APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1988, p. 2885–2889
0099-2240/88/122885-05$02.00/0

Vol. 54, No. 12

Biodegradation of Pentachlorophenol by the White Rot Fungus *Phanerochaete chrysosporium*

GERALD J. MILESKI,[1,2] JOHN A. BUMPUS,[1,3]* MARIA A. JUREK,[1] AND STEVEN D. AUST[1,2]

Biotechnology Center,[1] Chemistry and Biochemistry Department,[2] and Biology Department,[3] Utah State University, Logan, Utah 84322-4430

Received 7 March 1988/Accepted 6 June 1988

Extensive biodegradation of pentachlorophenol (PCP) by the white rot fungus *Phanerochaete chrysosporium* was demonstrated by the disappearance and mineralization of [^{14}C]PCP in nutrient nitrogen-limited culture. Mass balance analyses demonstrated the formation of water-soluble metabolites of [^{14}C]PCP during degradation. Involvement of the lignin-degrading system of this fungus was suggested by the fact that the time of onset, time course, and eventual decline in the rate of PCP mineralization were similar to those observed for [^{14}C]lignin degradation. Also, a purified ligninase was shown to be able to catalyze the initial oxidation of PCP. Although biodegradation of PCP was decreased in nutrient nitrogen-sufficient (i.e., nonligninolytic) cultures of *P. chrysosporium*, substantial biodegradation of PCP did occur, suggesting that in addition to the lignin-degrading system, another degradation system may also be responsible for some of the PCP degradation observed. Toxicity studies showed that PCP concentrations above 4 mg/liter (15 μM) prevented growth when fungal cultures were initiated by inoculation with spores. The lethal effects of PCP could, however, be circumvented by allowing the fungus to establish a mycelial mat before adding PCP. With this procedure, the fungus was able to grow and mineralize [^{14}C]PCP at concentrations as high as 500 mg/liter (1.9 mM).

Pentachlorophenol (PCP) has been used extensively as a wood preservative, fungicide, bactericide, herbicide, molluscicide, algicide, and insecticide (7). Although numerous reports have shown that PCP undergoes biodegradation, its biodegradation in the environment is, in fact, often slow (see references 7, 17, and 18 for reviews of PCP degradation). This, coupled with its extensive use, has led to the contamination of many terrestrial and aquatic ecosystems worldwide. Of concern to the public is the possibility that large numbers of humans may be nonoccupationally exposed to PCP (17).

The white rot fungus *Phanerochaete chrysosporium* has been shown to degrade a wide variety of environmentally persistent organopollutants, including a number of organohalides (1–6, 8). The ability to degrade such a diverse group of compounds has been shown to be dependent on the nonspecific and nonstereoselective lignin-degrading system which is expressed by this microorganism under nutrient (nitrogen, carbon, or sulfur)-limiting conditions (13, 15, 16). The lignin-degrading system consists, in part, of a family of lignin peroxidases (commonly known as ligninases), which are able to catalyze the initial oxidative depolymerization of the lignin polymer (16, 21, 22). It has recently been shown that ligninases are also able to catalyze the initial oxidation of a number of environmentally persistent xenobiotics (5, 10, 11, 19). In this study, we present evidence that the lignin-degrading system is also responsible for degradation of PCP. Additionally, we have developed conditions which allow *P. chrysosporium* to survive and degrade PCP at concentrations that are typically lethal to the fungus.

MATERIALS AND METHODS

Fungus. *P. chrysosporium* (BKM-F-1767) was obtained from the United States Department of Agriculture, Forest Products Laboratory (Madison, Wis.). The organism was maintained on malt agar slant cultures at room temperature and was subcultured every 30 to 60 days.

Chemicals. PCP and 2,3,5,6-tetrachloro-2,5-cyclohexadiene-1,4-dione (TCHD) were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Carbon-14-labeled PCP (10.57 mCi/mmol) and glucose (1.88 mCi/mmol) were obtained from Pathfinder Laboratories, Inc. (St. Louis, Mo.). Purity of [^{14}C]PCP was assessed by migration of the compound on thin-layer chromatography or high-pressure liquid chromatography (HPLC). Radiolabeled PCP was purified by thin-layer chromatography to at least 98% purity. [^{14}C]glucose required no further purification for use in these studies.

