Chlorophenol degradation in soil columns inoculated with Anthracophyllum discolor immobilized on wheat grains

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Abstract

The white-rot fungus Anthracophyllum discolor immobilized on wheat grains was evaluated for chlorophenol (2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol) degradation in allophonic soil columns present in kraft mill wastewater (Navia et al., 2003, 2005; Diez et al., 2005). However, no information is available regarding the biodegradation of chlorophenols by immobilized white-rot fungi in a fixed-bed column packed with allophonic soil. In inoculated soil columns, 2,4-dichlorophenol was highly degraded and this degradation is associated with a high production of manganese peroxidase. 2,4,6-trichlorophenol was degraded to a lesser extent compared with 2,4-dichlorophenol. Pentachlorophenol was first removed by adsorption and then through degradation by the fungus. Manganese peroxidase activity was lowest when the column was fed with pentachlorophenol and highest when the column was fed with 2,4-dichlorophenol. Laccase was also produced by the fungus but to a lesser degree.

Keywords:
Anthracophyllum discolor
Chlorophenols
Soil columns
Biodegradation

1. Introduction

The use of soil or natural adsorbents for the removal of organic compounds from contaminated wastewater is considered a beneficial method, as in this system the contaminants can be removed by both adsorption and degradation processes (Kookana and Rogers, 1995; Lin and Juang, 2009; Uddin et al., 2009). Taking into account both processes into account, adsorption and degradation processes, in inoculated soil columns, 2,4-dichlorophenol was highly degraded and this degradation is associated with a high production of manganese peroxidase. 2,4,6-trichlorophenol was degraded to a lesser extent compared with 2,4-dichlorophenol. Pentachlorophenol was first removed by adsorption and then through degradation by the fungus. Manganese peroxidase activity was lowest when the column was fed with pentachlorophenol and highest when the column was fed with 2,4-dichlorophenol. Laccase was also produced by the fungus but to a lesser degree.

(Kookana and Rogers, 1995; Diez et al., 2005; Cea et al., 2007). In a pH-dependent variable surface charge soil, Diez et al. (1999) demonstrated that phenolic compounds adsorption increased as the pH decreased, as a result of electrostatic repulsion between the compounds and the resulting negative surface charge.

White-rot fungi are microorganisms with a well-known capacity for degrading a wide range of organic compounds, attributed to their extracellular enzymatic system conformed by lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) involved in the degradation of lignin compounds. Phanerochaete chrysosporium and Trametes versicolor have been the most widely used fungi for chlorophenol degradation. However, several studies have been performed to evaluate new fungal strains with a high ability for degrading recalcitrant organic compounds (Levin et al., 2004; Tortella et al., 2008), new technological processes such as degradation of pentachlorophenol in soil slurry cultures by Bjerkandera adusta and Anthracophyllum discolor (Rubilar et al., 2007) and biodegradation of 2,4-dichlorophenol in columns packed with immobilized P. chrysosporium (Wu and Yu, 2008). Tortella et al. (2008) characterized several Chilean native wood-rotting fungi, and showed that the selected strains presented high lignin peroxidase (LiP) and manganese peroxidase (MnP) activity. Their ability to degrade 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and PCP especially by the fungus A. discolor (Sp4) was also shown.
Several aspects of white-rot fungi degradation properties have been reviewed recently (Tortella et al., 2005; Gianfreda and Rao, 2008; Rubilar et al., 2008). The application of white-rot fungi in a fixed-bed column has not been investigated in any depth (Wu and Yu, 2008), in spite of the fact that they can degrade some complex substances which are beyond the metabolic abilities of bacteria. The main purpose of this study was to evaluate chlorophenol degradation in columns packed with allophanic soil and inoculated with the white-rot fungus A. discolor immobilized on wheat grains.

