Bull. Environ. Contam. Toxicol. (2000) 65:28–34
© 2000 Springer-Verlag New York Inc.
DOI: 10.1007/s001280000090

Bulletin of Environmental Contamination and Toxicology

Mineralization of Trichloroethylene (TCE) by the White Rot Fungus Phanerochaete chrysosporium

J. S. Yadav,[2] C. Bethea,[1] C. A. Reddy[1]

[1] Department of Microbiology and the NSF Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824-1101, USA
[2] Department of Environmental Health, Division of Toxicology, University of Cincinnati, Cincinnati, OH 45267-0056, USA

Received: 7 October 1999/Accepted: 11 May 2000

Trichloroethylene (TCE), a volatile aliphatic halocarbon compound, is a common industrial degreasing solvent as well as a precursor for industrial chemical synthesis. Large scale production and usage of TCE each year, combined with improper waste disposal practices and landfill leaching, has led to the contamination of soils, air, and aquifers with this chemical (Storck 1987). TCE is a suspected carcinogen and prolonged exposure to it has been shown to cause blood and cardiac irregularities, as well as neurological problems (Bruckner et al. 1989). It is one of the top ten most commonly detected chemicals at the hazardous waste sites and has been classified as a priority pollutant regulated by the US Environmental Protection Agency.

TCE has been shown to be degraded by individual bacteria as well as bacterial consortia (Landa et al. 1994; Hyman et al. 1995; Chauhan et al. 1998). There is, however, little information on fungal metabolism of this chemical. The ligninolytic white rot fungus, *Phanerochaete chrysosporium*, is known to degrade a wide range of aromatic pollutants including dioxins, PCBs, chlorobenzenes, DDT, phenoxyherbicides, and chlorophenols (Bumpus and Aust 1987; Hammel 1992; Reddy 1995). Conventionally, ligninolytic peroxidases including lignin peroxidases (LiPs) and manganese dependent peroxidases (MnPs), produced under nutrient-limited culture conditions, have been implicated in the metabolism of a number of these environmental pollutants by *P. chrysosporium* (Hammel 1992; Valli et al. 1992). This report constitutes the first demonstration of degradation and mineralization of TCE to CO_2 by *P. chrysosporium* and highlights the importance of using nutrient-rich conditions for an enhanced degradation of this aliphatic pollutant.

MATERIALS AND METHODS

P. chrysosporium ME-446 (ATCC 34541) used in this study was maintained on malt extract (ME) agar slants described previously (Yadav and Reddy 1993). ME medium (8 m*M* N and 2% glucose), low-nitrogen medium (2.4 m*M* N, 1% glucose), and high-N medium (24 m*M* N, 1% glucose) have been described previously (Yadav and Reddy 1993). The fungus was grown as static cultures (10 mL) in sterile 125-mL serum bottles as previously described (Yadav and Reddy

Correspondence to: C. A. Reddy

1993). Immediately after inoculation, the bottles were flushed with O_2 for 1 min followed by the addition of liquid TCE at 10 ppm concentration, unless otherwise indicated. The bottles were then sealed immediately with Teflon-coated gray butyl rubber stoppers and aluminum crimps. The bottles were shaken for 30 min to disperse TCE in the liquid before incubating at 37°C for varying periods (see below). Each experiment included uninoculated and heat-killed controls in triplicate. The heat-killed controls consisted of autoclaved cultures that had been pregrown for 7 days under conditions identical to those of the experimental cultures. Percent degradation at a specified interval was calculated by comparing concentrations in the uninoculated blanks with those in the experimental bottles. All degradation values were corrected for the sorption values determined using the heat-killed controls.

TCE concentration in the headspace was measured by gas chromatography (GC) as described previously (Yadav and Reddy 1993; Yadav et al. 1995a). A 500 µL headspace sample was analyzed, following equilibration of the cultures at 25°C. Fungal biomass was measured as mycelial dry weight as previously described (Yadav and Reddy 1993).