Culture conditions. To determine PCP disappearance, mineralization, or toxicity, stationary-phase cultures of *P. chrysosporium* were incubated at 37 to 39°C in 10 ml of nutrient nitrogen-limited culture medium containing 56 mM glucose, trace elements, thiamine (1 mg/liter), and 1.2 mM ammonium tartrate in 20 mM 2,2-dimethylsuccinate buffer, pH 4.2 (9, 15). Nutrient nitrogen-sufficient cultures were grown in the same medium except that the ammonium tartrate concentration was 12 mM. Cultures were inoculated with a spore suspension (1 ml; 0.8 to 1.6 absorbance units at 600 nm), grown under ambient atmosphere for 3 days, and then flushed every 3 days with oxygen (99.9%). In one experiment (Fig. 2), [^{14}C]PCP was added to the cultures after inoculation. All other experiments with PCP were initiated by adding the PCP in a small amount of acetone or ethanol (100 μl or less) to cultures after 6 days of growth in order to overcome the toxicity of PCP to spore germination, which occurs at levels above 4 mg/liter (15 μM). Cultures were grown in medium bottles (250 ml) sealed with Teflon-lined screw caps. It should be noted that the solubility of PCP in water is 20 to 25 mg/liter (76 to 95 μM). Thus, at concentrations higher than 25 mg/liter (95 μM), the concentration of PCP must be regarded as nominal.

Mineralization of PCP and respiration of glucose. To measure the evolution of $^{14}CO_2$ from cultures containing either [^{14}C]PCP or [^{14}C]glucose, the caps of the incubation flasks

* Corresponding author.

were modified to include gas exchange manifolds as described (6). Every 3 days the headspaces of the incubation flasks were flushed for 20 min with oxygen (99.9%), and the CO_2 evolved was trapped in 10 ml of a solution containing ethanolamine–methanol–Safety-Solve scintillation cocktail (Research Products International Corp., Mt. Prospect, Ill.) (1:4:5). The amount of $^{14}CO_2$ trapped was determined by liquid scintillation spectrometry.

PCP concentration. The amount of PCP remaining in cultures was determined after homogenization and extraction by HPLC with a C-18 reverse-phase column (4.6 by 250 mm) packed with either R-Sil C-18 (10 μm) or Econosphere C-18 (5 μm) (Alltech Associates). PCP was eluted from the column with acetonitrile-H_2O-glacial acetic acid (75:25:0.125) at a flow rate of 1 ml/min. Elution was monitored at 238 nm and quantitated by peak area with a Hewlett-Packard digital integrator (model 3390A). A standard curve was determined daily by plotting the peak area obtained from the integrator versus known standard amounts of PCP injected.

At the time of harvest, 1 μmol of sodium azide was added to the cultures to inhibit enzyme activity. The harvested cultures were stored at −20°C until they were homogenized in a glass tissue homogenizer with a motor-driven Teflon pestle. Culture flasks were rinsed with absolute ethanol (5 ml), followed by water (5 ml), and the rinses were combined with the homogenates and stored at −20°C. PCP was extracted from 0.5- to 5-ml samples of culture homogenates with hexane (0.5 to 2 ml) following the addition of 1 ml of H_2O saturated with NaCl.

