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Biodegradation of TNT (2,4,6-Trinitrotoluene) by *Phanerochaete chrysosporium*

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Extensive biodegradation of TNT (2,4,6-trinitrotoluene) by the white rot fungus *Phanerochaete chrysosporium* was observed. At an initial concentration of 1.3 mg/liter, 35.4 ± 3.6% of the [^{14}C]TNT was degraded to $^{14}CO_2$ in 18 days. The addition of glucose 12 days after the addition of TNT did not stimulate mineralization, and, after 18 days of incubation with TNT only, about 3.3% of the initial TNT could be recovered. Mineralization of [^{14}C]TNT adsorbed on soil was also examined. Ground corncobs served as the nutrient for slow but sustained degradation of [^{14}C]TNT to $^{14}CO_2$ such that 6.3 ± 0.6% of the [^{14}C]TNT initially present was converted to $^{14}CO_2$ during the 30-day incubation period. Mass balance analysis of liquid cultures and of soil-corncob cultures revealed that polar [^{14}C]TNT metabolites are formed in both systems, and high-performance liquid chromatography analyses revealed that less than 5% of the radioactivity remained as undegraded [^{14}C]TNT following incubation with the fungus in soil or liquid cultures. When the concentration of TNT in cultures (both liquid and soil) was adjusted to contamination levels that might be found in the environment, i.e., 10,000 mg/kg in soil and 100 mg/liter in water, mineralization studies showed that 18.4 ± 2.9% and 19.6 ± 3.5% of the initial TNT was converted to $^{14}CO_2$ in 90 days in soil and liquid cultures, respectively. In both cases (90 days in water at 100 mg/liter and in soil at 10,000 mg/kg) approximately 85% of the TNT was degraded. These results suggest that this fungus may be useful for the decontamination of sites in the environment contaminated with TNT.

The compound TNT (2,4,6-trinitrotoluene) is the predominant conventional explosive used by military forces (27). Unfortunately, past practices for the disposal of TNT-containing wastes generated during the production of TNT and of military ordnance which use TNT have led to soil, sediment, and water contamination (24, 27). This is of concern because exposure to TNT is known to cause pancytopenia, a disorder of the blood-forming tissues characterized by a pronounced decrease in the number of leukocytes, erythrocytes, and reticulocytes in humans and other mammals (12). Also, TNT is toxic to fathead minnows (*Pimephales promelas*) and bluegills (*Lepomis macrochirus*) at concentrations of 2 to 3 μg/ml (13, 26). It is also toxic to certain green algae (*Selenastrum capricornutum*, *Microcystis aeruginosa*, *Chlamydomonas reinhardtii*), tidepool copepods (*Tigriopus californicus*), and oyster (*Crassostrea gigas*) larvae (13, 26, 28). Additionally, TNT is a mutagen as assayed by the Ames test (28).

Recent studies have shown that the wood-rotting fungus, *Phanerochaete chrysosporium*, possesses remarkable biodegradative properties (1–11, 20). This fungus is one of the relatively few microorganisms known to be able to degrade lignin, a naturally occurring and recalcitrant biopolymer, to carbon dioxide (3, 14, 16, 17, 21). *P. chrysosporium* is also able to degrade a wide variety of environmentally persistent xenobiotics to carbon dioxide, including a number of chlorinated hydrocarbons such as DDT [1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane], lindane (1,2,3,4,5,6-hexachlorocyclohexane), chloroanilines, and polychlorinated biphenyls (1–11). Recent evidence suggests that the ability to degrade such a diverse group of compounds is dependent on the nonspecific and nonstereoselective lignin-degrading system which is expressed by this organism under nutrient (nitrogen, carbon, or sulfur)-limiting conditions (1–11, 14, 17). It has recently been shown that lignin peroxidases from this fungus are also able to catalyze the initial oxidation of a number of environmentally persistent xenobiotics (7, 10, 11, 25). However, Kohler et al. observed that lignin peroxidases of *P. chrysosporium* have no role in the initial oxidation of DDT (19).