2. Materials and methods

2.1. Column preparation

Glass columns (35 cm long with 5 cm internal diameter) packed with a mixture of quartz sand and allophanic soil (1:1) were tested for 2,4-DCP, 2,4,6-TCP and PCP adsorption. The allophanic soil used was an Andisol, belonging to the Temuco Series, located in southern Chile. The soil sample was taken from 0 to 20 cm depth, air dried at room temperature and sieved through a 2 mm mesh. The allophanic soil used has a pH (w) of 5.9 and 14.6% organic matter (Cea et al., 2007). The quartz sand was washed with distilled water, dried at 100 °C and stored in a vacuum desiccator prior to use. Columns were packed with 300 g of total mass (bulk density (p) was 1.26 g/mL) and were pre-conditioned to pH 4.5 by eluting the columns with H2SO4 0.1 M so as to activate the soil surface (Diez et al., 1999). The columns were covered with aluminium foil to avoid oxidation processes.

2.2. Fungus immobilization

A white-rot fungus A. discolor, isolated from decayed wood in the rain forest of southern Chile (Tortella et al., 2008) was used in this study. The fungus was transferred from slant tubes (maintained at 4 °C) to glucose malt extract agar plates (15 g/L agar, 3.5 g/L malt extract, and 10 g/L glucose) and kept at 30 °C for 5–7 days before its use as inoculum. To immobilize the fungus, 30 g of wheat grains were put in a flask of 250 mL and moistened with 30 mL of distilled water and sterilized at 121 °C for 15 min. Then, the flask was inoculated with 5 agar disks (6 mm in diameter) of active mycelia of A. discolor from 5-day-old cultures on LBM medium (Tortella et al., 2008) and put in darkness at 25 °C for 6 days approximately (or until the mycelia covered completely the wheat grains). To evaluate the degree of immobilization, samples were analyzed using scanning electron microscope JEOL JSM-6380LV.

2.3. Columns operation

Columns were inoculated with white-rot fungus A. discolor previously immobilized on wheat grains. The colonized wheat grains were placed on the upper part of each column (5 cm). The columns were operated in continuous systems and were fed with the respective chlorophenol (100 mg/L) at a flow rate of 1.5 mL/min, at room temperature for approximately 29 days (until saturation point C/Co = 0.7). Samples were taken from the effluent of the columns throughout the time and analyzed for phenols (2,4-DCP, 2,4,6-TCP, PCP), manganese peroxidase (MnP) and laccase (Lac) enzymes. All the experiments were carried out in duplicate and the average values were used for further calculations. Columns without inoculation with A. discolor were used as the control to evaluate the adsorption capacity of the soil columns. The evaluation of the column performance was conducted by plotting chlorophenol concentration in effluent to chlorophenol concentration in influent (C/Co) as a function of flow time (min).

3. Results and discussion

3.1. Chlorophenol breakthrough curves without inoculation

Fig. 1 shows chlorophenol (2,4-DCP, 2,4,6-TCP and PCP) adsorption breakthrough curves in soil columns (pH 4.5) without A. discolor inoculation. The soil column fed with 2,4-DCP solution was rapidly saturated (90 h), showing a lower adsorption capacity for this contaminant; by contrast, the columns fed with 2,4,6-TCP and PCP were saturated after 160 and 250 h, respectively. In Fig. 1, it is clearly stated that the total area under the PCP breakthrough curve is much higher than those of 2,4-DCP and 2,4,6-TCP, suggesting a higher affinity of PCP to allophanic soil under these experimental conditions.
conditions. Indeed, under the experimental pH condition (4.5), PCP should be present at about 50% in anionic form, and likely adsorbed completely (Diez et al., 1999). This is not the case for 2,4-DCP and 2,4,6-TCP pollutants, which are present in their non-ionic form at pH 4.5, leaching through the column and decreasing their adsorption.

Previous studies have demonstrated that the presence of MnP and laccase enzyme activity in columns packed with allophanic soil without inoculation is not significant (Diez et al., 2006), suggesting that the removal of chlorophenols under these conditions may be associated mainly with adsorption processes.