A uniformly-labeled ^{14}C-TCE (sp. activity = 10 mCi/mmol; >99% purity), was obtained from Dr. Craig Criddle of the Environmental Engineering Department at Michigan State University and $4x10^5$ cpm was added to each 10 mL static culture, along with 10 ppm of the unlabeled TCE (Aldrich Chemical Co., Milwaukee, Wis). An uninoculated control with 1 m*M* sodium azide was run in parallel for comparison. At weekly intervals, the $^{14}CO_2$ generated was trapped in 1 *N* NaOH by flushing the culture headspace with CO_2-free air for 30 min and quantified by scintillation counter using Safety Solve (Yadav et al. 1995a). An organic trap (ORBO 32 S from Supelco Inc., Bellefonte, Pa) was used to trap volatile organic counts due to ^{14}C-TCE as described previously (Yadav et al. 1995a). Since the fungal cultures had acidic pH, further acidification of a selected set of flushed bottles by adding HCl to pH 2.0 did not yield additional $^{14}CO_2$ in the NaOH trap. The values were corrected for the background counts detected in the uninoculated controls.

RESULTS AND DISCUSSION

TCE degradation by *P. chrysosporium* (Fig. 1) in ME medium after three weeks incubation was 46.2%. Under identical conditions, TCE degradation in high N medium was 14.0 % while in low N medium it was 6.5 %. Since the ME medium supported maximum degradation, this became the medium of choice for the subsequent experiments. Time course of TCE degradation in ME medium (Fig. 2A) showed that, after a slight lag in the first two days, there was a steep increase in the rate of degradation, coincident with the increase in the fungal biomass, up to 6 days. This was followed by a slower rate during the remaining period of incubation.

The observed TCE disappearance in the degradation experiments (Fig. 1 and 2A)

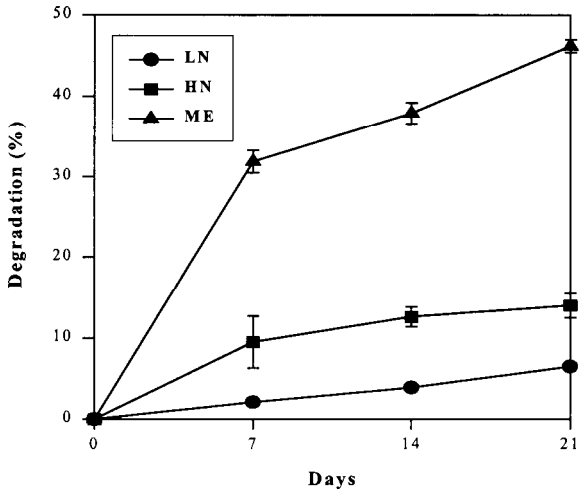

Figure 1. Degradation of trichloroethylene (TCE) by *P. chrysosporium* in different media. The fungus was grown as 10 mL-static cultures in low nitrogen (LN), high nitrogen (HN), and malt extract (ME) media, each of which was spiked with TCE (10 ppm). Cultures were incubated at 37°C for 3 weeks. All values were corrected for sorption values obtained with parallel heat-killed controls. Values presented are means ± standard deviations for triplicate cultures.

is due to fungal metabolic activity and not due to sorption to the fungal biomass because the values for the experimental cultures were corrected for sorption by subtracting the values for identical heat-killed control cultures. Moreover, there was a parallel relationship between the fungal growth and TCE degradation (Fig. 2A).

Degradation of a number of chlorinated hydrocarbons by *P. chrysosporium* has been shown to involve its extracellular ligninolytic peroxidases, LiPs and MnPs (Hammel 1992; Valli et al. 1992). Our results show higher level of TCE degradation in high N medium and ME medium, in which production of LiPs and MnPs by *P. chrysosporium* is known to be suppressed, than in low N medium in which *P. chrysosporium* is known to produce a full complement of LiPs and MnPs (Reddy 1995). These results indicate that TCE degradation by *P. chrysosporium* is not linked to the production of LiPs and MnPs and appear to involve alternate enzyme system(s) upregulated under these nutrient-rich conditions. Mineralization of TCE in the absence of LiPs and MnPs appears to be consistent with the pattern reported by us and others for the degradation of other aromatic pollutants by white-rot basidiomycetes (Kohler et al. 1988; Sutherland et al. 1991; Yadav and Reddy 1993; Yadav et al. 1995a and b; Kullman and Matsumura 1996; Jackson et al. 1999).

To determine the effect of TCE concentration on its degradability and growth of *P. chrysosporium,* the initial TCE concentration was varied from 5 to 100 ppm. The percent degradation of TCE decreased with the increase in TCE concentrations although the net degradation of TCE was still greater at the higher concentrations (Table 1). However, the effect on mycelial dry weight was relatively insignificant except that at a concentration of 100 ppm TCE, there was a slight inhibition of growth. Toleration of relatively high concentrations of TCE by *P. chrysosporium* without significant loss of growth suggests the potential usefulness of this organism for bioremediation of TCE-contaminated environments.