The purpose of this investigation was to determine whether *P. chrysosporium* would grow in the presence of a highly toxic contaminant like TNT at concentrations that occur in the environment and whether it would degrade the TNT. Because *P. chrysosporium* has the ability to degrade a wide variety of environmentally persistent organopollutants to carbon dioxide, we have suggested that this microorganism may be useful in certain hazardous waste treatment systems (8). In this study, the ability of *P. chrysosporium* to degrade TNT-contaminated water and soil was investigated by adjusting the TNT concentration equivalent to contamination levels found in the environment. The results of this study indicate that biological treatment systems that utilize *P. chrysosporium* could be used as effective and efficient methods for remediation of TNT-contaminated water and soil.

MATERIALS AND METHODS

Organism. *P. chrysosporium* BKM-F-1767 was obtained from the Forest Products Laboratory, U.S. Department of Agriculture, Madison, Wis. The organism was maintained at room temperature on 2% (wt/vol) malt agar slants. Subcultures were routinely made every 30 to 60 days.

Chemicals. Radiolabeled TNT (ring labeled; specific activity, 21.58 mCi/mmol) was purchased from Chemsyn Science Laboratories (Lenexa, Kans.), and the nonradioactive TNT was obtained from the U.S. Army Toxic and Hazardous Materials Agency (Aberdeen, Mo.) and from Chem Service,

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Inc. (West Chester, Pa.). Two dinitrotoluene congeners (2,4-dinitrotoluene and 2,6-dinitrotoluene) and 2-, 4-, and 6-nitrotoluene were purchased from Aldrich Chemical Co., (Milwaukee, Wis.). The purity of the chemicals was determined by thin-layer chromatography and high-performance liquid chromatography (HPLC) and was found to be greater than 98% in all cases.

Culture conditions. *P. chrysosporium* was incubated in liquid medium composed of 56 mM glucose, 1.2 mM ammonium tartrate (nitrogen limited), trace metals, and thiamine (1 mg/liter) in 20 mM 2,2′-dimethylsuccinate (sodium) buffer (pH 4.2) (6, 8, 17). The culture medium was sterilized by filtration through a cellulose acetate membrane filter (pore size, 0.22 μm). Culture bottles were sterilized by autoclaving at 121°C and 15 lb/in^2 for 20 min. Samples of the culture medium (9 ml) were dispensed into each of several 250-ml Wheaton bottles equipped with a gas exchange manifold with a Teflon seal (8). Cultures were inoculated with a spore suspension of *P. chrysosporium* (1 ml; 0.5 absorbance unit at 650 nm) and grown at 39°C. Control cultures contained culture medium minus *P. chrysosporium* inoculum. The cultures were grown under ambient atmosphere for 6 days. On day 6, 57.9 nmol (13 μg) of [^{14}C]TNT was added to each culture; then, at 3-day intervals thereafter, the headspaces were flushed with oxygen (99.9%) and the liberated CO_2 was passed through a volatile organic trap consisting of 10 ml of scintillation cocktail (Safety Solve; Research Products International Corp., Mt. Prospect, Ill.) prior to passage through a vial containing 10 ml of CO_2 trap. The CO_2 trap was a mixture of ethanolamine in methanol and scintillation cocktail (1:4:5; vol/vol/vol). The volatile organic trap was used to ensure that the radiolabeled material trapped in the CO_2 trap was not contaminated with volatile organics as a result of air stripping during flushing. The amount of radioactivity in each trap was determined by liquid scintillation spectrometry.

To perform mass balance analyses on liquid cultures incubated with [^{14}C]TNT, the contents of each 250-ml Wheaton bottle were extracted three times with 30 ml of dichloromethane and 30 ml of water (1:1; vol/vol). The dichloromethane extracts were combined and concentrated by evaporation under a gentle stream of nitrogen. Following these extractions, particulate matter, i.e., fungal mat, was separated from the aqueous fraction by filtration through glass wool. The amount of ^{14}C which was bound to the fungal mat and not extractable by organic solvent was determined by combustion in a Harvey Biological Oxidizer (R. J. Harvey Instrument Corp., Hillsdale, N.J.), and measurement of radioactivity in the evolved CO_2 was determined by liquid scintillation spectrometry. The aqueous fraction was also assayed for radioactivity by liquid scintillation spectrometry.

