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Assessing adsorption of polycyclic aromatic hydrocarbons on *Rhizopus oryzae* cell wall components with water–methanol cosolvent model

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ABSTRACT

The contribution of different fungal cell wall components in adsorption of polycyclic aromatic hydrocarbons (PAHs) is still unclear. We isolated *Rhizopus oryzae* cell walls components with sequential extraction, characterized functional groups with NEXAFS spectra, and determined partition coefficients of PAHs on cell walls and cell wall components with cosolvent model. Spectra of NEXAFS indicated that isolated cell walls components were featured with peaks at ~532.7 and ~534.5 eV energy. The lipid cosolvent partition coefficients were approximately one order of magnitude higher than the corresponding carbohydrate cosolvent partition coefficients. The partition coefficients for four tested carbohydrates varied at approximate 0.5 logarithmic units. Partition coefficients between biosorbents and water calculated based cosolvent models ranged from 0.8 to 4.2. The present study proved the importance of fungal cell wall components in adsorption of PAHs, and consequently the role of fungi in PAHs bioremediation.

1. Introduction

Microbial treatments of contaminated soils are more efficient, financially affordable, and adaptable than physicochemical treatment because of potential advantages: the complete degradation of the pollutants, lower treatment cost, greater safety, and less soil disturbance (Habe and Omori, 2003). Plenty of fungi (filamentous fungi, basidiomycetes, white-rot fungi, and deuteromycetes) have been shown to remove PAHs efficiently, even for five-ring PAHs which is difficult to be degradated (Peng et al., 2008). Therefore, an understanding of the fungi uptake and translocation mechanisms of PAHs is crucial for guidance of the field application of microbial remediation with fungi. There are two mechanisms involved in PAH degradation by fungi: one utilizes the cytochrome P-450 system (Yadav et al., 2006), the other uses the soluble extracellular enzymes of lignin catabolism, including lignin peroxidase, manganese peroxidase (Steffen et al., 2003) and laccase (Mayer and Staples, 2002). The cytochrome P-450 system degrades PAHs in cells after uptake by fungi, but extracellular enzymes will be released out through cell walls and degrade PAHs in surrounding regions. Both mechanisms are expected to associate with the cell wall of fungi, which entirely surrounds the exterior of cells. As the outermost surface against the environment, fungal cell walls control the transport of molecules into or out to the cells and may also provide functions affecting the transpiration and translocation of PAHs through the intra- and extra-cellular regions of fungal cells. The fungal cell walls are composed of lipid, protein, and polysaccharides, including chitin, chitosan, glucan, and mannan (Ruiz-Herrera, 1992). For hydrophobic organic compounds, the lipid fraction is the main storage site for adsorbates (Li et al., 2005). The partition coefficients of lipids were assumed to be linear correlations with the corresponding octanol water partition coefficients (K_{ow}), and polysaccharides water partition coefficients (K_{pw}) assumed to be low (Chiou et al., 2001). Previous studies provided evidence for the importance of the plant root cell walls in the partitioning of phenanthrene by plant roots, and the models for partition coefficients were significantly improved by including the contribution of polysaccharides instead of using root lipids alone (Chen et al., 2009; Zhang and Zhu, 2009). Therefore, elucidation of the fundamental functions of the cell wall in the interactions between cell walls and PAHs is of great importance. However, a thorough understanding of the role of fungal cell walls in the partitioning and uptake of PAHs is still lacking.

The concentrations of PAHs in water are often very low and difficult to measure accurately because of low solubility. Furthermore, dissolved organic matter (DOM) and particles present in the equilibration environment both disturb the determination of PAHs

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in aqueous phase (Jonker and Smedes, 2000). The addition of co-solvents such as methanol or acetone can increase the solubility of PAHs in the aqueous phase and stabilize the equilibrating solutions (Smedes et al., 2009). Therefore, partition coefficients determined in cosolvent mixtures may yield more accurate and more precise partition coefficients than direct measurements in cosolvent free water (Smedes et al., 2009). The cosolvent method has been applied to the measurement of sediment-water partition coefficients, for which the overestimation of solute concentrations in the aqueous phase is due to the abundance of DOM and particles in the sediments (Jonker and Smedes, 2000). The cosolvent method was also used for the measurements of partition coefficients for silicone rubber and a polymer of passive polymeric materials (Smedes et al., 2009; Yates et al., 2007). However, the cosolvent method has rarely been applied for estimating partition coefficient of biosorbent materials.

Numerous studies have reported the outstanding capacity of the worldwide distributed saprophyte, *Rhizopus oryzae*, for adsorbing organic pollutants (Das et al., 2006; Fu and Viraraghavan, 2001). The biopolymer component of cell walls mainly contributes to the adsorption behavior of *R. oryzae* (Das et al., 2008). The present study was done to determine the adsorptive characteristics of individual components in cell walls of *R. oryzae* for PAHs. The cell wall components were characterized with near-edge X-ray absorption fine structures (NEXAFS). Sorption of naphthalene, fluorene, phenanthrene, and pyrene as model PAHs on *R. oryzae* cell walls and cell wall components were quantitatively evaluated in methanol water cosolvent mixtures. Moreover, correlation models based on partial least regression approach for PAHs adsorption on each cell wall component and calculated molecular structural descriptors were estimated. The most contributing molecular structural descriptor was compared with K_{ow} to declare the role of molecular structural descriptors in predicting partition coefficients of PAHs on biosorbents. Cosolvent models base on the Bayesian cosolvent models in for extrapolating partition coefficients of PAHs on *R. oryzae* cell wall components were estimated in our previously study (Ma et al., 2011), the partition coefficients of PAHs on biosorbents in water solution were calculated in the present studies.

