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Degradation of pentachlorophenol in soil by *Phanerochaete chrysosporium*

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Abstract

Biodegradation of pentachlorophenol (PCP) by *Phanerochaete chrysosporium* was examined in nonsterile soil. The rate of mineralization of PCP was essentially linear for at least 27 d and increased almost linearly with increasing concentration of PCP (from 50 to 1600 ppm). At 100 ppm no PCP was found at 18 d while 40% of the added PCP was present as pentachloroanisole (PCA), and mineralization continued linearly. Both PCP and PCA were found after 18 d when the initial concentration of PCP was 800 ppm. The rate of mineralization of PCA also increased with increasing concentrations of PCA, however the increase was not linear. Essentially no radioactivity was found in either the aqueous or volatile organic fraction during the mineralization of either PCP or PCA.

1. Introduction

Phanerochaete chrysosporium, a white rot fungus, has the ability to degrade a variety of structurally diverse organopollutants, including a number of haloorganics. This ability of the fungus is dependent, at least in part, on the nonspecific and nonstereoselective lignin-degrading system which is induced by growing the fungus under nutrient-limiting conditions [1, 2]. The ability of the fungus to degrade a wide variety of organopollutants has led to the study of this fungus for bioremediation of contaminated groundwater and soil [1, 2]. Pentachlorophenol (PCP) has been used extensively as a fungicide, insecticide, and herbicide [3]. The accidental spillage and inappropriate disposal at many wood treatment facilities, along with its relative stability in the environment, has resulted in the contamination of terrestrial and aquatic ecosystems [4–6]. This has led the US Environmental Protection Agency to classify PCP as a priority pollutant.

Information on the ability of *P. chrysosporium* to mineralize and transform organopollutants to innocuous products might be necessary before the fungus is used for

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bioremediation in soil. Although there are some studies on degradation of PCP by *P. chrysosporium* [4, 7, 8], there is no systematic study on the effect of PCP concentration on its biodegradation in soil. A mass balance study may be important to get information of the fate of PCP during its degradation in soil by the fungus. Here we described the effect of PCP concentration on its biodegradation in soil.

2. Materials and methods

2.1. Reagents

PCP was obtained from Sigma Chemical Co. (St. Louis, MO). Pentachloroanisole (PCA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). [^{14}C]PCP, with a specific activity of 10.56 mCi/mmol and a purity of >98%, was obtained from Pathfinder Laboratories, Inc. (St. Louis, MO). [^{14}C]PCA was prepared by the reaction of [^{14}C]PCP with diazomethane in ether and purification by using thin-layer chromatography [9]. Ground corncobs were purchased from Mt. Polaski Products Inc. (Pine Bluff, AR). Other chemicals were of reagent grade.

2.2. Mass balance studies of PCP degradation

Ten grams of nonsterile soil (a silt loam) [10] was placed in Wheaton bottles (250 ml) equipped with gas exchange manifolds. Then, 22.2 nmol of [^{14}C]PCP and various concentrations of PCP dissolved in 100 µl of acetone were added to the soil, the acetone allowed to evaporate and the samples thoroughly mixed. The soil water potential of the cultures was adjusted to − 1.7 kPa by adding sterile deionized water. The fungus was added by adding 6.6 g of corncobs (moistened to 50% water) that had been previously inoculated with *P. chrysosporium* BKM-F-1767 [10, 11] and allowed to grow at 39 °C for 14 d. The incubation temperature for all experiments was 39 °C. The cultures were then flushed with pure oxygen every 3 d, and the radioactivity in the volatile organics and CO_2 evolved were determined as described in [10, 11].

2.3. Analytical procedures

For mass balance analyses, complete cultures were harvested at 18 d and extracted with hexane–acetone (1 : 1) as described in [10]. Radioactivity in the solvent, particulate, and aqueous fractions were determined by liquid scintillation spectrometry as described in [10]. Extracts were analyzed by gas chromatography-electron capture detection (a Varian model 3700 gas chromatography with a DB-5.625 fused silica capillary column from J & W Scientific, Folsom, CA) for quantification of PCP and PCA.

2.4. Mineralization of PCA in soil

Pentachloroanisole has been identified as a major extractable metabolite of PCP metabolism in soil by *P. chrysosporium* [7, 8]. Therefore the mineralization of PCA

was also examined. The cultures were prepared as described above. All cultures contained 22.2 nmol of [^{14}C]PCA and various concentrations of PCA. The mineralization of PCA was performed for 18 d as described above. The radioactivity in the volatile organics and CO_2 evolved was determined as described previously [10, 11].

