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Final Report

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Bioaugmentation using the white rot fungus Phanerochaete chrysosporium was found to be effective in enhancing TNT removal in lightly (i.e., below 100 mg/kg) contaminated soil. The percent reduction in TNT increased from 15% to 53% when soils were treated with fungal inoculated organic material. Maximum removal rates improved from 0.33 mg TNT/kg-day to 0.76 mg TNT/kg-day during the same treatment. Neither fungal inoculation nor organic amendment addition resulted in improving TNT removal kinetics in highly contaminated soils (i.e., above 1000 mg/kg).

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EXECUTIVE SUMMARY

Laboratory scale compost studies were conducted to evaluate the effect of bioaugmentation with the white rot fungus, *Phanerochaete chrysosporium*, on remediating explosives contaminated soil. All studies involved the use of explosives contaminated soil obtained from the Umatilla Army Depot Activity (UMDA)

Short term (*e.g.*, 25 days) batch studies demonstrated that in lightly contaminated soils (*e.g.*, below 100 ppm), addition of organic amendments suitable for fungal growth increased the rate and extent of TNT removal compared to unamended conditions. The extent of removal increased from 15% to 33% when organic amendment was added. In systems that also received fungal inoculation, the extent of removal increased to 53%. Estimation of removal rates indicated that fungal inoculation did not improve TNT removal kinetics suggesting that fungal inoculation improves the initial removal rate but not the long term rate compared to systems receiving organic amendment only. Maximum TNT removal rates of 0.78 mg/kg-day, 0.76 mg/kg-day and 0.33 mg/kg-day were observed for systems receiving lightly contaminated soil plus organic amendment, organic amendment plus fungal inoculum and no amendment nor inoculum.

In soils containing high concentrations of TNT (*e.g.*, above 1000 ppm), the rate and extent of TNT removal did not vary between treatment conditions. Approximately 35 to 40% of TNT was removed under all conditions. Irreversible sorption of TNT to organic amendment and/or chemical reaction may explain the observed TNT removal. Soils containing high concentrations of TNT may also have been moisture limited resulting in microbial inhibition. Independent analysis of soil moisture content as a function of soil pressure potential indicated that the field moisture capacity of the highly contaminated soil was zero.

No traces of the intermediates 2-amino 4,6 dinitrotoluene, 4-amino 2,6 dinitrotoluene or dinitrobenzene were observed in any soil extracts suggesting that aerobic conditions prevailed throughout the test period.

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INTRODUCTION

The production and processing of explosives have resulted in widespread soil contamination at ordinance manufacturing and storage facilities. The current treatment method for remediation of these soils is incineration. While effective, the costs associated with excavation, transportation, fuel requirements and ash handling have made this option economically prohibitive even when large volumes of soil are treated. An alternative method being considered for remediation of explosives contaminated soil is composting. Previous laboratory and pilot scale studies directed by the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) have demonstrated that composting is a cost effective method to remediate soils contaminated by the explosives Trinitrotoluene (TNT), Hexahydro-1,3,5 trinitro-1,3,5-triazine (RDX) and Octohydro-1,3,5,7-tetranitro-1,3,5,7-tetraazaocine (HMX) (Williams and Myler, 1990). Approaches to enhance the rate and extent of explosives soil composting have focused on the use of bioaugmentation. Bioaugmentation is the enrichment of the compost system by addition of microorganisms known to degrade specific explosive compounds. One such organism which transforms TNT in pure culture systems is the white rot fungus, *Phanerochaete chrysosporium* (Spiker *et al.*, 1992; Tsai, 1991). Although demonstrated in pure liquid culture, fungal enhanced degradation of TNT in soils has never been reported. Fungal composting technology varies little from the standard soils composting technology developed by USATHAMA. The key variables are the type of organic amendment chosen, moisture levels, aeration and temperature maintained in the compost pile (Haug, 1980).

Goal and Objectives

The goal of this research program was to evaluate the effect of bioaugmentation of explosives soil composting with the white rot fungus *Phanerochaete chrysosporium*. Because of unforeseen delays pertaining to the procurement of explosives contaminated soil, the specific research objectives were modified from the original proposal.

The modified objectives included:

1. Evaluation of the effect of fungal inoculation (*Phanerochaete chrysosporium*) on the rate and extent of TNT removal during explosives soil composting.
2. Monitoring the rate of production and utilization of TNT chemical intermediates (*e.g.*, 2 amino 4, 6 dinitrotoluene, 4 amino 2,6 dinitrotoluene) during soil composting operation.
3. Identification of the presence or absence of the intermediate, dinitrobenzene, in pre and post composted soil using gas chromatography/mass spectrometry (GC/MS).