Table 1. Effect of initial TCE concentration on its degradation and mycelial dry weight in cultures of *P. chysosporium* in ME medium[a]

Initial TCE concentration (ppm)	Net TCE degradation[b,c] (ppm)	Percent degradation[c]	Mycelial dry weight (mg)[c]
5	1.81 ± 0.24	36.2 ± 4.8	120 ± 0
10	3.15 ± 0.51	31.5 ± 5.1	110 ± 10
20	4.58 ± 0.34	22.9 ± 1.7	117 ± 20
50	6.35 ± 1.6	12.7 ± 3.2	110 ± 0
75	6.98 ± 2.25	9.3 ± 3.0	107 ± 6
100	6.50 ± 1.0	6.5 ± 1.0	97 ± 10

[a]The fungus was grown in static cultures spiked with varying concentrations of TCE (5 to 100 ppm) and incubated at 37°C for 7 days. Mycelial dry weights are given in mg per 10 mL culture.

[b]Degradation values are corrected for sorption values obtained with parallel heat-killed controls.

[c]Values presented are means ± standard deviations for triplicate cultures.

Results presented in Fig. 2B on mineralization of ^{14}C-TCE to $^{14}CO_2$ in ME cultures show that most of the mineralization (27.4%) occurred during the first week of incubation. This was only slightly less than the total degradation of 31.9% observed during the same period. Cultures that were reoxygenated after the first week showed higher level of mineralization (38.5%) than control cultures suggesting that cultures may be oxygen-limited after the first week of incubation. Moreover, weekly reoxygenation resulted in better mineralization than biweekly reoxygenation (data not shown). These observations indicate the importance of available oxygen for the activity of the TCE degrading system of this organism.

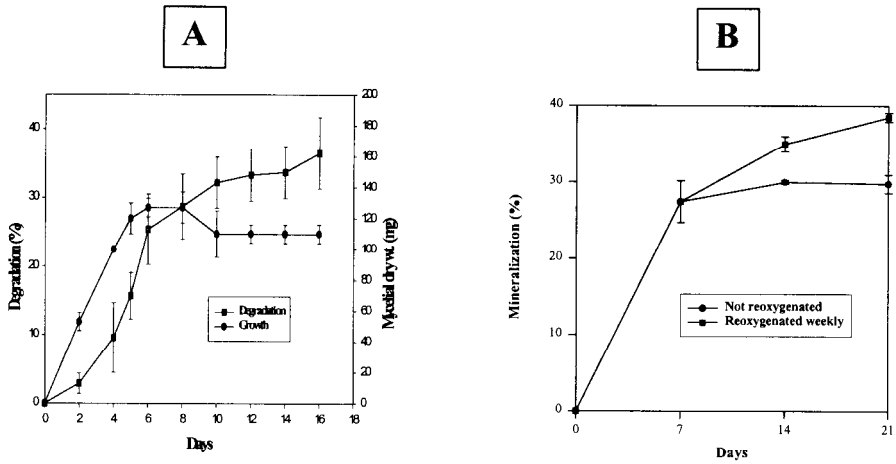

Figure 2 A. Rate of growth and degradation of TCE by *P. chrysosporium* in ME medium. The fungus was grown in 10 mL static ME cultures with TCE (10 ppm) at 37°C for 16 days. Growth was measured as mycelial dry weight (Yadav and Reddy 1993). Degradation was measured by headspace analysis at 2-day intervals. All values are corrected for sorption values obtained with parallel heat-killed controls. Values presented are means ± standard deviations for triplicate cultures.

B. Rate of mineralization of ^{14}C-TCE in ME medium by *P. chrysosporium.* The fungus was grown as described above. Each culture received $4x10^5$ cpm of ^{14}C -TCE along with 10 ppm of unlabeled TCE. Duplicate cultures were sacrificed every week for 3 weeks for headspace flushing to quantify the $^{14}CO_2$ production as described in Materials and Methods. Another set of duplicate cultures was reoxygenated at weekly intervals after $^{14}CO_2$ trapping.

Since much of the primary growth of this fungus occurs in the first week of incubation (Fig. 2A), most of the oxygen gets depleted during this time leading to reduced TCE mineralization in the remaining incubation period. Plateauing of degradation of BTEX (benzene, toluene, ethyl benzene, and xylenes) after 5 days incubation in identically-designed fungal cultures in sealed serum bottles was ascribed to depleted oxygen levels in earlier studies (Yadav and Reddy 1993). Those studies showed 95.4% consumption of available oxygen in 5 day-old cultures in these serum bottles.