HPLC analyses were performed by using a system equipped with a Spectra-Physics model SP 8810 pump (Spectra-Physics, San Jose, Calif.), a Rheodyne injector (Rheodyne Inc., Cotati, Calif.), a reverse-phase column (5 μm; 4.6 by 250 mm; Rsil C-18; Beckman Instruments, Inc., San Ramon, Calif.), and a Spectra-Physics model SP 8450 (Spectra-Physics) variable wavelength absorbance detector. Isocratic elution was performed with methanol-water (50:50, vol/vol) at a flow rate of 1 ml/min. The retention time for TNT was determined by monitoring elution at 254 nm. For the mass balance studies, 20-μl samples of the concentrated organic extracts were used for injection into the HPLC. Fractions (1 ml) were collected in scintillation vials. Safety Solve (9 ml) was added to each fraction, and radioactivity was determined as described above.

The ability of *P. chrysosporium* to degrade [^{14}C]TNT in soil was also examined. An agricultural silt loam soil (sand, 19%; silt, 54%; and clay, 27%) was used in this study. The organic matter content of the soil was 3.62%, of which 2.10% was organic carbon. Total nitrogen was 0.19%, and the soil pH was 6.4. The cation exchange capacity was 23.6 meq/100 g. Ten grams of soil was placed in 250-ml Wheaton bottles. Then, 57.9 nmol of [^{14}C]TNT dissolved in 160 μl of acetone was added to the soil. The acetone was allowed to evaporate, and the soil was then mixed with 6.7 g of corncobs that had been previously inoculated (10 days earlier) with *P. chrysosporium*. Preinoculated corncobs were made by autoclaving the corncobs (121°C; 15 lb/in^2) for 30 min and inoculating them aseptically with *P. chrysosporium*. The moisture content of the soil was adjusted (40%, wt/wt) by adding unsterilized water. Unlike the conditions used in the liquid culture experiment, sterile conditions were not used in the soil culture experiment. Also, cultures were not buffered or supplemented with trace metals or other nutrients. Cultures were incubated at 39°C for 30 days. Control cultures contained identical culture parameters, and, instead of inoculated corncobs, they contained 6.7 g of uninoculated corncobs. Every 3 days, the headspaces of the culture bottles were flushed with oxygen, liberated CO_2 was trapped, and the amount of radioactivity in the CO_2 and in the volatile organic trap was determined by liquid scintillation spectrometry as described above.

Mass balance analyses were also performed on soil cultures by extraction procedures previously described by Jenkins and Walsh (15). The contents of each 250-ml Wheaton bottle were extracted three times with 30 ml of acetonitrile. The soil-corncob-acetonitrile mixtures were dispersed by mixing on a vortex mixer (Scientific Industries, Inc., Bohemia, N.Y.) for 10 min, followed by sonication in an ultrasonic bath (Branson Equipment Company, Shelton, Conn.) for 18 h. The acetonitrile extracts were combined and concentrated by evaporation under a gentle stream of nitrogen. The concentrated extract was then centrifuged for 5 min at 1,500 rpm, and the clear supernatant (10 ml) was removed with a volumetric pipette and mixed with an equal volume of water in a glass scintillation vial (15). The contents of the vial were thoroughly mixed, allowed to stand for 15 min, and then filtered through a Gelman Sciences 0.45-μm-pore-size ARCO LS-25 disposable filter assembly. Twenty microliters of the organic extract was used for HPLC analysis as described above. Fractions (1 ml) were collected, and radioactivity was determined as described above. Radiolabeled compounds which were retained in the soil-corncob-fungal matrix and which were not recovered by organic solvent extraction were combusted to CO_2 in a Harvey Biological Oxidizer, and radioactivity was measured by liquid scintillation spectrometry.

In other experiments, *P. chrysosporium* was tested for its ability to mineralize [^{14}C]TNT in both liquid and soil at levels that might be encountered in the environment, i.e., 100 mg/liter in water (22, 24) and 10,000 mg/kg in soil. Culture conditions were as described above except for the concentration of TNT and the time of incubation. Unless otherwise stated, all studies were performed in quadruplicate. Rates of mineralization were obtained, and mass balance analyses were performed as described above after 30, 60, and 90 days for the liquid and the soil cultures. One culture was extracted at each time except for the 90-day time point for soils, in which case two cultures were used.