Mass balance experiments. Nutrient nitrogen-limited and nutrient nitrogen-sufficient cultures of *P. chrysosporium* were established in 250-ml Wheaton medium bottles equipped with a gas exchange manifold and allowed to grow for 3 days under an atmosphere of air and 3 days under an atmosphere of O_2, after which time [^{14}C]PCP (13.6 nmol) was added in 10 μl of acetone. The cultures were then flushed with oxygen and incubated for 4 more days, after which the amount of $^{14}CO_2$ evolved was assayed as described above. The cultures were then homogenized in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The homogenized material was then extracted with two 50-ml portions of hexane, which were then pooled (hexane fraction). Following the hexane extraction, the aqueous phase was acidified to pH 2.0 with concentrated HCl and extracted with two 50-ml portions of methylene chloride, which were then pooled (acidic CH_2Cl_2 fraction). The mycelium was separated from the aqueous fraction by filtration. Safety-Solve (10 ml) was then added to the recovered mycelium in a scintillation vial. Safety-Solve (10 ml) was also added to 1-ml samples of the hexane, methylene chloride, and aqueous fractions. The radioactivity of all fractions was determined by liquid scintillation spectrometry.

Enzyme assay. Ligninase activity was measured by the veratryl alcohol oxidase activity assay described previously (22).

Ligninase purification. Ligninases were produced by 1-liter agitated cultures of *P. chrysosporium* in 2.8-liter Fernbach flasks. Inoculum for the agitated cultures was obtained from 2-day-old cultures as previously described (12) except that the medium used to grow the inoculum contained 10 mM dimethylsuccinate (sodium), pH 4.5, in addition to 56 mM glucose, 1.2 mM ammonium tartrate, thiamine (1 mg/liter), and a mineral supplement (15) and the inoculating cultures were grown under ambient atmosphere instead of 100% oxygen. The medium used to grow agitated cultures contained 10 mM dimethylsuccinate (sodium), pH 4.5, 56 mM glucose, 1.2 mM ammonium tartrate, 0.4 mM veratryl alcohol, 0.6 mM benzyl alcohol, 0.1% (vol/vol) Tween 80, thiamine (1 mg/liter), and a sevenfold-greater concentration of mineral supplement (12). The extracellular fluid was harvested after incubating the cultures for 5 days at 37°C on a rotary shaker (200 rpm) under ambient atmosphere. The mycelium was removed by filtering the extracellular fluid with a Buchner funnel under a slight vacuum. The extracellular fluid was frozen overnight at −20°C, filtered, and centrifuged (10,000 × *g*, 15 min) to remove mucilaginous material, which interfered with concentration and chromatography. The resultant fluid was concentrated to 500 ml and 132 ml with a Millipore Minitan (Bedford, Mass.) and an Amicon concentrator, respectively, both equipped with a membrane having a 10,000-molecular-weight exclusion limit. This concentrate was dialyzed overnight in two 1-liter changes of 20 mM sodium succinate, pH 6.0, and then applied to a DEAE-Sepharose column (1 by 12.5 cm) and eluted with the same buffer. Two peaks having veratryl alcohol oxidase activity were eluted with 20 mM sodium succinate, pH 6.0, and the second peak which eluted between 180 and 312 ml was selected for further purification. The pooled fractions were concentrated (8 ml) and dialyzed against 10 mM sodium acetate, pH 6.0, the buffer used in the subsequent fast protein liquid chromatography. The preparation was applied to a Mono Q HR 10/10 column (Pharmacia, Uppsala, Sweden), washed with 25 ml of 10 mM sodium acetate, pH 6.0, and eluted with a gradient of 10 mM to 0.7 M sodium acetate, pH 6.0 (1 ml/min, 50 min).

A peak which eluted at 0.18 M sodium acetate was collected and analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing under denaturing conditions, both of which showed only one band of protein. This peak eluted at the same ionic strength as one previously designated H-2 (14).

PCP oxidation. Incubations (4 ml) to monitor the oxidation of PCP and formation of product consisted of 20 mM sodium tartrate, pH 3.0, 25 nM ligninase, 25 μM PCP, 20 μM H_2O_2, and 10% *N*,*N*-dimethylformamide. Reactions were initiated by adding H_2O_2. This mixture was incubated at room temperature for 10 min, and the reaction was terminated by addition of 1.4 g of NaCl and 1 ml of hexane. The PCP and TCHD were extracted by mixing vigorously on a Vortex mixer for 30 s, after which the phases were separated by centrifugation (900 × *g*, 5 min). Portions of the extract were analyzed by HPLC as described above except that elution of TCHD and PCP was monitored at 290 nm for the first 5 min and then at 238 nm for the remaining time. TCHD and PCP were quantitated by extrapolation by using their peak areas obtained by a digital integrator from a standard curve of peak area versus standard amounts of TCHD and PCP.