TECHNICAL DISCUSSION

Materials and Methods

Explosives contaminated soil was obtained from the U.S. Army Munitions Depot in Umatilla, Oregon (UMDA). Three five gallon plastic buckets of the contaminated soil (arbitrarily designated soils I, II, and III) were transported from UMDA and stored at 4 °C at the Utah Water Research Laboratory (UWRL) walk-in freezer until used in composting experiments. All three buckets of soil were evaluated by Utah State University (USU) soil testing laboratory for standard soil physical and chemical properties. Results from these tests are provided in Tables 1 and 2. Chemical analyses of soils indicated that soil I had an average TNT concentration above 1000 ppm while soils II and III had TNT concentrations below 100 ppm. Due to the chemical and physical similarity between soils II and III, it was decided that evaluation of only soils I and II would be necessary since they reflect the range of TNT concentrations found at the UMDA facility.

Table 1. Properties of Soil Samples I, II and III

Sample	Sand %	Silt %	Clay %	Texture	pH	EC[1]	%OC[2]	CEC[3]	% Moisture @ 1/3 Bar
I	92	5	3	Sand	7.3	0.2	0.23	6.2	4.3
II	85	10	5	Loamy Sand	7.1	0.2	1.91	7.0	0
III	85	11	4	Loamy Sand	7.1	0.2	1.94	6.6	0

[1]EC: Electrical Conductivity in mm hos/cm

[2]OC: Organic Carbon %

[3]CEC: Cation Exchange Capacity in meq/100g

Table 2. Elemental Analysis for Soils I, II, and III*

Soil	P	K	Zn	Fe	Cu	Mn	Cd	Cr	Ni	Pb
I	3.2	114	0.8	5.6	0.1	6.1	<	<	<	<
II	4.6	101	2.4	10.4	0.2	6.9	0.1	<	<	0.7
III	4.3	97	2.2	18.2	0.2	7.0	0.1	<	0.1	<

Soil	Ca	Mg	Na	K	B	SO_4
I	39.52	8.48	15.11	7.3	0.06	38.8
II	45.86	9.63	18.53	6.8	0.04	23.9
III	48.97	10.11	21.99	7.5	0.06	25.2

*mg per kg of soil

Compost-Laboratory Study

Mixing Requirements

Preliminary investigations by Utah Water Research Laboratory (UWRL) personnel and others have demonstrated that the spatial variations in TNT concentration in UMDA soils are significant. To reduce the heterogeneity in TNT concentration, contaminated soils were mixed before initiation of the bench scale composting study. The mixing procedure consisted of placing one kilogram of both soils I and II in separate aluminum containers. Each container was mixed for approximately twenty-four hours in a tumble shaker system. After mixing, soils were ready for laboratory compost experiments.

Composting Microcosm Operation

Soil composting was initiated by placing four grams of mixed contaminated soil into a series of compost microcosms. Each microcosm consisted of a 250 milliliter beaker covered with polyethylene to facilitate oxygen transfer while minimizing moisture loss (Figure 1). In all, three separate treatment systems were evaluated to estimate the significance of fungal bioaugmentation on explosive soil composting (Table 3).

Table 3. Composting Treatments Evaluated in Laboratory Microcosms*

Treatment A. - Soil + corn cobs (no fungal inoculum)
Treatment B. - Soil + fungus inoculated corn cobs
Treatment C. - Soil only (no organic)