PCP presents a high affinity with soil organic matter (14.6% in the allophanic soil used in this study), associated with its log \( K_{\text{ow}} \) value of 5.01 compared to the 2,4,6-TCP \( K_{\text{ow}} \) value of 3.08. The \( pK_a \) values of PCP, 2,4,6-TCP and 2,4-DCP are 4.75, 6.15 and 7.85, respectively, with their adsorption being strongly affected by soil pH in allophanic soil (Diez et al., 1999; Cea et al., 2007).

Chlorophenol adsorption capacity of allophanic soil in batch processes has been studied under different operational and environmental conditions (Diez et al., 1999, 2005; Cea et al., 2005; Cea et al., 2007), and it has been demonstrated that its use is technically feasible with a high removal efficiency. Cea et al. (2007) described the adsorption capacity of this allophanic soil in three depths for 2,4-DCP and PCP, showing that PCP adsorption was higher than that observed for 2,4-DCP, and it decreased as organic matter fell with soil depth. The multiple regression analysis between \( K_d \) and various soil properties showed that soil organic carbon content is a strong indicator of chlorophenol adsorption. In addition to organic carbon, pH is an important parameter controlling adsorption behavior (Cea et al., 2007). Columns assays using this allophanic soil for chlorophenol removal from contaminated wastewater have also been reported (Navia et al., 2003, 2005, 2006), and the operational conditions, the irrigation model, and the kinetic parameters have been established, leading to the conclusion that the adsorption rates are comparable to other adsorption systems and adsorbent materials.

3.2. Chlorophenol breakthrough curves with inoculation

Fig. 2 shows chlorophenol (2,4-DCP, 2,4,6-TCP and PCP) breakthrough curves in soil columns (pH 4.5) inoculated with \( A. \ discolor \) immobilized on wheat grains. The adsorption of the chlorophenols into the \( A. \ discolor \) mycelium was about 2%; therefore, it was not considered in the evaluation. In general, it can be observed that the operational time of the inoculated columns increased compared to the non-inoculated columns, showing the following trend: 2,4-DCP > PCP > 2,4,6-TCP. The 2,4-DCP breakthrough curve shows high degradation of the contaminant compared with the 2,4-DCP breakthrough curve in the soil column without inoculation (Fig. 1). When using the inoculated column, 2,4-DCP degradation was constant between 100 and 320 h with \( C/C_0 \) of approximately 0.2 in this period of time. Then, the \( C/C_0 \) ratio increased until complete saturation of the column after 600 h of operation. The high 2,4-DCP removal was associated with degradation processes and with the high production of the ligninolytic enzyme manganese peroxidase (MnP) produced by fungus \( A. \ discolor \) (Fig. 3). MnP activity increased during the operation of the column, with the maximum value of 70 U/L being attained after 280 h.

The 2,4,6-TCP breakthrough curve in the column inoculated with the fungus \( A. \ discolor \) shows degradation of the contaminant (Fig. 3), but to a lesser extent than 2,4-DCP. The removal of 2,4,6-TCP was higher compared with the 2,4,6-TCP retention in the soil column without inoculation (Fig. 1). 2,4,6-TCP degradation was almost constant between 80 and 185 h with \( C/C_0 \) of approximately 0.4 in this period. Then, the \( C/C_0 \) ratio increased until complete saturation of the column after 270 h of operation. 2,4,6-TCP degradation using the inoculated column was associated with the production of the ligninolytic enzyme manganese peroxidase (MnP) produced by the fungus \( A. \ discolor \). MnP activity increased during the operation of the column, with the highest value of 50 U/L being attained after 150 h.

The breakthrough curves of PCP in both columns (with and without inoculation) were similar until 150 h of operation (Figs. 1 and 2), indicating that the adsorption process for PCP removal is predominant in this period. After 150 h, PCP was degraded in the inoculated column, and the degradation remained constant between 150 and 350 h (\( C/C_0 \) of 0.4). The total saturation of this column was obtained after 420 h of operation. The degradation of PCP was associated with MnP production by the fungus during the column operation (Fig. 3). MnP activity increased during the operation of the column, with the maximum value of 30 U/L being attained after 300 h.