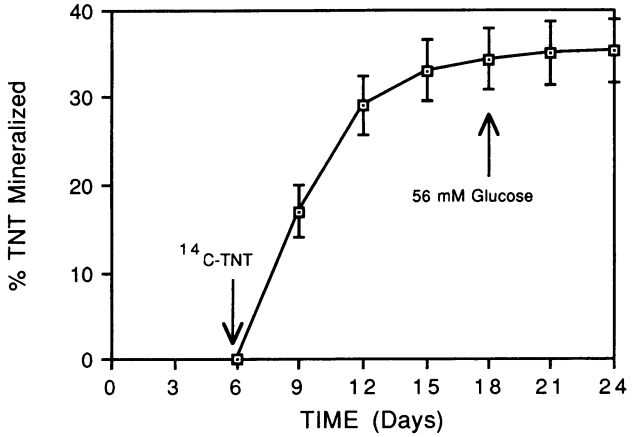

FIG. 1. Mineralization of [^{14}C]TNT in nutrient nitrogen-limited cultures of *P. chrysosporium*. Each culture contained 57.9 nmol of [^{14}C]TNT. Datum points represent the mean ± standard deviation (n = 4). Arrows indicate addition of [^{14}C]TNT and supplemental glucose.

RESULTS

P. chrysosporium mineralized 35% of the [^{14}C]TNT during 12 days of incubation (Fig. 1). Supplemental glucose (equivalent to 56 mM), added to the cultures on day 18, did not affect the evolution of $^{14}CO_2$, so the experiment was terminated after 24 days of incubation and a mass balance analysis was performed. A total of 35.4 ± 3.6% of the total radioactivity was evolved as $^{14}CO_2$, 25.1% was present as water-soluble metabolites, 15.7% was found in the methylene chloride fraction, and 17.3% was associated with the mycelial fraction. A total mass recovery of 93.5% was achieved. HPLC analysis (Fig. 2) of the methylene chloride extract demonstrated that only about 3.3% of the [^{14}C]TNT initially present might be identified as undegraded TNT. The remaining 12.4% represented unidentified metabolites formed during the 18-day incubation period. Almost all of the unidentified metabolites remaining in the methylene chloride extract were more polar than TNT. None of the metabolites corresponded to mono- or dinitrotoluenes. In control cultures incubated under the same culture conditions but not inoculated with *P. chrysosporium*, 98% of the radioactivity added was found in the methylene chloride fraction and was unmetabolized [^{14}C]TNT.

Biodegradation was also examined in a system in which [^{14}C]TNT was adsorbed onto soil and mixed with corncobs previously inoculated (10 days earlier) with *P. chrysosporium*. In this soil-corncob mixture, 6.3 ± 0.6% of the [^{14}C]TNT initially present was degraded to $^{14}CO_2$ during 30 days (Fig. 3). Mass balance analysis of cultures of *P. chrysosporium* incubated with [^{14}C]TNT in a soil-corncob matrix for 30 days revealed that 6.3 ± 0.6% of the recovered radioactivity was evolved as $^{14}CO_2$, 63.6% was present in the acetonitrile extract, and 25.2% was unextractable and was present in the soil-corncob matrix. This material could not be identified as it could not be extracted from the matrix. A total mass recovery of 95.1% was achieved. HPLC analysis (Fig. 4) of the radiolabeled material in the acetonitrile extract revealed that only about 2.2% of the [^{14}C]TNT initially present might be identified as undegraded TNT.