*All compost reactors were evaluated in triplicate

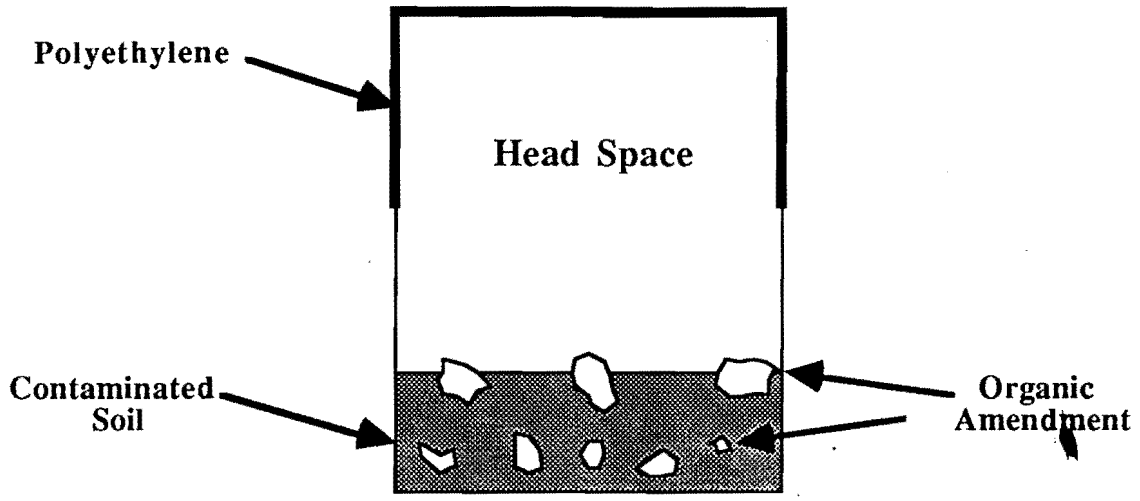

Figure 1. Schematic Diagram of Laboratory Scale Microcosm

toxic, explosive

$CH_3C_6H_2(NO_2)_3$ = TNT

Two treatment approaches evaluated in the present study involved the use of added organic amendments (*i.e., Treatments* A and B) while the third received no additional organic material (*Treatment* C). Since trinitrotoluene cannot serve as a growth substrate for the white rot fungus, the organic amendment chosen had to meet both the carbon and energy requirements for the fungus. Moreover, the white rot fungus is known to be sensitive to low oxygen tensions with atmospheric oxygen concentrations of less than 5% having been shown to completely shut down the enzymatic pathway responsible for TNT degradation (Dosoretz *et al.*, 1990; Haemmerli *et al.*, 1986; Sanglard *et al.*, 1986). Due to the oxygen sensitivity, measures must be taken to minimize oxygen transfer limitations during fungal compost reactor operation.

Corn cobs were selected as the organic amendment since they: 1) were known to be utilized by *Phanerochaete chrysosporium* as an energy source, 2) act as an effective bulking agent to enhance oxygen transfer in the compost mixture and 3) are inexpensive.

Prior to addition of organic amendment to soil in Treatment A, corn cobs were saturated with distilled deionized water (DDW). After allowing the corn cobs to soak in DDW for two hours, cobs were drained and added to the appropriate compost microcosms. For Treatment B, fungal

inoculated corn cobs were prepared by adding a fungal conidia suspension to saturated corn cobs. The white rot fungus, *Phanerochaete chrysosporium* BKMF-1767, which was obtained from the Utah State University Biotechnology Center, was maintained at 39°C on 4% malt agar slants in a 250 milliliter Wheaten bottle with an aerobic head space. Conidia taken from a four-week old slant were shaken with 100 milliliters of distilled deionized water. The fungal suspension was used to inoculate two kilograms of moistened corn cobs. Once fungal growth was confirmed by visual inspection, the corn cobs were added to the appropriate microcosms (Treatment B). The saturated corn cobs were added to contaminated soil in Treatments A and B on a 1:1 basis (w/w). After addition of corn cobs, compost reactors were mixed with a glass rod and incubated at a constant temperature of 20°C.

To maintain aerobic conditions, compost microcosms were aerated every other day with pure oxygen at a rate of 200 mL/minute for five minutes. The moisture level of the compost systems were maintained at 40 - 60% of soil field capacity by the periodic addition of distilled deionized water (DDW).

Quality Assurance and Quality Control

The batch kinetic study was conducted in accordance with the Quality Assurance/Quality Control (QA/QC) plan of the Environmental Quality Laboratory at Utah State University. Six sampling events were used to monitor TNT and metabolite concentrations over time. These sampling events occurred on days 0, 4, 7, 18, 21 and 25. All sampling analyses were done in triplicate with sufficient numbers of blanks and standards to confirm the accuracy of instrument response. Best fit curves for data were plotted using Cricket™ Graph (MacIntosh, Inc.) computer program.

Extraction of Compost Samples

At the selected sampling intervals, triplicate samples were extracted and analyzed for trinitrotoluene (TNT) and metabolites. Compost samples were extracted by addition of sixty

milliliters of high performance liquid chromatography (HPLC) grade methanol (Baker, Inc.) to each microcosm. In all cases, the entire microcosm was sacrificed and analyzed for chemical contaminants.

After addition of methanol, the solvent saturated slurry was sonicated for thirty minutes. After allowing the sample to settle for approximately thirty minutes, the slurry was centrifuged at 7500 rpm for one hour. Twenty milliliters of the slurry supernatant solution was filtered using a syringe filter (0.22 um, 25 mm diameter Nucleopore Brand). The filtered extracts were collected and analyzed for TNT and its chemical intermediates.

TNT and Metabolite Concentrations in Compost Reactors

Compost extracts were examined for TNT and metabolites by a SHIMADZU liquid chromatograph (LC-6A) equipped with a C_8 Supelco column (3.5 cm x 4.6 mm inner diameter). Analytical samples of 2,4,6-trinitrotoluene, 2-amino 4,6-dinitrotoluene and 4-amino 2,6-dinitrotoluene were provided by the U.S. Army Analytical Chemical Laboratory located in Indianhead, MD.

HPLC operation employed a mobile phase of methanol containing a 2% solution of Tetrahydrofuran:water (ratio of 30:70). The wavelength for the UV detector (SHIMADZU SPD-6A) was maintained at 230 nanometers. The flow rate through the HPLC column was held constant at two milliliters per minute. The injection volume was 30 µl for lightly contaminated soil extracts (soil II) whereas for the highly contaminated soil extracts (soil I), the injection volume was 1µl. The HPLC procedure employed was a modification of the method provided by the U.S. Army Analytical Chemical Laboratory located in Indianhead, MD. Their method, which employed an acetonitrile:water mobile phase, did not produce the peak separation or clarity desired for the samples from UMDA.