The enzyme activity of manganese peroxidase (MnP) and laccase during the operation of the inoculated columns are shown in Figs. 3 and 4. In general, it can be observed that MnP activity was
higher (up to 70 U/L) when the column was fed with 2,4-DCP and lower (up to 30 U L⁻¹) when the column was fed with PCP. However, the highest MnP activity was obtained during the constant degradation period for the three chlorophenols. Laccase was also produced by the fungus but to a lesser extent, reaching less than 10 U/L values for the three chlorophenols.

The degradation capacity of white-rot fungi has been attributed to enzyme ligninolytic activity (Gianfreda and Rao, 2008). In our work, the highest MnP activity was obtained in the column fed with 2,4-DCP and was lower when the column was fed with PCP. This addition inhibited MnP production by A. discolor in the soil column; however, its degradation was not affected (Fig. 2). These results agree with the results obtained by Rubilar et al. (2007). The authors reported that the PCP degradation capacity of A. discolor was not affected when MnP activity decreased in a soil slurry culture, and that MnP activity was negatively affected (up to 75% reduction) when initial PCP concentrations were increased from 100 to 250 mg/kg of soil. The growth and colonization of wheat grains promoted chlorophenol degradation in the allophanic soil columns, coupled with ligninolytic enzyme production. The growth and colonization of wheat grains by A. discolor completely covered the lignocellulosic support after 7 days of incubation. The effect caused by wheat grains on fungus growth is probably due to their high content of carbohydrates and starch, which are not present in the other supports, and it provides a major source of energy for the fungus.

4. Conclusions

Chlorophenols were removed using allophanic soil columns with and without A. discolor inoculation by both adsorption and degradation processes. Chlorophenol degradation increased in allophanic soil columns inoculated with the fungus A. discolor immobilized on wheat grains, increasing the capacity of allophanic soil columns to adsorb and eliminate these contaminants. Equilibrium sorption capacity and retention time increased when inoculated soil columns were used. The higher degradation capacity of the inoculated soil columns was associated with the presence of the ligninolytic enzymes manganese peroxidase and laccase.

Acknowledgements

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References


Table 1

<table>
<thead>
<tr>
<th>Column</th>
<th>Chlorophenol</th>
<th>qₑₑₑ (mg/g)</th>
<th>tₛ (h)</th>
<th>R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non inoculated</td>
<td>2,4-DCP</td>
<td>1.69</td>
<td>90</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>2,4,6-TCP</td>
<td>3.23</td>
<td>160</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>PCP</td>
<td>9.46</td>
<td>250</td>
<td>61.2</td>
</tr>
<tr>
<td>Inoculated</td>
<td>2,4-DCP</td>
<td>20.25</td>
<td>600</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>2,4,6-TCP</td>
<td>8.09</td>
<td>270</td>
<td>52.1</td>
</tr>
<tr>
<td></td>
<td>PCP</td>
<td>16.5</td>
<td>420</td>
<td>46.2</td>
</tr>
</tbody>
</table>

$q_{eq}$ = equilibrium sorption capacity.

$t_{sp}$ = saturation time.

R = total removal efficiency.

Fig. 4. Laccase activity in soil column (pH 4.5) inoculated with A. discolor.

show that Wu and Yu (2008) studied the biosorption of 2,4-DCP from aqueous solutions by immobilized P. chrysosporium biomass in a fixed-bed column. The authors found values of $q_{eq}$ between 5.2 and 12.2 mg/g and, total removal capacity between 22.3 and 72.6 (%) depending on the flow rate (1.0–3.0 mL/min), influent concentration (20.9–80 mg/L) and bed depth (15–26 cm) used in their study.

The use of A. discolor immobilized on wheat grains promoted chlorophenol degradation in the allophanic soil columns, coupled with ligninolytic enzyme production. The growth and colonization of wheat grains by A. discolor completely covered the lignocellulosic support after 7 days of incubation. The effect caused by wheat grains on fungus growth is probably due to their high content of carbohydrates and starch, which are not present in the other supports, and it provides a major source of energy for the fungus.