RESULTS

Biodegradation of PCP. Figure 1 demonstrates that PCP underwent rapid and extensive degradation in nutrient nitrogen-limited cultures of *P. chrysosporium*. At an initial concentration of 1.1 mg/liter (4.2 μM), a 97% decrease in PCP concentration was observed in 28 h of incubation relative to uninoculated controls.

Biodegradation of PCP by *P. chrysosporium* was also demonstrated by mineralization of [^{14}C]PCP. Figure 2 shows that 23 ± 2% of the [^{14}C]PCP had been mineralized after 30 days of incubation in nutrient nitrogen-limited cultures of *P. chrysosporium*. It should be noted that the amount of $^{14}CO_2$ evolved from [^{14}C]PCP in nutrient nitrogen-limited cultures

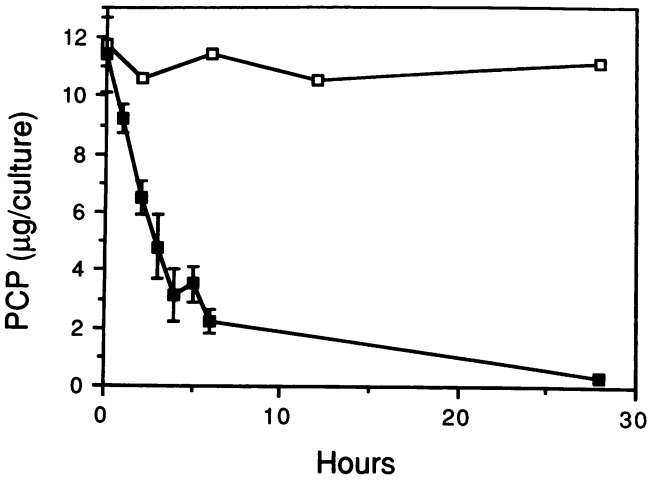

FIG. 1. PCP degradation by cultures of *P. chrysosporium*. Nutrient nitrogen-limited (1.2 mM ammonium tartrate) cultures of *P. chrysosporium* (■) containing PCP were incubated for the specified times, homogenized, extracted, and analyzed by HPLC with UV absorbance at 238 nm used to detect and quantitate PCP. Cultures inoculated with *P. chrysosporium* were analyzed in triplicate, and the data plotted are the means ± standard deviation. A single uninoculated sterile control (□) containing PCP was analyzed by the same method at various times during the experiment.

was variable between experiments. In some experiments the amount of $^{14}CO_2$ evolved was greater than 50%. This type of variation is similar to that observed during [^{14}C]DDT mineralization (3) under these conditions. Figure 2 also shows that, like [^{14}C]lignin mineralization (6, 13), mineralization of [^{14}C]PCP was suppressed (10.1 ± 4.7% mineralized) when nutrient nitrogen was not limiting.

Mass balance experiments were also performed after [^{14}C]PCP (13.6 nmol) had been incubated in nutrient nitrogen-limited and nutrient nitrogen-sufficient cultures of *P. chrysosporium*. Results (Table 1) showed that, in nutrient nitrogen-limited cultures, extensive degradation of [^{14}C]PCP occurred, as evidenced by the fact that 50.5% of the recovered radioactivity was present as $^{14}CO_2$ and 24.8% was present as water-soluble metabolites of [^{14}C]PCP. Only 2.8% of the recovered radioactivity had been incorporated into the fungal mycelium. Mass balance results also showed that substantial biodegradation of [^{14}C]PCP occurred in nutrient nitrogen-sufficient cultures of *P. chrysosporium*, since 10.2% of the recovered radioactivity was present as $^{14}CO_2$ while 26.8% was present as water-soluble metabolites and 7.6% had been incorporated into fungal mycelium.

