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Biosorption of pentachlorophenol by *Anthracophyllum discolor* in the form of live fungal pellets

Research Paper

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Pentachlorophenol (PCP) is an extremely dangerous pollutant for every ecosystem. In this study we have detected how PCP concentration and pH levels can influence PCP adsorption by *Anthracophyllum discolor* in the form of live fungal pellets. PCP adsorption was evaluated after 24 hours in KCl 0.1 M electrolyte solution with initial PCP concentrations of 5 and 10 mg L^{-1} and with pH values between 4 and 9 (at intervals of 0.5). Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups of fungal biomass that can interact with PCP. The amount of PCP that was adsorbed by *A. discolor* was >80% at pH values between 5 and 5.5, whatever the concentration tested. PCP adsorption significantly decreased in liquid medium of pH > 6.0. FTIR results showed that amides, alkanes, carboxylates, carboxyl and hydroxyl groups may be important to the PCP adsorption for pellets of *A. discolor*. Live fungal pellets of *A. discolor* may be used as a natural biosorbent for liquid solutions contaminated by PCP.

Introduction

Pentachlorophenol (PCP) is considered to be one of the most hazardous contaminants of soil and water. It was widely used for many years in different areas of the world as a low cost biocide [1]. An economic method to remove PCP is the biological treatment described as microbial degradation [2–7] and adsorption [8,9].

Removal of PCP by adsorption of live or dead microorganisms uses the biomass capacity as an effective adsorbent for the particular attraction between the cellular component and the pollutant compound. The use of microorganisms for PCP adsorption is increasing because it offers an economical, practical and efficient alternative to the commonly used methods [8,9]. Recent literature has shown many microorganisms have proven performance in PCP adsorption. PCP adsorption by the non-viable biomass of *Aspergillus niger* was pH-dependent [10]. Moreover, the biomass of *A. niger* treated with cetyltrimethylammonium showed a 100% removal at 1 mg PCP L^{-1} at all pH levels from aqueous solutions. Brandt *et al.* [11] detected that *Mycobacterium chlorophenolicum* can effectively be used for PCP adsorption. Jianlong *et al.* [12] obtained improved results in regard to the biosorption ability of PCP by the microbial biomass in aqueous solution. Fungi such as *Phanerochaete chrysosporium, Trametes versicolor, Ganoderma lobatum* and several other species were used in experimental PCP adsorption using dead biomass [13]. All species showed a good adsorption capacity at acidic pH levels. Rubilar *et al.* [14] also used dead biomass from the white-rot fungus *Anthracophyllum discolor* in a fixed-bed column reactor for PCP adsorption at different concentrations and pH values. They concluded that *A. discolor* dead biomass was an effective adsorbent of PCP at acid pH values.

Almost all studies of pollutant adsorption using biomass are developed with dead biomass. This happens because dead microbial cells avoid toxicity concerns and do not require growth media or nutrients. Moreover, this biomass can be stored for a long time

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and can be used in several cycles of adsorption and desorption of the pollutant compound. However, live biomass has, in many cases, shown better performance in pollutant adsorption. Adsorption and biodegradation processes of live microorganisms can be used together to aid contaminant removal [13,15,16]. Furthermore, there are few studies that are focused on the effect of pH levels and PCP concentrations on adsorption capacity from living fungal biomass.

The principal objective of the present study was to evaluate the adsorptive capacity of *A. discolor* in the form of live fungal pellets in a liquid medium which can be affected by different pH values and initial PCP concentrations. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out on *A. discolor* biomass to determine functional groups that are active during PCP adsorption.

Materials and methods

Fungal strain and cultivation conditions

A. discolor is a white-rot fungus isolated from decayed wood in the temperate forest of southern Chile [17]. The strain was stored on malt extract agar (MEA) (15 g L^{-1} agar; 3.5 g L^{-1} malt extract; 10 g L^{-1} glucose) in slant tubes at 4°C in the Environmental Biotechnology Laboratories of the Universidad de La Frontera (Temuco, Chile).

Preparation of the fungal pellets

Seven plugs (diameter 6 mm) of *A. discolor* mycelium cultured on MEA for 7 days in Petri dishes, were placed in a flask which contained 100 ml of modified Kirk medium (10 g L^{-1} glucose; 2 g L^{-1} peptone; 2 g L^{-1} KH_2PO_4; 0.5 g L^{-1} $MgSO_4$; 0.1 g L^{-1} $CaCl_2$; 3.3 g L^{-1} sodium acetate; 5 ml L^{-1} Tween 80 to 10%; 2.11 ml L^{-1} $MnSO_4$) and incubated at 25°C for 7 days. The fungal mycelium in the culture broth was homogenized with a blender for 2 min to prepare the inoculum. Finally, 2 ml of inocula (1.5 mg L^{-1} fungus dry weight) were placed in the flasks which contained 100 ml of modified Kirk medium and incubated at 25°C on a rotating shaker incubator at 120 r.p.m. for 10 days in order to obtain the pellets. The formed pellets were washed with distilled water and stored in an empty falcon at 25°C for 3 days.

