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Reclamation and Reuse of Waste from Wood Based Industries: First year report to the RRTTC

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Reclamation and Reuse of Waste From Wood Based Industries

Research Progress Report

for

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Submitted by

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Overall objective

The overall objective of this research is to use a wood-based waste generated in Iowa to serve as a growth substrate in a treatment system designed to remediate soils that are contaminated with hazardous organic compounds.

Specific objectives

1. To assess the ability of wood based wastes to promote growth of selected white rot fungi to be used in the bioremediation of soil contaminated with TNT, HMX and RDX.
2. To assess the effectiveness of a wood based waste to promote bioremediation of soil contaminated with TNT, HMX and RDX.
3. To determine if aqueous extracts of treated soils are less toxic that those of control soils.

Introduction

Soil that is contaminated with synthetic environmentally persistent toxic organic chemicals represents a potential health risk. There are several ways in which this problem may be addressed. For example, contaminated soils may be placed in secure landfills. Alternatively they may be incinerated. Soils may also be decontaminated by bioremediation. In some cases, indigenous microorganisms effectively metabolize the contaminating chemicals. This, however, is often a slow process that can sometimes be enhanced by adding nutrients to promote microbial growth and metabolism. In some cases indigenous microorganisms do not possess the metabolic capability to degrade the contaminating chemicals. In such cases, exogenous microorganisms that do possess the requisite metabolic capabilities are added.

The present investigation centers on assessing the efficacy of using a wood-based waste generated by Weyerhauser Corporation to enhance the ability of white rot fungi to remediate soil that is contaminated with munitions waste at the Naval Weapons Station Yorktown (NWSY). The NWSY was established in 1918, on a 10,624 tract of land on the Virginia Peninsula in James and York Counties in the State of Virginia. It was originally called the US Naval Mine Depot. During its time of operation normal activities involved in weapons manufacture and processing resulted in contamination of soil and water with the high explosives TNT, RDX and HMX. In 1992, the NWSY was

placed on the National Priorities List and slated for environmental cleanup.

Methods and Materials

Microorganisms. Phanerochaete chrysosporium (BKM-F-1767) was obtained from the American Type Culture Collection. This fungus and a proprietary strain (strain F-600) of another white rot fungus were adapted to grow in the presence of high concentrations of TNT by Mycotech Corporation. Cultures were maintained on malt agar slants.

Chemicals. Analytical grade TNT was purchased from Chem Service (Westchester, PA) and ^{14}C-TNT (specific activity =21.58 mCi/mmol) was purchased from ChemSyn Science Laboratories (Lenexa, KS). A proprietary cellulose based industrial byproduct was supplied by Weyerhauser Corporation (Cedar Rapids, IA).

Substrate Utilization Studies. Strain F-600 and *P. chrysosporium* were cultured on agar plates containing 2% (w/w) noble agar containing 1% (w/w) industrial byproduct and on agar plates containing 2% (w/w) noble agar and 1% (w/w) industrial byproduct extract. The industrial byproduct extract was produced by stirring 500 g of this material with 1000 mL of water for 30 min. The mixture was then filtered and the filtrate was used to prepare the agar plates described above. Agar plates were inoculated with fungi and growth was measured daily until the mycelium reached the edge of the Petri plate.

Biodegradation of ^{14}-TNT by white rot fungi. Five 20 mL stationary liquid cultures of strain F-600 and both strains of P. *chrysosporium* were prepared using previously described nutrient nitrogen limited media (Fenn and Kirk, Kirk *et al.* 1978). Both strains of *P. chrysosporium* and strain F-600 were incubated at 37^{o} and 25^{o}C, respectively. After three days 60 µL of a ^{14}C-TNT solution (260,000 dpm) in acetone was added to cultures containing *P. chrysosporium.* After six days of incubation, the same amount of ^{14}C-TNT was added to strain F-600. Flasks containing uninoculated media served as controls. Evolution of $^{14}CO_2$ was assessed after three more days of incubation and at three day intervals for a total incubation time of 30 days at which time a mass balance

analysis was performed as described by Bumpus (1989).

Remediation of contaminated soil-Pilot Studies. Soil from two areas on Site 7 at the NWSY was collected, screened through a one-half inch sieve and mixed together. In 3.5 gallon containers was placed a two inch layer of gravel. Several treatments containing various amounts of fungi, solid and liquid nutrients were studied. Fungal inoculation was achieved by the addition of either a 20% or a 40% (wt/wt) inoculum from spawn bags. Samples were collected before addition of nutrients (i.e., baseline samples), at time zero (immediately after addition of nutrients), and at one month, two month and four month intervals. these samples were frozen and shipped on dry ice to Mycotech Corporation for analysis. EPA method 8330 was used to determine the concentrations of TNT, RDX and HMX present.

Remediation of contaminated soil-Field Studies. For the field demonstration, two plots with wooden frames 36 ft long, 8 ft wide and 1 ft in depth were constructed. A 40 mil liner was placed on the bottom of each plot and 2 inches of coarse gravel was placed on the liner to provide appropriate drainage. Approximately six cubic yards of contaminated soil from Site 7 was added to each plot and spread uniformly using a back hoe. To each plot was added two cubic yards of the cellulose based industrial byproduct, 300 lbs of a proprietary fungal specific nutrient developed by Mycotech Corporation and 240 lbs of gypsum. One plot was inoculated with one cubic yard of strain F-600 on a solid substrate. The other plot received one cubic yard of the uninoculated solid substrate. Each plot was mixed using a rototiller. One-hundred gallons of a proprietary liquid amendment were then added and the plots were covered with a 7 mil tarp. Soil samples were obtained before addition of nutrients (baseline), at time zero and after two weeks, four weeks and two months of incubation. Ten samples were taken at a depth of one foot for each timepoint and analyzed using EPA method 8330 to determine the concentrations of TNT, RDX and HMX.

