bearing *P. catenlannulatus* were prepared under room temperature in the glove box as the following protocols: 100 mL *P. catenlannulatus* with 0.01 mol/L $NaNO_3$ were added into 250 mL flask bottles and then pre-equilibrated for 24 h. Then U(VI) solutions were dropwise added under vigorous stirring conditions in order to avoid the precipitation of U(VI) solids, and the solution were adjusted to pH 4.5 by 0.01–1.0 mol/L HNO_3 or NaOH solution. Samples were then gently agitated on a shaker for 2 days. The solid phase was separated from liquid phase by centrifuging it at 6000 rpm for 15 min. The wet pastes of U(VI)-bearing *P. catenlannulatus* were mounted in Teflon sample holders with Kapton tape.

The U L_{III}-edge XANES and EXAFS spectra were conducted into Shanghai Synchrotron Radiation with Si(111) double crystalline monochromator mode with 32-Ge element detector. The spectrum of crystalline $U^{(IV)}O_2(s)$ sample was conducted in transmission mode, whereas the other spectra were collected in fluorescence mode.

The pre-treatment and fitting of EXAFS spectra were fitted by ATHENA and ARTEMIS interfaces to IFFEFIT 7.0 software, respectively (Newville, 2001; Ravel and Newville, 2005). The fitting of paths (i.e., U–Oax, U–Oeq, U–U and U–C shell) was generated from the crystal structures of rutherfordine (UO_2CO_3) (Finch et al., 1999).

3. Results and discussion

3.1. Characterization

The change in surface functional groups of *P. catenlannulatus* after addition of U(VI) before and after U(VI) addition was characterized by FT-IR and XPS analysis. Fig. 1A shows the FT-IR spectra of *P. catenlannulatus* after addition of U(VI). The strong and wide peaks at 3490 cm^{-1} was attributed to the strength vibration of –OH groups for adsorbed water (Sun et al., 2017a; Cheng et al., 2015). The characteristic band of C=O groups was observed at 1705 cm^{-1} (Sun et al., 2012a; Ding et al., 2016). The FT-IR bands at 1620 and 1020 cm^{-1} could be ascribed to the stretching vibration of aromatic C=C groups (Song et al., 2016) and C-O groups (Jin et al., 2015), respectively. The peaks at 680 and 940 cm^{-1} were C-H out-of-plane and in-of-plane on 1,2-ring, respectively (Sun et al., 2013). As shown in Fig. 1B, the high resolution O 1s spectra of *P. catenlannulatus* can be deconvoluted four bands at $\sim$531.2, 531.9, 532.4 and 536.3 eV, respectively, which can be attributed to C-O, P/S-O, O=C-O and –OH of adsorbed water, respectively (Sun et al., 2017b; Ding et al., 2014; Hu et al., 2017; Cheng et al., 2016). The minimum inhibitory concentration of U(VI) against fungus *P. catenlannulatus* was 142 mmol/L in liquid culture conditions, indicating that fungus *P. catenlannulatus* presented an excellent potential for the detoxification of U(VI) in contaminated environments. The characteristic results indicated that *P. catenlannulatus* presented a variety of functional groups.

3.2. Effect of pH on U(VI) reduction

The pH in aqueous solutions was of great important parameter, which influenced the surface charge of adsorbent and speciation of adsorbate (Sun et al., 2015; Sheng et al., 2016; Cheng et al., 2017; Wang et al., 2015; Zou et al., 2016; Yin et al., 2017; Chen et al., 2017). Fig. 2A shows the effect of pH on U(VI) reduction by *P. catenlannulatus* under anoxic conditions. No change in reduction of U(VI) to U(IV) was observed at pH 1.0–3.0, and then reduction of U(VI) significantly increased with increasing pH from 4.0 to 5.5, Approximate 75% of U(VI) was reduced to U(IV) by *P. catenlannulatus* at pH 5.5, whereas noticeable decreased reduction of U(VI) was observed at pH > 6.0. This trend could be explained by the proportion of biomass in dry weight. As