Mass Spectrometry Analysis for Intermediates

A Varian 3700 Gas Chromatograph was used to estimate optimum conditions required for gas chromatographic-mass spectrometry (GC-MS) analysis. The GC-MS analyses were performed on an automated gas chromatograph and mass spectrometer (Finnigan Model IE-EC) at the Biotechnology Center of the Utah State University. A total of 2000 scans were obtained over the m/z range of 30 to 500 for each extract.

SUMMARY OF RESULTS

Quality Assurance/Quality Control

Quality assurance and quality control measures indicated that both extraction procedures and instrument response were within acceptable levels. The standard curves for the two ranges of TNT investigated (*i.e.*, 0-100 ppm and 0-2000 ppm) are contained in Figures 2 and 3, respectively. The reproducibility in HPLC standards provided confidence in the TNT concentration estimates of compost samples.

GC-MS scans are provided in Figures 4 and 5 for soils I and II, respectively. Neither the highly contaminated sample (soil I) nor the lightly contaminated soil (soil II) appeared to have any traces of the compounds dinitrobenzene, 2-amino 4, 6 dinitrotoluene or 4-amino 2, 6 dinitrotoluene. Both soils contained detectable amounts of TNT with a molecular ion peak occurring at a m/z of 210.

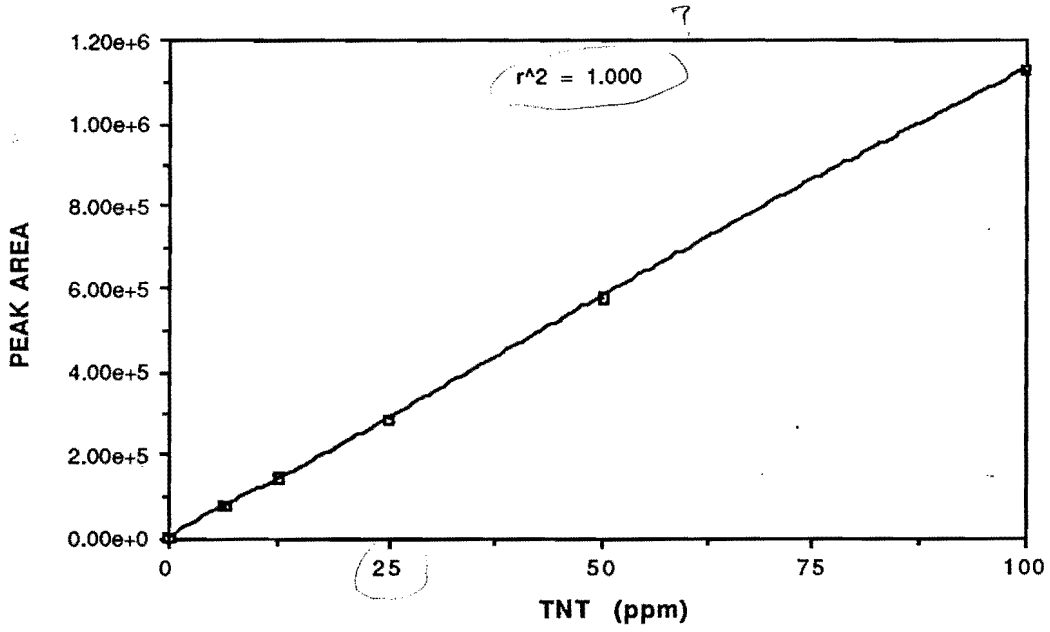

Figure 2. Standard Curve for Low Concentrations of TNT.