2. Material and method

2.1. Materials

R. oryzae (3.5842) was obtained from China General Microbiological Culture Collection Center. The different PAHs, methanol, hexane and all other chemicals were purchased from Sigma USA, Tedia USA, and Sinopharm Chemical reagent China, respectively.

2.2. Isolation of of cell walls

Potato-dextrose broth was used for the cultivation of *R. oryzae*. The media were dispensed in aliquots of 75 mL in 250 mL Erlenmeyer flasks and sterilized by autoclaving at 121 °C for 15 min. The flasks containing the medium were inoculated with 3.5×10^7/mL *R.oryzae* spores. Inoculated media were incubated under submerged condition (130 rpm) at 30 °C for 96 h. At the end of incubation, fungal mycelia were harvested by centrifugation (Sigma laboratory centrifuge 3K18, Germany) and washed with Milli-Q water.

The cell walls of *R. oryzae* were prepared following the procedure described by Das et al. (2008) with little modification. In brief, *R. oryzae* biomass was suspended in Milli-Q water and disrupted by an ultrasonic disruptor (Sonics, USA) for 1 min (pulse of 5 s, interval of 5 s, output level of 5). The cell suspension was centrifuged at 4000*g* for 8 min at 4 °C and the supernatant decanted off. The cell wall materials were thoroughly washed with Milli-Q water followed by increasing concentrations of ethanol (50%, 70% and 100%) to remove the excess water. This was then dried by lyophilization and kept under refrigerated condition till use.

2.3. Isolation of cell wall components

Lipids were extracted from the cell wall matrix with a modified method of Li et al. (2005). Chitosan and crude chitin were isolated from the lyophilized *R. oryzae* cell walls following slight modification of the procedure of Synowiecki and Al-Khateeb (1997). Glucan and mannan were extracted from the lyophilized fungal cell walls following the methods of Northcote and Horne (Northcote and Horne, 1952). The isolated cell wall components were dried by lyophilization. The details are available in supporting information.

2.4. NEXAFS using scanning transmission X-ray microscopy

Near-edge X-ray absorption fine structures (NEXAFS) were obtained using scanning transmission X-ray microscopy (STXM) at beamline 08U of the Shanghai Synchrotron Radiation Facility (SSRF). This beamline produces a soft X-ray beam from the 3.5 GeV electron storage ring and illuminates a monochromator that is tunable over 250–2000 eV. The maximum spatial resolution of the STXM is 50 nm. The STXM obtains a map of a sample from 528 to 538 eV in 0.25 eV steps with 2 ms dwell time. The spectra for each point were then reconstructed over the entire region of the aligned stack with Stack analyze (Jacobsen and Flynn, 2000). Principal component and cluster analysis was performed to orthogonalize and noise-filter the data and to classify regions in the sample according to spectral similarities with PCA_GUI (Lerotic et al., 2004). Spectra from clusters were smoothed with a PSPLINE package for R.

2.5. Equilibration experiments

Cell wall components were equilibrated with a constant proportion of 20% v/v methanol, 10 mL water and 2.5 mL methanol added to 25 mL Erlenmeyer flasks with glass-ground stoppers to prevent volatilization. Subsequently $CaCl_2$ (0.005 M), NaN_3 (25 mg L^{-1}) and 1 mg L^{-1} of the PAHs solution (each compounds in methanol) were added. The individual cell wall components ranging from 0.005 g to 0.25 g were transferred to the flasks to allow 30–70% of the added solute to be adsorbed at equilibrium. All flasks were shaken upright for 24 h using orbital shakers at 130 rpm in a constant temperature of 25 °C.

Adsorption of PAHs to lipids was determined in a different way. About 0.2 g of extracted lipids was dissolved by 2 mL of hexane as stock solution. Subsequently 1–2 μL of lipids solution was transferred to 25 mL Erlenmeyer flasks described above with the same liquid condition. Hexane was evaporated under a gentle stream of nitrogen to a constant weight. During the evaporation of the hexane, the lipids formed an adhesive thin film on the inner surface of flasks. Flasks were kept undisturbed for 72 h during the sorption experiment to avoid dislodging the lipid film.

2.6. Analysis

After equilibration, solvent and cell wall components were firstly separated with centrifugation. Then 10 mL solvent was filtered through a 0.22 μm polyethersulfone filter membrane and transferred to a 50 mL centrifugal tube. 10 μL 1 mg/mL internal standard Acenaphthene-D10 and 10 mL hexane were added and