At the end of 30, 60, and 90 days, liquid and soil cultures contaminated with 100 mg of TNT per liter and 10,000 mg of TNT per kg, respectively, were extracted and mass balance

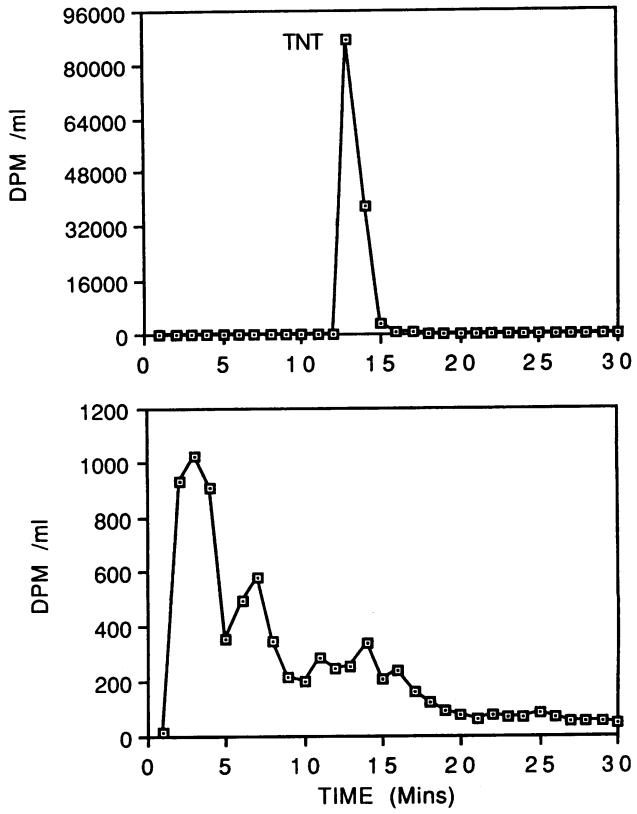

FIG. 2. HPLC elution profile of a methylene chloride extract of a nutrient nitrogen-limited culture of *P. chrysosporium* that was incubated with [^{14}C]TNT (57.9 nmol) for 18 days. Authentic [^{14}C]TNT eluted from the column at about 13 min. DPM, Disintegrations per minute.

analyses were performed as described above. The results (Table 1) of mass balance analysis of 100 mg of TNT per liter of contaminated liquid cultures showed that 19.6 ± 3.5% of the recovered radioactivity was evolved as $^{14}CO_2$, 22.7% was found in the methylene chloride extract, 50.1% was present as water-soluble compounds, and 2.2% was bound to

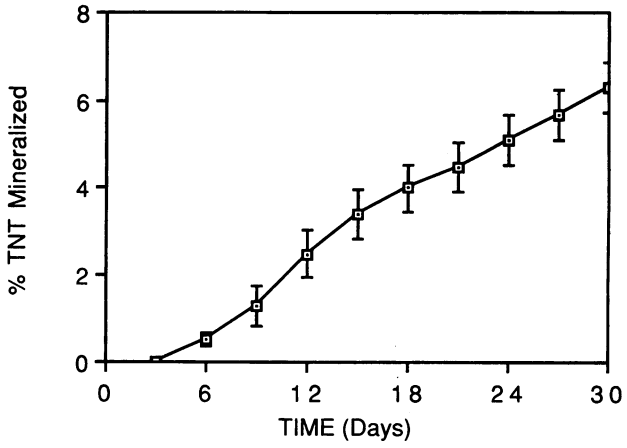

FIG. 3. Mineralization of [^{14}C]TNT in soil-corncob cultures of *P. chrysosporium*. Each culture contained 57.9 nmol of [^{14}C]TNT. Datum points represent the mean ± standard deviation (n = 4).

the fungal mat after a period of 90 days of incubation. A total mass recovery of 94.6% was achieved. When the methylene chloride fraction was analyzed by HPLC, the amounts of unmetabolized [^{14}C]TNT remaining in liquid cultures were 22.1, 14.9, and 12.3% over a period of 30, 60, and 90 days of incubation, respectively. In control cultures, which were incubated under the same conditions but which were not inoculated with *P. chrysosporium*, greater than 99% of the radioactivity was found in the methylene chloride extract and was identified as TNT by HPLC.