Comparison of the values for total degradation (46.2%) and mineralization (38.5%) of TCE suggests that most of the degraded TCE is mineralized to CO_2 and a part may accumulate as metabolic intermediate (s). Ability of *P. chrysosporium* to substantially degrade TCE under nutrient-rich conditions (ME and HN media), indicates the potential for using this fungus for TCE bioremediation particularly in nutrient-rich environmental matrices.

Acknowledgments. We thank Craig Criddle for providing the radiolabeled TCE and Jim Tiedje for the use of the gas chromatography unit. This work was supported in part by grant DE-FG02-85 ER 13369 from the U.S. Department of Energy and grant BIR 912 006 from the NSF Center for Microbial Ecology.

REFERENCES

Bruckner JV, Davis BD, Blancato JN (1989) Metabolism, toxicity, and carcinogenicity of trichloroethylene. Crit Rev Toxicol 20:31-50

Bumpus JA, Aust SD (1987) Biodegradation of environmental pollutants by the white rot fungus *Phanerochaete chrysosporium:* involvement of the lignin degrading system. Bioessays 6: 166- 170

Chauhan S, Barbieri P, Wood TK (1998) Oxidation of trichloroethylene, 1,1-dichloroethylene, and chloroform by toluene/o-xylene monooxygenase from *Pseudomonas stutzeri* OX1. Appl Environ Microbiol 64:3023-3024

Hammel KE (1992) Oxidation of aromatic pollutants by lignin-degrading fungi and their extracellular peroxidases, In: Sigel H, Sigel A (ed) Metal ions in biological systems, vol 28. Degradation of environmental pollutants by microorganisms and their metalloenzymes. Marcel Dekker, Inc., New York, p. 41-60

Hyman MR, Russell SA, Roger LE, Williamson KJ, Arp DJ (1995) Inhibition, inactivation, and recovery of ammonia-oxidizing activity in cometabolism of trichloroethylene by *Nitrosomonas europaea.* Appl Environ Microbiol 61: 1480-1487

Jackson MM, Hou L-H, Banerjee HN, Sridhar R, Dutta SK (1999) Disappearance of 2,4-dinitrotoluene and 2-amino,4,6-dinitrotoluene by *Phanerochaete chrysosporium* under non-ligninolytic conditions. Bull Environ Contam Toxicol 62:390-396

Kohler A, Jager A, Willershausen H, Graf H (1988) Extracellular ligninase of *Phanerochaete chrysosporium* Burdsall has no role in the degradation of DDT. Appl Microbiol Biotechnol 29:618-620

Kullman S, Matsumura F (1996) Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan. Appl Environ Microbiol, 62:593-600

Landa AS, Sipkema EM, Weijma J, Beenackers AACM, Dolfing J, Janssen DB (1994) Cometabolic degradation of trichloroethylene by *Pseudomonas cepacia* G4 in a chemostat with toluene as the primary substrate. Appl Environ Microbiol 60: 3368-3374

Reddy CA (1995) The potential of white rot fungi for the treatment of pollutants. Curr Opinion Biotechnol 6:320-328.

Storck W (1987) Chlorinated solvent use hurt by federal rules. Chem Eng News 65:ll

Sutherland JB, Selby AL, Freeman JP, Evans FE, Cerniglia, CE (199 1) Metabolism of phenanthrene by *Phanerochaete chrysosporium.* Appl Environ Microbiol 57:3310-3316

Valli K, Wariishi H, Gold MH (1992) Degradation of 2,7-dichlorodibenzo-p-dioxin by the lignin-degrading basidiomycete *Phanerochaete*

chrysosporium. J Bacterial 174:2131-2137

Yadav JS, Reddy CA (1993) Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium.* Appl Environ Microbiol 59:756-762

Yadav JS, Wallace RE, Reddy CA (1995a) Mineralization of mono- and di-chloro-benzenes and simultaneous degradation of chloro- and methyl-substituted benzenes by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 61:677-680

Yadav JS, Quensen III JF, Tiedje JM, Reddy CA (1995b) Degradation of polychlorinated biphenyl mixtures (Aroclors 1242, 1254, and 1260) by the white rot fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 61:2560-2565