3. Results and discussion

It has been known that PCP is toxic to microorganism since PCP is an inhibitor of oxidative phosphorylation [3, 4]. Thus it was expected that increasing concentrations of PCP might inhibit its mineralization by the fungus. To test the effect of PCP concentration on its own mineralization, initial PCP concentrations were varied from 50 to 1600 ppm. The mineralization of PCP continued almost linearly for 27 d (Fig. 1, shown at the initial concentrations of 100, 400 and 1600 ppm). The rate of mineralization of PCP increased almost linearly with increasing concentrations of PCP (from 50 to 1600 ppm), suggesting that the mineralization is subject to a first-order kinetics (Fig. 2). There was a trend for a decrease in the percentage mineralized at higher concentrations of PCP (Fig. 2). In liquid, the rate of PCP mineralization increased linearly up to about 14 ppm of PCP [8]. Table 1 shows the mass balance of PCP degradation by *P. chrysosporium* after 18 d at initial concentrations of 100 and 800 ppm. Other metabolites besides PCA were not observed. No PCP was found when the initial concentration was 100 ppm, and 40% of the original PCP was present as PCA. There was still a linear rate of mineralization beyond 18 d (Fig. 1), suggesting that the chemical being mineralized during this time was PCA. The effect of PCA concentration on its mineralization was examined at various initial concentrations of PCA. The rate of mineralization of PCA increased with increasing concentration of PCA (Fig. 3), however the increase was not linear. Thus the percentage of PCA

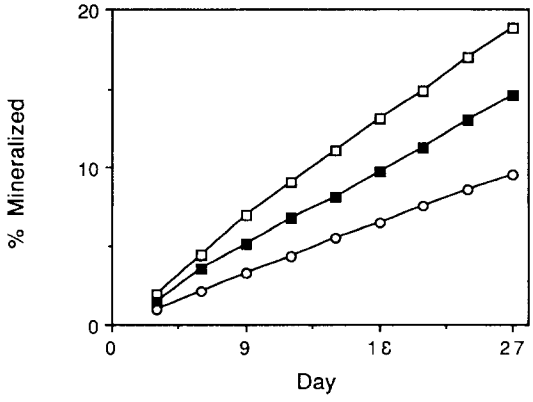

Fig. 1. Mineralization of PCP in nonsterile soil by *P. chrysosporium*. Mineralization are shown for 100 (□), 400 (■), and 1600 ppm (○) PCP. Values are the means for triplicate cultures. Standard deviations were generally about 25%.

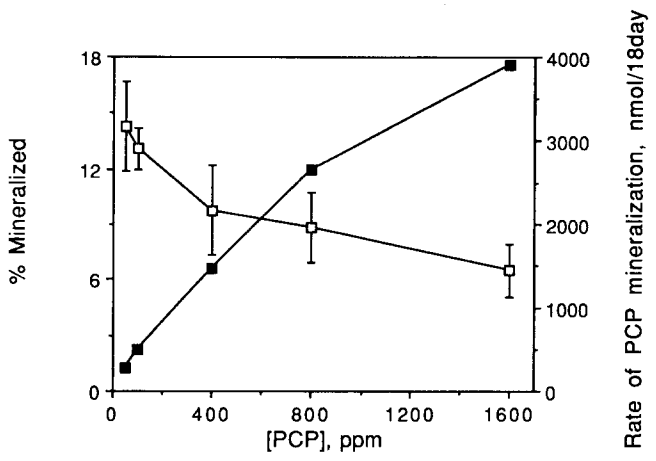

Fig. 2. Effect of PCP concentration on its rate of mineralization by *P. chrysosporium*. Various concentrations of PCP in nonsterile soil were incubated with *P. chrysosporium* and assayed for the percentage of the PCP mineralized (□) and the rate of PCP mineralization for 18 d (■). Values for the percent mineralized are the means ± standard deviations for triplicate cultures.

mineralized in 18 d varied from 9 ± 2% (100 ppm) to 2.4 ± 1.1% (1600 ppm). The nonextractable radioactivity (particulate) was 17 ± 1 and 12 ± 1% of the initial-concentrations of 100 and 800 ppm PCP, respectively (Table 1). These values were about half of what was observed in the particulate fraction in both controls. A very small amount of the radioactivity was found in the aqueous fractions.