The results of mass balance analysis of soil cultures contaminated with 10,000 mg of TNT per kg showed that 18.4 ± 2.9% was evolved as $^{14}CO_2$, 62.6% was found in the acetonitrile extract, and 11.5% was bound to the soil-corncob-fungal matrix after 90 days (Table 1). The total mass recovery was 92.5% after a period of 90 days of incubation. When the acetonitrile extracts of the 30-, 60-, and 90-day cultures were analyzed by HPLC, they showed that the amounts of residual [^{14}C]TNT that was not degraded to $^{14}CO_2$ or intermediates were 50.8, 29.3, and 14.9%, respectively. In control cultures incubated under the same nonsterile conditions but not inoculated with *P. chrysosporium*, greater than 99% of the radioactivity was found in the acetonitrile fraction and was unmetabolized [^{14}C]TNT. The assay for radioactivity in the volatile organic trap revealed that less than 0.5% of the [^{14}C]TNT was volatilized or air stripped during the flushing of the cultures with oxygen. In some experiments, CO_2 was trapped in $Ba(OH)_2$ rather than in the ethanolamine-based scintillation cocktail to show that the radioactivity was quantitatively precipitated with $BaCO_3$ (data not shown).

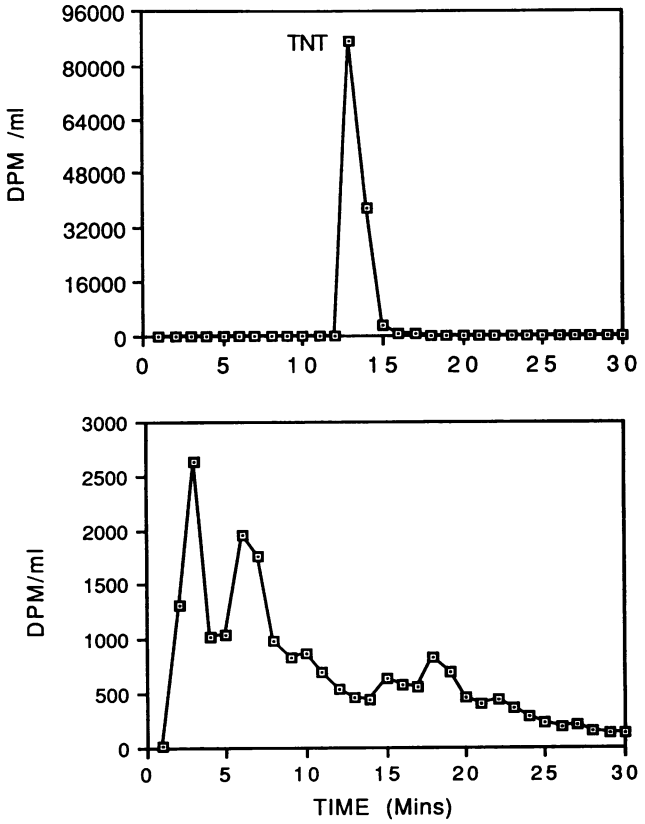

FIG. 4. HPLC elution profile of an acetonitrile extract of a soil-corncob culture incubated with *P. chrysosporium* and [^{14}C]TNT (57.9 nmol) for 30 days. Authentic [^{14}C]TNT eluted from the column at 13 min. DPM, Disintegrations per minute.

DISCUSSION

This study shows that the wood-rotting (white rot) fungus *P. chrysosporium* is able to cause extensive degradation of [^{14}C]TNT in a reasonably short period of time. Degradation was demonstrated by mineralization of [^{14}C]TNT, metabolite formation, and mass balance analyses. Biodegradation of [^{14}C]TNT was also shown to occur in a soil-corncob mixture inoculated with *P. chrysosporium*. Compared with liquid cultures, in soil cultures, substantially less [^{14}C]TNT was converted to $^{14}CO_2$. However, it is worth noting that in liquid cultures, mineralization of [^{14}C]TNT virtually ceased after 15 days, whereas in soil cultures, it continued throughout the 90-day incubation period at a nearly continuous, albeit relatively slow, rate. This suggests that the extent of mineralization in soil-corncob matrices could be extended simply by increasing the incubation period. However, in liquid cultures, addition of supplemental glucose after 12 days of incubation did not stimulate the mineralizations as we observed with the degradation of other xenobiotics by *P. chrysosporium* (3, 6). Xenobiotic degradation studies in liquid cultures with *P. chrysosporium* and glucose have revealed that after 9 to 12 days of incubation, the rate of mineralization is reduced. The reason for this could be either depletion of glucose or the chemical being degraded from the culture medium. The results of TNT degradation in liquid cultures verified the possibility of depletion of the chemical from the culture medium instead of glucose when the mass balance results revealed that the amount of TNT remaining

