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Differences of cell surface characteristics between the bacterium *Pseudomonas veronii* and fungus *Ophiostoma stenoceras* and their different adsorption properties to hydrophobic organic compounds

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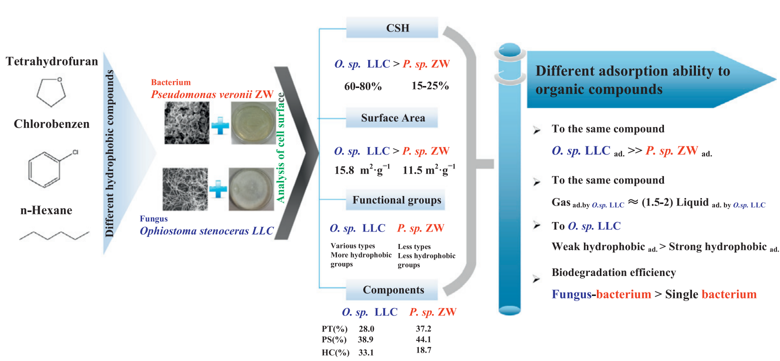
HIGHLIGHTS

- *Ophiostoma stenoceras* exhibits higher adsorption capacity than *Pseudomonas veronii.*
- Adsorption of organic compounds by *O. stenoceras* is a physical monolayer adsorption.
- Phase partition reveals that gaseous compounds are favorably adsorbed by *O. stenoceras*.
- BET and XPS analyses show *O. stenoceras* has more surface area and adsorption sites.
- Fungal-bacterial symbiotic system needs less time for degradation of organic compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

The first step of microbial biodegradation is the adsorption of pollutants on the microorganisms' surface, which is determined by the microorganism type and pollutant hydrophobicity. One fungus *Ophiostoma stenoceras* LLC and one bacterium *Pseudomonas veronii* ZW were chosen for the investigation of cell surface hydrophobicity and adsorption abilities to various organic compounds. Results showed that the fungus could better capture and adsorb organic compounds in liquid and gas phases, and the adsorption was a physical monolayer adsorption process. Much smaller partition coefficient for gas-fungus suggested that direct gaseous adsorption was preferred. The XPS (X-ray photoelectron spectroscopy) characterization further confirmed that several functional groups changed after the adsorption of compounds. The time taken for complete degradation of hexane, tetrahydrofuran and chlorobenzene was shorter with the addition of *O. stenoceras* LLC. Such findings are useful in exploring the special cell surface of fungus in adsorption and bioenhancement for organic treatment of organic contaminants using bacteria.

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1. Introduction

Most volatile organic compounds (VOCs) (including α-pinene) are raw materials for various industries (e.g., paper, pharmaceutical and

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paint industry), and the gaseous emissions usually contain these VOCs, which cause a major environmental problem. Although the traditional technologies (such as incineration, catalytic oxidation, adsorption and absorption) could achieve efficient treatments for VOCs, they may be expensive, especially with the purpose of complete purification. On the other hand, biopurification is a low cost and environmentally friendly process, and has received increasing interests among researchers (Rene et al., 2015; Schiavon et al., 2016; Zhang et al., 2018).

As the core material of the biopurification system, the type of microorganism determines the removal rate of VOCs. Bacteria are the most commonly used microorganisms in the biopurification systems. However, when dealing with hydrophobic VOCs, the performance of bacteria is relatively low. This is attributed to the lower cell surface hydrophobicity (CSH) of bacteria and more humid environment around the bacteria. Both of them inhibit the availability of VOCs to the bacteria (Lebrero et al., 2010). Compared with bacteria, fungi have better cell surface characteristics and better adaptabilities under undesirable conditions (dry, weak acid, etc.), making them more promising for the treatment of hydrophobic VOCs (Y. Cheng et al., 2016; Z.W. Cheng et al., 2016). Several studies have demonstrated that the fungi-dominated biofilter had better removal for hydrophobic VOCs, such as styrene (Rene et al., 2010), toluene (Estrada et al., 2013), hexane (Saucedo-Lucero et al., 2014). α-Pinene is a typical moderately hydrophilic compound (with the Henry coefficient of 0.271, dimensionless) (Y. Cheng et al., 2016; Z.W. Cheng et al., 2016), and mostly selected as the model compound for microbial biodegradation and biofiltration (Miller and Allen, 2005; Bagherpour et al., 2005; Jin et al., 2007; Cheng et al., 2011). There are several reported microorganisms, which have the ability to biodegrade α-pinene, such as *Bacillus pallidus* BR425 (Savithiry et al., 1998), *Aspergillus niger* (Agrawal and Joseph, 2000), *Pseudomonas veronii* ZW (Cheng et al., 2011), *Ophiostoma* sp. (Jin et al., 2006). The reported critical elimination capacities by the fungus *Ophiostoma* sp. dominated-biofilters was 143 g m^{-3} h^{-1} (Jin et al., 2006), much higher than the one by the bacterium *Pseudomonas veronii* dominated-biofilter (only 44 g m^{-3} h^{-1}) (Chen and Ding, 2012). These differences suggested that the fungus has better ability to biodegrade α-pinene.