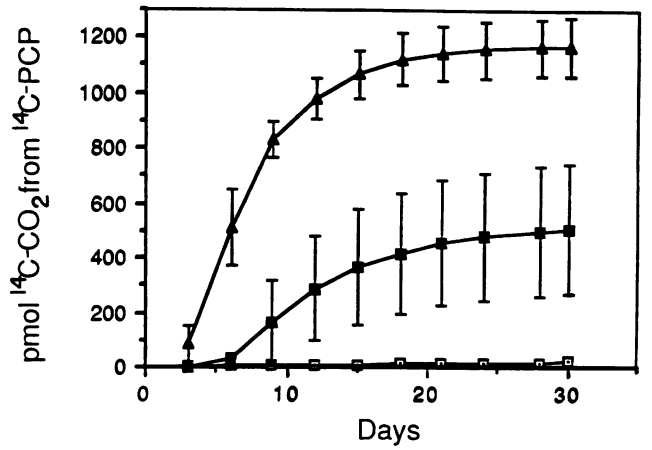

FIG. 2. Effect of nutrient nitrogen on PCP mineralization by *P. chrysosporium*. Cultures containing either 12 mM ammonium tartrate (■; nitrogen sufficient) or 1.2 mM ammonium tartrate (▲; nutrient nitrogen limited) were incubated with 5.0 nmol of [^{14}C]PCP. Values are the means ± standard deviations for triplicate cultures. A single uninoculated sterile control (□) was incubated with 5.0 nmol of [^{14}C]PCP.

TABLE 1. Mass balance analysis of *P. chrysosporium* cultures 4 days after addition of [^{14}C]PCP to 6-day-old cultures

Fraction	% of radioactivity recovered[a]	
	Nitrogen-limited culture	Nitrogen-sufficient culture
Hexane	8.9	51.1
Acidic CH_2Cl_2	13.0	4.3
Aqueous	24.8	26.8
Mycelium	2.8	7.6
CO_2	50.5	10.2

[a] The total radioactivity recovered from nutrient nitrogen-limited and nutrient nitrogen-sufficient cultures was 70 and 67%, respectively.

PCP toxicity. Initial results showed that PCP concentrations of ~4 mg/liter (15 μM) or higher prevented growth when cultures of *P. chrysosporium* were initiated with spores. However, it was found that if cultures were allowed to establish a mycelial mat before the addition of PCP, the lethal effects of PCP could be circumvented. To examine possible toxic effects of PCP on fungal respiration (as measured by metabolism of [^{14}C]glucose to $^{14}CO_2$), cultures of *P. chrysosporium* were incubated with [^{14}C]glucose and concentrations of PCP between 0 and 500 mg/liter (1.9 mM). Table 2 shows that, except for nitrogen-limited cultures containing 500 mg of PCP per liter (1.9 mM), increasing PCP concentration had remarkably little ability to inhibit fungal respiration, as determined by measuring the total amount of [^{14}C]glucose metabolized to $^{14}CO_2$ during the 24-day incubation period.

The effect of PCP concentration on its own mineralization is presented in Table 3. The amount of PCP mineralized increased with increasing PCP concentrations under both nutrient nitrogen-limited and -sufficient conditions.

PCP oxidation by a purified ligninase. Extraction and HPLC analysis of an incubation mixture containing PCP, ligninase, H_2O_2, and 10% *N,N*-dimethylformamide resulted in the loss (9.3 μM) of PCP and the appearance of TCHD (7.3

TABLE 2. Effect of PCP on respiration of *P. chrysosporium*[a]

PCP concn, mg/liter (mM)	Mean amt of [^{14}C]glucose converted to $^{14}CO_2$ during 24 days of incubation[b]	
	N-limited cultures	N-sufficient cultures
0 (0)	31.6 ± 11.9	38.7 ± 3.4
1 (0.0038)	42.6 ± 3.5	39.6 ± 1.0
10 (0.038)	28.1 ± 16.8	38.5 ± 2.8
100 (0.38)	25.9 ± 12.6	39.6 ± 1.9
500 (1.9)	0.5 ± 0.03	39.1 ± 2.2

[a] [^{14}C]glucose (53.2 nmol, 100 nCi) and the indicated amount of PCP were added to 6-day-old cultures. Cultures were then incubated for another 24 days.