TABLE 1. Mass balances for 2,4,6-trinitrotoluene metabolism by *P. chrysosporium* in soil and liquid cultures

Matrix	Incubation period (days)	% Mineralized	% Metabolites in water fraction	% Extracted in acetonitrile[a] or methylene chloride[b] fraction	% Adsorbed to soil-corncob[a] or fungal mat fraction	% Mass recovery	% TNT remaining
Soil[a] (10,000 mg/kg)	30	9.8 ± 1.9		69.5	14.4	93.7	50.8
	60	17.1 ± 2.2		59.8	15.3	92.2	29.3
	90	18.4 ± 2.9		62.6	11.5	92.5	14.9
Liquid[b] (100 mg/liter)	30	18.4 ± 2.4	52.0	12.1	11.0	93.5	22.1
	60	19.0 ± 3.0	51.6	19.5	5.1	95.2	14.9
	90	19.6 ± 3.5	50.1	22.7	2.2	94.6	12.3

[a] In soil cultures, 57.9 nmol of [^{14}C]TNT and 100 mg of TNT, dissolved in acetone, were adsorbed onto 10 g of nonsterile soil. The acetone solvent was allowed to evaporate, 6.7 g of preinoculated corncobs was added, and the water content was adjusted to 40% (wt/wt). Mass balances were quantitated as described in Materials and Methods.

[b] In liquid cultures, 6-day-old ligninoytic cultures of *P. chrysosporium* contained 57.9 nmol of [^{14}C]TNT and 1 mg of TNT. Mass balances were quantitated as described in Materials and Methods.

in the culture medium was less than 3%, and the additional supply of glucose had no effect on the restoration of mineralization.

Previous research has shown that, in general, TNT is quite resistant to biodegradation by most bacteria and fungi (22). Typically, the aromatic ring is not cleaved and degradation to CO_2 does not occur. This is not to say that TNT is totally refractory to biodegradation, since a number of microorganisms have been isolated that mediate substantial biodegradation of this compound. For example, Won et al. (29) have reported that pseudomonas-like bacteria could cause extensive degradation of TNT to several intermediates, all but two (2-amino-4,6-dinitrotoluene and 2-nitro-4,6-diaminotoluene) of which were further degraded.

Similarly, a number of fungi have been shown to be able to degrade TNT as assayed by TNT disappearance from culture (18, 23). Of particular note is the fungus *Rhizopus stolonifer*, which was able to mediate near complete disappearance of TNT from cultures containing 100 mg of TNT per liter (18).

A major objective of our research is the development of bioremediation systems by using *P. chrysosporium* to treat water, soils, sediments, and other materials that are contaminated with toxic or recalcitrant organopollutants or both. In this study, we have shown that *P. chrysosporium* is able to extensively degrade [^{14}C]TNT. Of particular interest is the fact that substantial amounts of [^{14}C]TNT can be converted to $^{14}CO_2$ by this fungus.

The concentration of [^{14}C]TNT used in the liquid culture experiments was of the same magnitude as that found in waters contaminated by TNT in the environment (22, 24). The concentration of TNT in effluents from TNT manufacturing processes is, on average, about 20 mg/liter (22), and the concentration of TNT in contaminated soil may be as high as 10,000 mg/kg. Interestingly, high concentrations of TNT were not lethal to the fungus in these experiments and considerable quantities of TNT were degraded. We have suggested that *P. chrysosporium* may be useful in the biodegradation of hazardous wastes in waste treatment systems (8). When compared with costly and tedious physical decontamination processes, the *P. chrysosporium* system used in this study reveals that a versatile organism such as *P. chrysosporium* may provide a more economical biological treatment system that could be applied to in situ decontamination processes when the conditions are adapted for the growth of the fungus. The enzymes involved in the degradation of TNT and the identities of the intermediates are under investigation.