Toxicity Testing. Toxicity of aqueous extracts of treated and untreated soils was assessed using the *Daphnia magna* method and the Microtox® assay.

Results

The proprietary cellulose based byproduct and its aqueous extract promoted growth of strain F-600. When grown on Noble agar alone, this fungus reached the edge of the Petri plate in fifteen days. In the presence of the proprietary cellulose byproduct this took eleven days whereas in the presence of the aqueous extract it took eight days for this to occur. The effect of these materials had little influence on the growth of *P. chrysosporium* . In the absence of these materials, *P. chrysosporium* reached the edge of the Petri plate in three days. In their presence, it took four days.

P. chrysosporium and strain F-600 both metabolized ^{14}C-TNT. However, *P. chrysosporium* was more effective in degrading ^{14}C-TNT to $^{14}CO_2$. HPLC analysis revealed that both fungal strains were effective in degrading ^{14}C-TNT to more polar metabolites. The initial step in the biodegradation of TNT by both fungi was the reduction of this compound to 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene. It is worth noting that strain F-600 converted a substantial amount of ^{14}C-TNT to particulate material that was not soluble in water or in methylene chloride, suggesting that it was covalently incorporated into structural components of the fungus.

Results of the pilot studies were disappointing. Only minimal amounts of TNT, RDX and HMX disappearance were observed. This, however, was attributed to the high concentrations of these chemicals in the soil and their toxicity to the fungi. Because, TNT metabolites were discovered in these studies, the decision was made to proceed with the field studies. However, for the field studies soils with lower concentrations of TNT, RDX and HMX were used. In soil amended with growth substrates and strain F-600 concentrations of TNT, HMX and RDX were reduced from 194.0 $\pm$ 50, 61 $\pm$ 20 and 118 $\pm$ 30 mg/Kg to 3 $\pm$ 4, 18 $\pm$ 7 and 5 $\pm$ 3 mg/Kg , respectively during 62 days of incubation. Interestingly, in soils that were amended with nutrients but not strain F-600, the concentrations of TNT, HMX and RDX were reduced from 283 $\pm$ 10, 67 $\pm$ 20 and 144 $\pm$ 50 mg/Kg to 10 $\pm$ 10, 34 $\pm$ 20 and 12 $\pm$ 10 mg/Kg, respectively, during the same incubation period.

Aqueous extracts of soil samples from the field studies before and after incubation

revealed that these treatments reduced the level of toxicity. For example, in the *Daphnia magna* assay a 50:50 mixture of the aqueous extract from untreated samples with water was required to cause a 50% mortality of the target species whereas a 70:30 mixture of the aqueous extract of plot 1 (*i.e.*, the plot treated with nutrients and strain F-600) with water caused a 50% mortality thus indicating that this treatment had decreased the toxicity of the soil. Interestingly, aqueous extracts from plot 2 (*i.e.*, the plot treated with nutrients only) were not toxic to *Daphnia magna*. Aqueous extracts from plots 1 and 2 were not toxic to the target organism in the Microtox® assay.

Discussion

These investigations demonstrate that *P. chrysosporium* and strain F-600 both are able to mediate extensive biodegradation of ^{14}C-TNT, the most recalcitrant of the high explosives under investigation. *P. chrysosporium* mediated more extensive degradation to $^{14}CO_2$ whereas F-600 was more effective in immobilizing metabolites of ^{14}C-TNT in fungal biomass. Because of its inability to degrade ^{14}C-TNT to $^{14}CO_2$, it is likely that incorporation of carbon atoms originally in TNT into biomass is the major route for detoxification of this compound by strain F-600.

Field studies demonstrated that when soils contaminated with TNT, HMX and RDX are amended with fungal growth substrates and strain F-600 substantial decontamination of the soil occurs. However, more significant is the observation that similar levels of decontamination occurs in soils that are amended with the nutrient but not with the fungus, thus indicating that indigenous microorganisms are able to effectively degrade these compounds if provided with suitable growth amendments.

Graduate Student Participation

Much this research was performed by Ms. Catherine A. Axtell in partial fulfillment of the requirements for the Master of Science degree in Environmental Science at the University of Northern Iowa. It is expected that this degree will be awarded in December 1997. Ms. Axtell is currently a doctoral student at Iowa State University.

Publications, reports and presentations resulting from this research.

Axtell, C.A. (1997) Bioremediation of Soil Contaminated with Explosives. Master's Thesis, Environmental Sciences Program, University of Northern Iowa (In progress).

Axtell, C., C. Johnston and J.A. Bumpus (1997) Biodegradation of explosives by wood-rotting fungi. Annual Meeting of the Iowa Academy of Sciences, April 26, 1997, Clarke College.

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Johnston, C.G. (1997) Degradation of Ordnance Ingredients by Different Strains of White Rot Fungi. Final Report and Proposal for Commercial Bioremediation. Prepared for the Indian Head Division, Naval Surface Warfare Center, Indianhead, MD.

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Kirk, T.K., E. Schultz, W.J. Connors, L.F. Lorenz and J.G. Zeikus (1977) Influence of Culture Parameters on Lignin Metabolism by Phanerochaete chrysosporium. Arch. Microbiol. **117**:277-285.