[b] Values (nanomoles) represent means ± the standard deviations for quadruplicate cultures.

TABLE 3. Effect of initial PCP concentration on the rate of its mineralization by *P. chrysosporium*

Initial concn of PCP, mg/liter (mM)[a]	Mean amt (nmol, ±SD) of PCP mineralized in 24 days of incubation		% of initial PCP concn mineralized[b]	
	N-limited cultures	N-sufficient cultures	N-limited cultures	N-sufficient cultures
1 (0.0038)	19.0 ± 1.7	5.5 ± 0.5	50.0 ± 4.6	14.6 ± 1.3
10 (0.038)	160.4 ± 43.7	41.8 ± 13.7	42.2 ± 11.5	11.0 ± 3.6
100 (0.38)	851.2 ± 623.2	285.0 ± 14.4	22.4 ± 16.4	7.5 ± 3.8
500 (1.9)	1,349.0 ± 342	931.0 ± 285.0	7.1 ± 1.8	4.9 ± 1.5

[a] The indicated amount of PCP (containing 155 nCi of [^{14}C]PCP) was added to 6-day-old cultures. Cultures were then incubated for another 24 days.
[b] Values (nanomoles) represent mean ± standard deviation for quadruplicate or triplicate cultures.

μM) (Fig. 3). The amount of TCHD formed accounted for 78% of the PCP oxidized. The peak eluting at 4.11 min had the same retention time and UV spectrum as authentic TCHD.

DISCUSSION

These results demonstrate that *P. chrysosporium* is able to cause extensive degradation of PCP. Biodegradation was demonstrated by disappearance, intermediary product formation, mineralization of [^{14}C]PCP, and mass balance analyses. We have also demonstrated that degradation of PCP is dependent on the lignin-degrading system of this fungus, as evidenced by the fact that the temporal onset, time course, and eventual decrease in the rate of mineralization were similar to those seen for [^{14}C]lignin in nutrient nitrogen-limited cultures of *P. chrysosporium* (6, 15). Similarly, we have shown that, like lignin mineralization (6, 15), mineralization of PCP is promoted in nutrient nitrogen-limited cultures of *P. chrysosporium* and suppressed in nutrient nitrogen-sufficient cultures. Direct evidence for the involvement of the lignin-degrading system in PCP degradation was demonstrated by the fact that a purified ligninase (isolated from nutrient nitrogen-limited cultures of *P. chrysosporium*) catalyzed the initial oxidation of PCP.

It is interesting that, relative to other environmentally persistent organohalide pollutants, [^{14}C]PCP underwent a greater degree of mineralization under the same culture conditions (4). It is also interesting that even under nutrient nitrogen-sufficient conditions, substantial mineralization of [^{14}C]PCP occurred. In fact, the amount of [^{14}C]PCP mineralized in nutrient nitrogen-sufficient cultures (10.1 ± 4.7%) typically was similar in magnitude to the amount of mineralization observed for a number of other environmentally persistent chemicals under nutrient nitrogen-limited conditions. For example, we have shown that [^{14}C]DDT, [^{14}C]lindane, and [^{14}C]chlordane underwent 10.5 ± 5.8%, 15.2 ± 6.2%, and 10.2 ± 1% mineralization, respectively, during 30 days of incubation with *P. chrysosporium* under nutrient nitrogen-limited conditions (3, 4, 6). These results may indicate that, in addition to the lignin-degrading system, another degradative system exists in this fungus that is also able to degrade PCP. An alternative explanation may be that, in nutrient nitrogen-sufficient cultures, the carbon source that serves as a growth substrate (i.e., glucose) declines to levels sufficiently low that the lignin-degrading system is induced by carbon limitation. In support of this hypothesis is the fact that the ligninases are synthesized under some culture conditions in response to carbon limitation (16).