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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. **Arjmand, M., and H. Sandermann, Jr.** 1986. Plant biochemistry of xenobiotics. Mineralization of chloroaniline/lignin metabolites from wheat by the white rot fungus *Phanerochaete chrysosporium*. Z. Naturforsch. Teil C **41**:206–214.
3. **Bumpus, J. A.** 1989. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **55**:154–158.
4. **Bumpus, J. A., and S. D. Aust.** 1987. Mineralization of recalcitrant environmental pollutants by a white rot fungus, p. 146–151. Proceedings of the National Conference on Hazardous Wastes and Hazardous Materials, Hazardous Materials Control Research Institute, Silver Spring, Md.
5. **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.
6. **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.
7. **Bumpus, J. A., and B. Brock.** 1988. Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **54**:1143–1150.
8. **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.
9. **Eaton, D. C.** 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb. Technol. **7**:194–196.
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. **Harris, J. W., and R. W. Kellermeyer.** 1970. The red cell: production, metabolism, destruction, normal and abnormal. Rev. ed. Harvard University Press, Cambridge, Mass.
13. **Hudock, G. A., and D. M. Gring.** 1970. Biological effects of trinitrotoluene. Contract NOO164-69-CO822. Naval Environmental Health Center, Norfolk, Va.
14. **Jeffries, T. W., S. Choi, and T. K. Kirk.** 1981. Nutritional regulation of lignin degradation by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **42**:290–296.
15. **Jenkins, T. F., and M. E. Walsh.** 1987. Development of an analytical method for explosive residues in soil. Report 87-7. Cold Regions Research and Engineering Laboratory. U.S. Government Printing Office, Springfield, Va.
16. **Kirk, T. K., and H. M. Chang.** 1975. Decomposition of lignin by white rot fungi. II. Characterization of heavily degraded lignins from decayed spruce. Holzforschung **29**:56–64.
17. **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.
18. **Klausmeier, R. E., J. L. Osmon, and D. R. Walls.** 1974. The effect of trinitrotoluene on microorganisms. Dev. Ind. Microbiol. **15**:309–317.
19. **Kohler, A., A. Jager, H. Willershausen, and H. Graf.** 1988. Extracellular ligninase of *Phanerochaete chrysosporium* Burdsall has no role in the degradation of DDT. Appl. Microbiol. Biotechnol. **29**:618–620.
20. **Leatham, G. F., R. L. Crawford, and T. K. Kirk.** 1983. Degradation of phenolic compounds and ring cleavage of catechol by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **46**:191–197.
21. **Lundquist, K., T. K. Kirk, and W. J. Connors.** 1977. Fungal degradation of kraft lignin and lignin sulfonates prepared from synthetic ^{14}C-lignins. Arch. Microbiol. **112**:291–296.
22. **Nay, M. W., Jr., C. W. Randall, and P. H. King.** 1974. Biological treatability of trinitrotoluene manufacturing wastewater. J. Water Pollut. Control Fed. **46**:485–497.
23. **Parrish, I. W.** 1977. Fungal transformation of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene. Appl. Environ. Microbiol. **34**:232–233.
24. **Pereira, W. E., D. L. Short, D. B. Manigold, and P. K. Ross.**

1979. Isolation and characterization of TNT and its metabolites in groundwater by gas chromatograph-mass spectrometer-computer techniques. Bull. Environ. Contam. Toxicol. **21**:554–562.

25. **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.
26. **Smock, L. A., D. L. Stoneburner, and J. R. Clark.** 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. Water Res. **10**:537–543.
27. **Williams, R. T., P. S. Ziegenfuss, and P. J. Marks.** 1988. Field demonstration composting of explosives-contaminated sediments at the Louisiana Army Ammunition Plant (LAAP). Final report. Contract DAAK-11-85-D-007; Report AMXTH-IR-TE-88242.
28. **Won, W. D., L. H. DiSalvo, and J. Ng.** 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. Appl. Environ. Microbiol. **31**:576–580.
29. **Won, W. D., R. J. Heckley, D. J. Glover, and J. C. Hoffsommer.** 1974. Metabolic disposition of 2,4,6-trinitrotoluene. Appl. Microbiol. **27**:513–516.