These results demonstrate that *P. chrysosporium* is able to mineralize high concentrations of PCP and PCA, a major extractable metabolite of PCP, in soil. The percent mineralization can be increased by simply extending the incubation period and/or by increasing fungal cell mass. It has been shown that the fungal cell mass had a significant effect on the mineralization [8, 12]. Mineralization is the best objective of biodegradation. However, the first step of degradation of organopollutants may require detoxification. The methylation of PCP to PCA may be a detoxification mechanism, since PCA is not an inhibitor of oxidative phosphorylation and less toxic than PCP to wood-rotting fungi, other microbes, and fish [12]. The solubility of PCA (~ 0.2 ppm) is less than that of PCP (~ 25 ppm), thus preventing the contamination of groundwater [4, 13].

Incorporation of PCP or its metabolites into soil organic matter, which may explain the radioactivity in the particulate fraction, might be an abiotic process since it was also observed in the control with no microbial activity. Much less incorporation was found with the inoculated cultures. Hider and Martin demonstrated that chlorinated phenols were mineralized from hybrid polymers of humus and chlorophenols by *P. chrysosporium* at about the same rate as the carbon from the humus [14]. Essentially no radioactivity (below 0.05%) was found in the volatile organic trap suggesting that PCP, PCA, or other intermediates were not volatile. This and the very

Table 1
Mass balances for pentachlorophenol degradation by *P. chrysosporium* in nonsterile soil at day 18

Cultures	Concentration (ppm)	Percentage recovery	Percentage of total radioactivity added				
			Solvent		Particulate	Aqueous	$^{14}CO_2$
			PCP	PCA			
P. chrysosporium	100	70 ± 10	0	40 ± 8	17 ± 1	0.45 ± 0.07	13 ± 1
	800	75 ± 12	44 ± 5	10 ± 4	12 ± 1	0.22 ± 0.06	9 ± 2
Control A[a]	100	79 ± 12	30 ± 3	9 ± 3	40 ± 6	0.55 ± 0.13	0.87 ± 0.23
	800	74 ± 12	33 ± 1	9 ± 2	31 ± 8	0.65 ± 0.32	0.65 ± 0.07
Control B[b]	100	70 ± 11	36 ± 4	0.65 ± 0.13	33 ± 6	0.71 ± 0.39	0.21 ± 0.05
	800	72 ± 7	42 ± 3	0.74 ± 0.07	28 ± 3	1.50 ± 0.44	0.20 ± 0.03

[a] The controls were received sterilized corncobs.
[b] The controls were received sterilized corncobs and 2 ml of formalin.

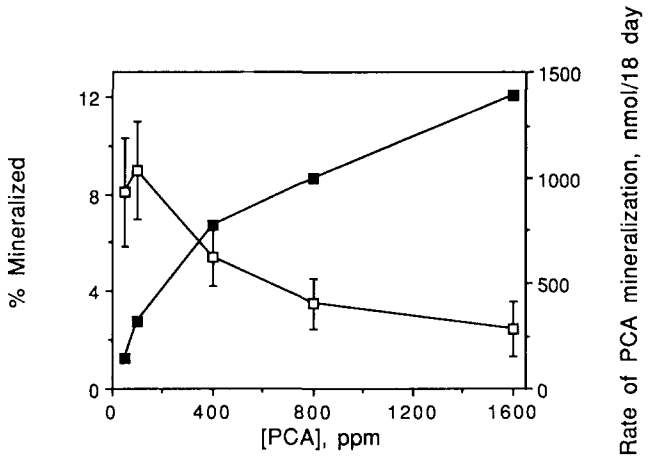

Fig. 3. Effect of PCA concentration on its rate of mineralization by *P. chrysosporium*. Various concentrations of PCA in nonsterile soil were incubated with *P. chrysosporium* and assayed for the percentage of the PCA mineralized (□) and the rate of PCA mineralization for 18 d (■). Values for the percent mineralized are the means ± standard deviations for triplicate cultures.

low radioactivity at the aqueous fractions would be a benefit of using *P. chrysosporium* for bioremediation because the contamination of air and groundwater should be minimized.

We conclude that *P. chrysosporium* can degrade PCP efficiently and produce no harmful intermediates during degradation of PCP in soil and that both PCP and PCA are readily mineralized in soil.

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