Filamentous fungi, which have a mycelium characterized by large specific surface area, can easily capture substrates in liquid phase (Vergara-Fernández et al., 2016). In addition, owing to some functional groups distributed on the cell surface, fungi can directly adsorb substrates from the gas phase (Rene et al., 2012). There are also some disadvantages of fungal treatments, such as the slow initial growth, which can extend the start-up period of reactors, as well as the large accumulation of mycelium, which can lead to the blockage of packing material and a high pressure drop (Vergara-Fernández et al., 2012). In addition, some organic compounds, which are toxic to fungi, can be converted to intermediates by special bacteria, and then become the carbon sources for fungal growth (Wang et al., 2012).

Most microbial degradation occurs when the substrates are adsorbed by microbial cell surfaces (Z.W. Cheng et al., 2017; M. Cheng et al., 2017). Marx and Aitken (2000) showed that the bacterial chemotaxis (the ability to sense and move along the chemical concentration gradients) was important for their biodegradation, especially when they utilize hydrophobic organic compounds. As bacteria require continuous liquid films for their movements, directed chemotactic dispersion is restricted in relative dry environment (Or et al., 2007). In some bioreactors for gaseous pollutant treatments, the lack of continuous liquid distribution may affect the bioaccessibility of gaseous substrates to bacteria, because the distance between bacteria and gaseous substrates is higher than the value required by them. Unlike bacteria, fungi can easily stretch their hyphae through gas–liquid interfaces to form dense networks, connecting the gas phase and themselves with a shorter substrate transfer path (Patel et al., 2017; Rodriguez et al., 2017). Other research studies also showed that fungal hypha was a better micro-environment for the transport of nutrients and diffusion of gaseous substrates to bacteria (Bending et al., 2006; Vergara-Fernández et al., 2016). Therefore, the symbiotic system of bacteria and fungi for the treatment of pollutants is a novel and efficient bioremediation process. Several studies have shown that the removal performances for low-solubility VOCs by the symbiotic systems were much better than those by the single bacterial or fungal ones (Jin et al., 2007; Estrada et al., 2013; Y. Cheng et al., 2016; Z.W. Cheng et al., 2016).

As both fungi and bacteria are major degraders of organic pollutants, the basic understanding of their interactions is most important for the development of such a new symbiotic system. However, most studies focused only on the removal performance and microbial community structure analysis for the fungal-bacterial system. Others used fungal hyphae loaded on nanoparticles as materials for adsorption of organic compounds without enough attention being paid to the fungus' adsorption (Zhang et al., 2014; Wang et al., 2015). The reasons behind the enhanced mass transfer by fungal hyphae have not been adequately investigated, and the differences of cell surface between fungi and bacteria have also not yet been discussed.

This paper reports, for the first time, the differences in cell surfaces between the fungus and bacterium and cell adsorption to different hydrophobic organic compounds. The fungus *Ophiostoma stenoceras* LLC and the bacterium *Pseudomonas veronii* ZW, both of which can use α-pinene for their growths, were chosen as the microorganisms to study the synergistic process. Based on their different CSHs, their adsorption characteristics to various hydrophobic compounds were investigated, both in liquid and gas phases. Brunauer-Emmett-Teller (BET) method was used to get information about the specific surface area and pore size distribution of fungal or bacterial cells. X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR) techniques were employed to analyze the element composition and functional groups on their surfaces. Based on these observations, the enhanced biodegradation of three hydrophobic compounds by the addition of the fungus *O. stenoceras* LLC was also investigated. Such results lay foundations for further research on fungi as adsorption materials or transporters of organic compounds to bacteria, providing a more efficient symbiotic system for pollutant removal.

2. Materials and methods

2.1. Strains and its medium

One fungus, *O. stenoceras* LLC, and four bacteria, namely *P. veronii* ZW, *Pseudomonas oleovorans* DT4, *Pseudomonas mendocina* NX-1 and *Ralstonia pickettii* L2, which are isolated by our groups, are being stored in the China Center for Type Culture Collection (CCTCC) under NO. M 208182, M 2015114, M 209250, M 209151 and M 2012235, respectively. The strains *O. stenoceras* LLC and *P. veronii* ZW, both having the ability to degrade α-pinene, were chosen for the cell surface analysis and adsorption investigation; while other three bacteria (without the ability to degrade α-pinene) were used for biodegradation enhancement tests only. The fungus and bacteria were enriched in sterilized (394 K, 20 min) Potato–Dextrose Agar (PDA) medium and Luria–Bertani (LB) medium, respectively. Another mineral inorganic medium used for batch biodegradation experiments was sterilized at 383 K for 40 min before being used (Y. Cheng et al., 2016; Z.W. Cheng et al., 2016).

2.2. Adsorption to organic compounds in liquid or gas phase

Six organic compounds, with different solubility coefficients and polarity indexes (shown in Supplementary Material Table S1), were selected for the measurement of adsorption ability by these two microbial cells. For liquid adsorption, the same mass of *O. stenoceras* LLC or *P. veronii* ZW was added into the bottles containing 100 mL deionized water and one selected organic compound (100 mg·L^{-1}). All the bottles were sealed and oscillated at 303 K. The concentration of each organic compound in the headspace was measured at designed time intervals. Their concentrations in the liquid phase were calculated

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