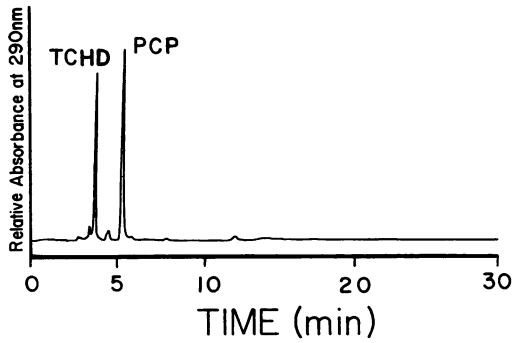

FIG. 3. HPLC chromatogram of the reaction mixture obtained after PCP oxidation by a purified ligninase. The incubation mix contained 20 mM sodium tartrate, pH 3.0, 10% dimethylformamide, 25 nM ligninase, and 25 μM PCP, and reactions were initiated with H_2O_2 (20 μM). After 10 min, the incubation mix was extracted with hexane. TCHD and PCP were identified by comigration with authentic standards.

PCP has been shown to be present in wood treatment plant wastewater effluents at concentrations ranging from 25 to 150 mg/liter (95 to 570 μM) (20). Concentrations of up to 500 mg/kg of a technical mixture of chlorophenols (including PCP) in soil from a wood treatment facility have been reported (23), and even higher concentrations may be present in soils at other wood treatment facilities.

We have suggested that *P. chrysosporium* may be useful in the biodegradation of hazardous organochemical wastes in some waste treatment systems (6). However, it seems paradoxical to suggest the use of this wood-rotting fungus for PCP degradation, since PCP has been used quite successfully as a wood preservative for many years by inhibiting the growth of such fungi. Cultures of *P. chrysosporium* could not be established by inoculation with fungal spores in the presence of PCP concentrations greater than 4 mg/liter (15 μM). The lethal effects of PCP were circumvented by allowing the fungus to establish a mycelial mat before PCP was added. The precise mechanism by which the lethal effects of PCP were circumvented is unknown. However, since PCP is an inhibitor of oxidative phosphorylation, it is reasonable to suggest that the minimal energy requirements for new growth from spores cannot be met in the presence of PCP concentrations greater than 4 mg/liter (15 μM). In contrast, the minimal energy requirements for established fungal mats would be expected to be substantially less than those of newly formed, rapidly dividing hyphae. Thus, the fact that PCP was not lethal to previously established fungal mats might be due to the inability of PCP to cause lethal inhibition of oxidative phosphorylation in these cells. Because initial mineralization experiments were performed with low (nonlethal) levels of [^{14}C]PCP, it was of interest to demonstrate that PCP concentrations of up to 500 mg/liter

were not lethal and that the fungus retained the ability to degrade substantial amounts of PCP. This, and the facts that the lethal effects observed above 4 mg/liter (15 μM) could be circumvented and that *P. chrysosporium* could be cultured at PCP concentrations as high as 500 mg/liter (1.9 mM), suggests that waste treatment systems can be designed which should be able to accommodate PCP concentrations at least this high.

ACKNOWLEDGMENTS

This research was supported in part by Cooperative Agreements CR813369 and CR814162, U.S. Environmental Protection Agency, Office of Research and Development, Hazardous Waste Engineering Research Laboratory, Cincinnati, Ohio (P. R. Sferra, Project Officer).

We thank Terri Maughan for her expert secretarial assistance during the preparation of this manuscript. We also thank Marnie Atkinson, Jon Hennebold, Rob Morse, and Walter Runkle for technical assistance.