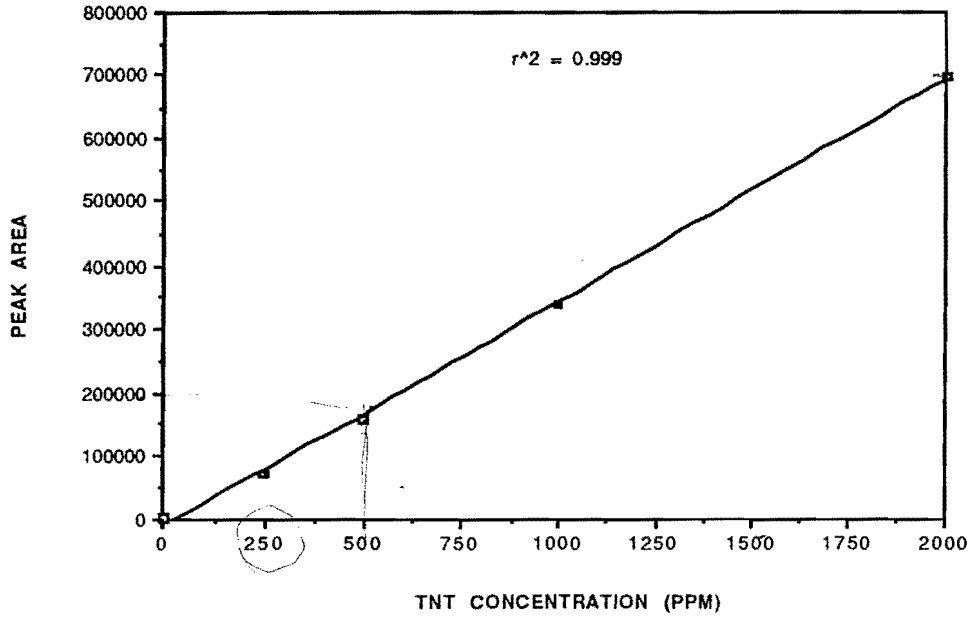

Figure 3. Standard curve for high concentration of TNT.

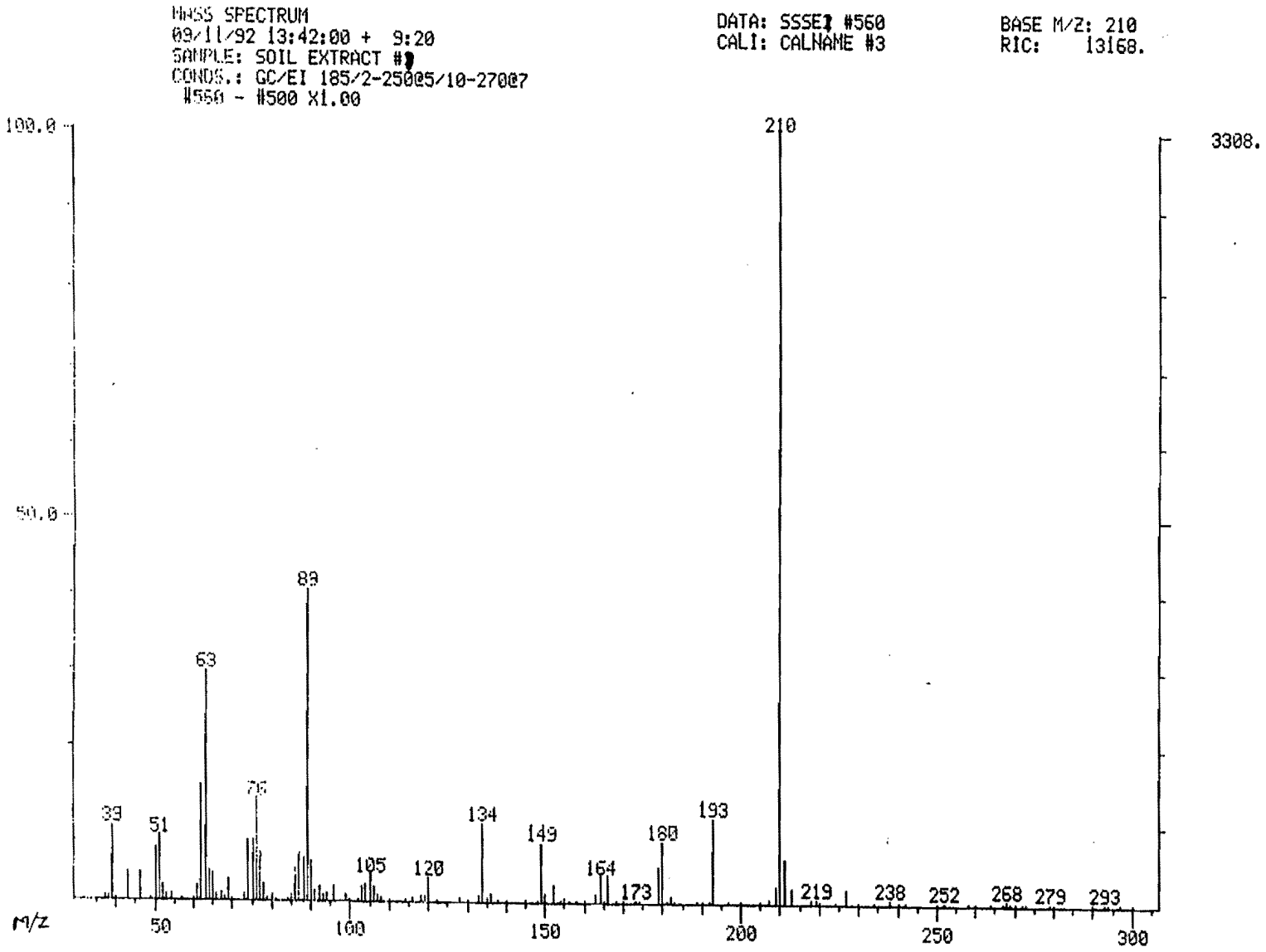

Figure 4. Mass Spec Scan for Soil Contaminated with Low Concentration of TNT.