Potentiometric titration of A. discolor cell wall

Surface charge behavior was assessed by acid–base titration in a N_2 atmosphere using 0.01 N NaOH and 0.01 N HCl. An amount of 20 *A. discolor* pellets (20 ± 0.2 mg fungus dry weight) were added to two Teflon vessels containing 100 ml of 0.001, 0.01 or 0.1 M KCl as a background solutions. The titrations were carried out by adding 200 μl of titrant after 15 min reaction time between additions to allow stabilization of the pH. To measure the pH levels was used the pH meter Thermo Scientific Orion 3-Star. This instrument accurately measure pH to 2 decimal points. The experiments were carried out at 20°C in rooms with permanent air conditioning. Finally, the surface charge behavior of the fungus was evaluated in the presence of PCP at two concentrations (5 and 10 mg L^{-1}) using KCl 0.1 M as a background electrolyte.

PCP adsorption

PCP adsorption experiments were performed in 10 ml amber glass bottles to determine the adsorptive capacity of pellets as a function of pH level. In each bottle, 5 ml of 0.1 M KCl (as background electrolyte) was added with either of the two PCP concentrations (5 and 10 mg L^{-1}) and 20 pellets of *A. discolor*. The pH levels of the solutions were adjusted with HCl or NaOH (0.1 M) and stabilized from a level of 4 to a level of 9 at intervals of 0.5 unit of pH. The control was carried out in the same procedure described previously only without the addition of PCP. The bottles were incubated at 25°C on a rotating shaker incubator at 120 r.p.m. for 24 h in darkness to avoid PCP photodegradation. The assays were conducted in triplicate. After incubation the samples were analyzed for pH levels. The amount of PCP adsorbed was determined by the difference of PCP added and the residual PCP present in the solution after 24 h. An aliquot (1 ml) of the liquid phase was filtered with the Syringe Filters Chromotech (pore size 0.45 μm; filter size 25 mm) and analyzed by using High Performance Liquid Chromatography (Hitachi Lachrom Elite) by way of a Chromolith RP-18e, 100 mm × 4.6 mm column. The injection volume was 20 μL. The mobile phase consisted of acetonitrile and phosphoric acid (1% aqueous solution) 1:1 (v/v) with a flow rate of 1 mL min^{-1}. Instrument calibration and quantifications were performed against the pure reference standard (5 and 10 mg L^{-1}). The detector wavelength was set at 215 nm with a retention time of 4.5 min.

FTIR analysis

The analysis of the fungal pellets were performed by FTIR only after adsorption of PCP. Briefly, 20 *A. discolor* pellets were added to a flask with 100 ml of 0.1 M KCl and 10 mg L^{-1} PCP. A control solution without PCP was obtained. The flasks were incubated at 25°C on a rotating shaker incubator at 120 r.p.m. for 24 h. The fungal biomass was separated from the KCl medium using filter paper (Whatman no. 1), washed with distilled water and dried in an oven at 30°C for 24 h. Samples of 1 mg were mixed with 100 mg of KBr. The FTIR spectrum was determined using a Bruker Tensor 27 with the following parameters: resolution 2 cm^{-1}; 32 scans min^{-1}; transmittance spectrum from 4000 cm^{-1} to 500 cm^{-1}; open setting 6 mm; scanner rate 10 kHz.

Statistical analysis

Each experiment was conducted in triplicate and the mean value is represented by one data point in the figures. All data were subjected to ANOVA by using XLSTAT 2013.1 for Windows. The assumptions of normality and homogeneity of the variants were tested by the Kolmogoroff–Smirnoff method. The significant differences between means of $P < 0.05$ were assessed in accordance with Tukey's multiple comparison test.

Results and discussion

Potentiometric titration

The potentiometric titration curve (Fig. 1) showed that as the pH level of the solution increased, the net surface charge became more negative, as previously demonstrated [10,18]. At a pH > 7.0, the net charge of the fungal wall was negative. The point at which the line representing different ionic strengths intersect is referred to as the point of zero salt effect (PZSE). The potentiometric titration curve of *A. discolor* pellets, for all KCl solutions, showed pronounced flexes, as in [19]. This allowed us to locate the area of