LITERATURE CITED

1. **Arjmand, M., and H. Sandermann, Jr.** 1985. Mineralization of chloroaniline/lignin conjugates and of free chloroanilines by the white rot fungus *Phanerochaete chrysosporium*. J. Agric. Food Chem. **33**:1055–1060.
2. **Bumpus, J. A., and S. D. Aust.** 1987. Biodegradation of environmental pollutants by the white rot fungus *Phanerochaete chrysosporium*: involvement of the lignin degrading system. BioEssays **6**:166–170.
3. **Bumpus, J. A., and S. D. Aust.** 1987. Biodegradation of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **53**:2001–2008.
4. **Bumpus, J. A., and S. D. Aust.** 1987. Mineralization of recalcitrant environmental pollutants by a white rot fungus, p. 146–151. *In* Proceedings of the National Conference on Hazardous Wastes and Hazardous Materials. Control Research Institute, Silver Spring, Md.
5. **Bumpus, J. A., and B. Brock.** 1988. Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **54**:1143–1150.
6. **Bumpus, J. A., M. Tien, D. Wright, and S. D. Aust.** 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science **228**:1434–1436.
7. **Crosby, D. G.** 1981. Environmental chemistry of pentachlorophenol. Pure Appl. Chem. **53**:1051–1080.
8. **Eaton, D. C.** 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb. Technol. **7**:194–196.
9. **Fenn, P., and T. K. Kirk.** 1979. Ligninolytic system of *Phanerochaete chrysosporium*: inhibition of *o*-phthalate. Arch. Microbiol. **123**:307–309.
10. **Haemmerli, S. D., M. S. A. Leisola, D. Sanglard, and A. Fiechter.** 1986. Oxidation of benzo[*a*]pyrene by extracellular ligninases of *Phanerochaete chrysosporium*: veratryl alcohol and stability of ligninase. J. Biol. Chem. **261**:6900–6903.
11. **Hammel, K. E., B. Kalyanaraman, and T. K. Kirk.** 1986. Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]-dioxins by *Phanerochaete chrysosporium* ligninase. J. Biol. Chem. **261**:16948–16952.
12. **Jager, A., S. Croan, and T. K. Kirk.** 1985. Production of ligninases and degradation of lignin in agitated submerged cultures of *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **50**:1274–1278.
13. **Jeffries, T. W., S. Choi, and T. K. Kirk.** 1981. Nutritional regulation of lignin degradation by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **42**:290–296.
14. **Kirk, T. K., S. Croan, M. Tien, K. E. Murtagh, and R. L. Farrell.** 1986. Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of a mutant strain. Enzyme Microb. Technol. **8**:27–32.
15. **Kirk, T. K., E. Schultz, W. J. Connors, L. F. Lorenz, and J. G. Zeikus.** 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. **117**: 277–285.
16. **Leisola, M. S. A., B. Kozulic, F. Meussdoerffer, and A. Fiechter.** 1987. Homology among multiple extracellular peroxidases from *Phanerochaete chrysosporium*. J. Biol. Chem. **262**:419–424.
17. **Rao, K. R. (ed.).** 1978. Pentachlorophenol: chemistry, pharmacology, and environmental toxicology. Plenum Press, New York.
18. **Rochkind, M. L., J. W. Blackburn, and G. S. Sayler.** 1986. Microbial decomposition of chlorinated aromatic compounds. Publ. no. EPA/600/2-86/090. U.S. Environmental Protection Agency Hazardous Waste Engineering Research Laboratory, Cincinnati, Ohio.
19. **Sanglard, D., M. S. A. Leisola, and A. Fiechter.** 1986. Role of extracellular ligninases in biodegradation of benzo[*a*]pyrene by *Phanerochaete chrysosporium*. Enzyme Microb. Technol. **8**: 209–212.
20. **Thompson, W. S., and J. V. Dust.** 1971. Pollution control in the wood preserving industry. I. Nature and scope of the problem. Forest Products J. **21**:70–75.
21. **Tien, M., and T. K. Kirk.** 1983. Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science **221**:661–663.
22. **Tien, M., and T. K. Kirk.** 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc. Natl. Acad. Sci. USA **81**:2280–2284.
23. **Valo, R., V. Kitunen, and M. Salkinoja-Salonen.** 1984. Chlorinated phenols as contaminants of soil and water in the vicinity of two Finnish sawmills. Chemosphere **13**:835–844.