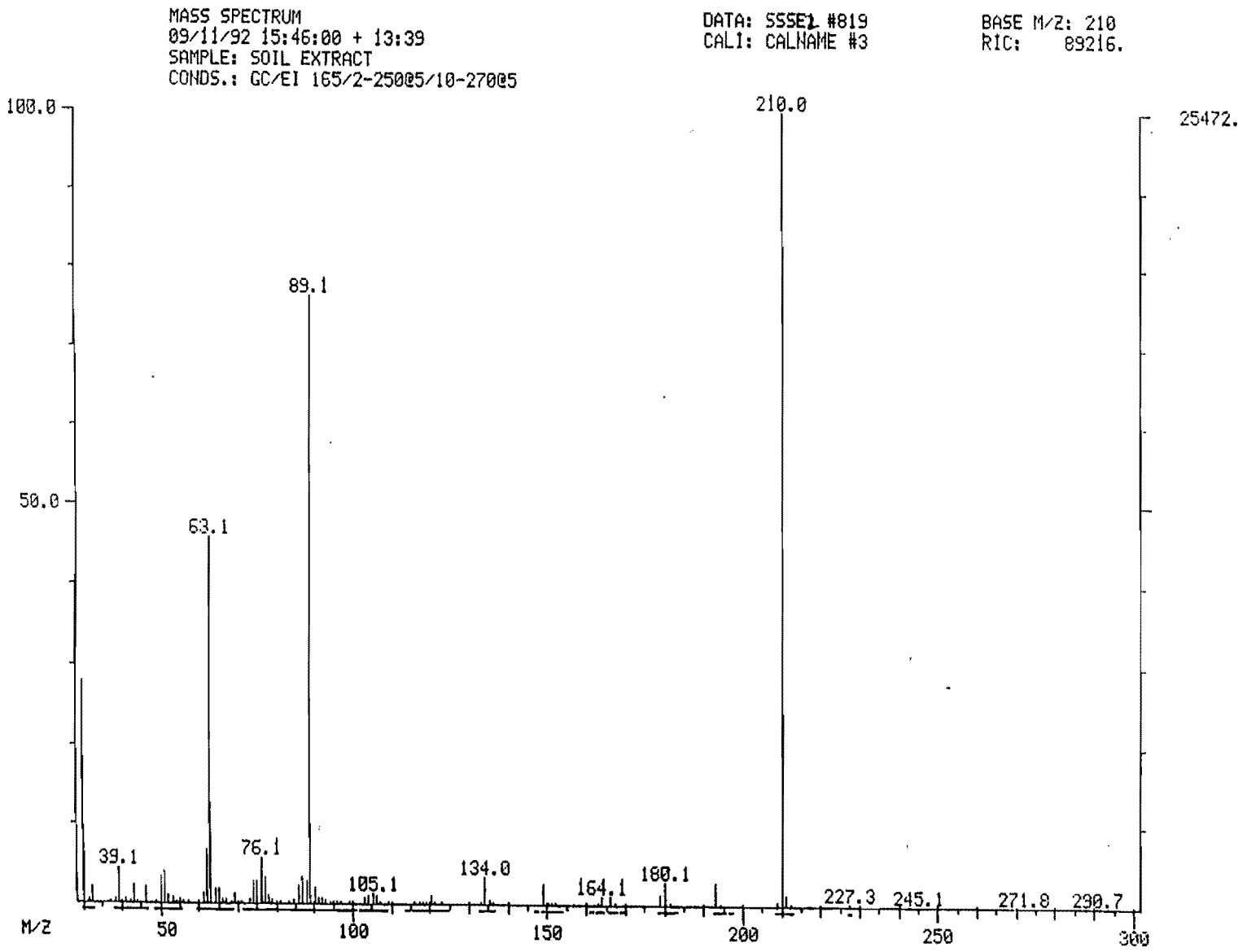

Figure 5. Mass Spec Scan for Soil Contaminated with High Concentration of TNT.

Soil Composting Study

The results of a twenty-five day soil composting study for both soils I and II are presented in Figures 6 and 7, respectively. Mixing the soils prior to initiation of the composting study significantly reduced the variability of TNT concentrations compared to unmixed preliminary experiments (data not shown).

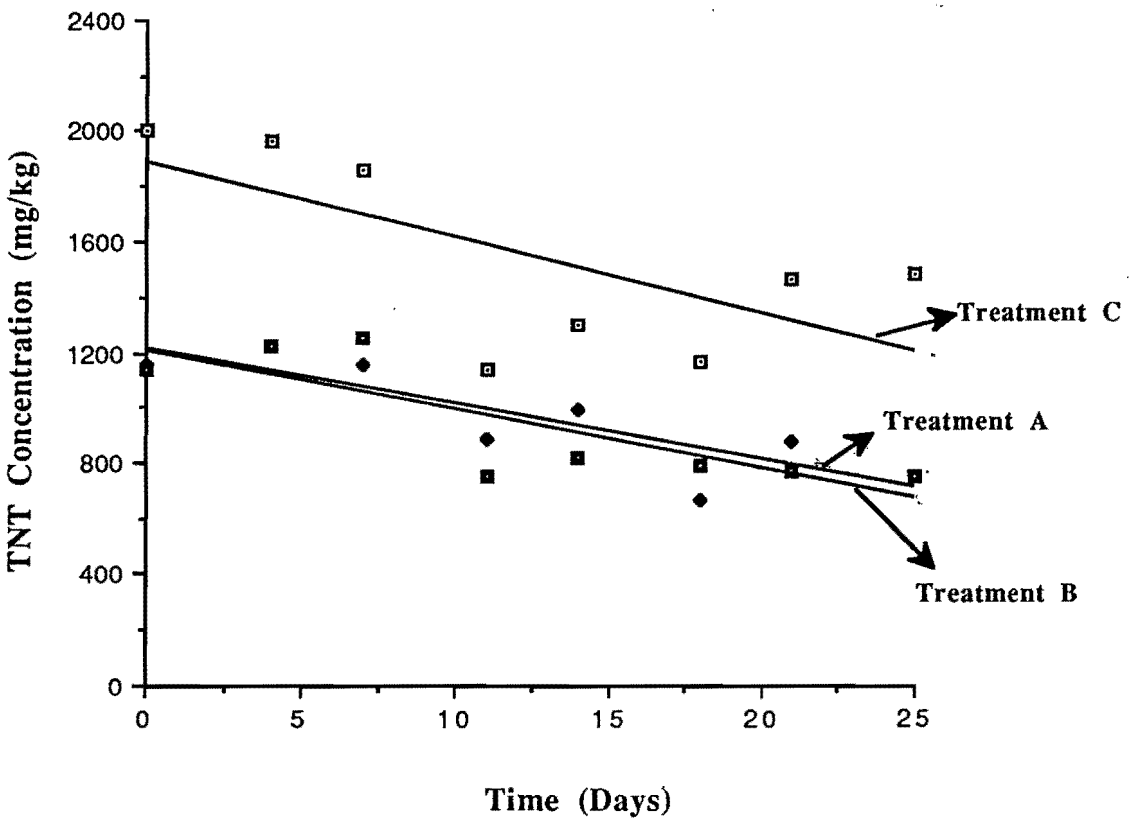

Figure 6. TNT Concentrations in Soil I (highly contaminated soil)

Note: Treatment A- Soil plus uninoculated corn cobs
Treatment B - Soil plus Fungal Inoculated Corn Cobs
Treatment C- Soil Only

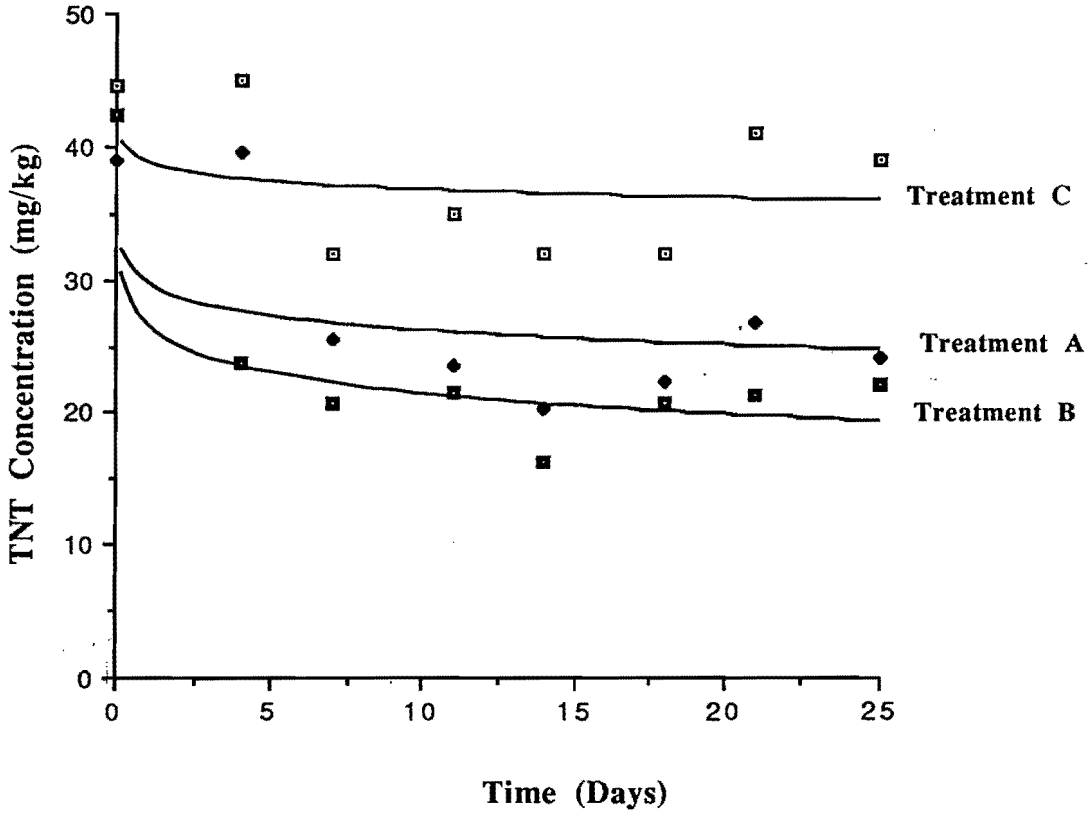

Figure 7. TNT Concentrations in Soil II (lightly contaminated soil)

Note: Treatment A- Soil plus uninoculated corn cobs
Treatment B - Soil plus Fungal Inoculated Corn Cobs
Treatment C- Soil Only

For the heavily contaminated soil (*e.g.*, soil I), the reduction in TNT concentration appeared to be zero order (*i.e.*, linear) for all treatment conditions (Figure 6). The extent of TNT removal from Treatments A, B, and C was 40%, 38% and 35%, respectively. The similarity in rates and extent of removal for all conditions evaluated suggests that fungal bioaugmentation did not enhance the transformation of TNT.

Although the rates of removal appeared to be independent of TNT concentration, the day zero TNT concentrations observed between the various treatment conditions were significantly different. The initial soil concentration for Treatment C was approximately 2000 mg/kg while Treatments A and B had initial concentrations of approximately 1200 mg/kg. These observations seem to suggest that an appreciable quantity of TNT may have been sorbed to the corn cobs. This sorption of contaminants could not be reversed through methanol extraction. The irreversible binding of contaminants to compost material or soil in fungal inoculated systems has been reported by UWRL personnel and others (Berry and Boyd, 1985). Bound residue formation is one of the primary mechanisms by which the white rot fungus, *P. chrysosporium*, reduces the toxicity of hazardous compounds (Qiu and McFarland, 1991).

The absence of enhanced biological removal may be explained by the unusual soil characteristics of soil I. From Table 1, it is clear that that the moisture content of the soil may have limited biological removal. The field capacity of soil I was estimated to be zero by the Utah Soil Science Department. Independent observations by UWRL personnel confirmed that soil I was a non-wetting soil. This suggests that soil I was either a sodic soil (*i.e.* high salt content) or contained a nonwetting organic contaminant. In either case, the inability of soil I to absorb and retain water would limit the soil's biological activity and ultimately the biodegradation of TNT.

In the lightly contaminated soil (*e.g.*, soil II), the effect of fungal bioaugmentation was more pronounced. The percent reduction in TNT concentration was 33%, 53%, and 15% in the treatment systems consisting of soil plus corn cobs, soil plus fungal inoculated corn cobs and soil only systems, respectively. The nonlinear behavior of TNT removal with time suggested a first order kinetic relationship. First order modeling efforts led to rate constants of 0.02 day^{-1}, 0.018

day^{-1} and 0.005 day^{-1} for Treatments A, B, and C, respectively. Using these rate constants, the maximum removal rates for the three treatment conditions were 0.78 mg/kg-day, 0.76 mg/kg-day and 0.33 mg/kg-day for Treatment A, B, and C, respectively.

Addition of corn cobs in soil II appears to enhance the rate and extent of TNT removal compared to the soil only system. The addition of fungus, however, did not appear to increase the removal rate compared to the addition of the organic amendment alone. This suggests that stimulation of indigenous organisms by the proper choice and addition of organic amendments is a more rationale approach to bioremediating TNT contaminated soils rather than microbial inoculation of non-indigenous species

As in the highly contaminated soils (*e.g*, soil I), sorption of TNT onto corn cobs appears to be the dominating TNT removal mechanism occurring during fungal compost treatment of soil II. However, sorption may not be the only mechanism responsible for TNT removal. Addition of organic amendment may, in fact, encourage the removal of TNT through the activity of indigenous microorganisms. Use of radiolabeled TNT together with poisoned control systems would be necessary to clarify the mechanism(s) of removal during compost treatment.

CONCLUSIONS

Short term batch kinetic studies demonstrated that in lightly contaminated soils (*e.g.*, TNT concentrations less than 100 mg/kg), addition of organic amendments suitable for fungal growth effectively increased the rate and extent of TNT removal compared to unamended conditions. Bioaugmentation efforts, which were characterized by fungal inoculation of organic amendment, did not increase the TNT removal rates over organic amendment alone. Maximum TNT removal rates of 0.78 mg/kg-day, 0.76 mg/kg-day and 0.33 mg/kg-day were observed for soil plus uninoculated corn cobs, soil plus inoculated corn cobs and soil only conditions, respectively.

In soils containing high concentrations of TNT (soil I), the rate and extent of TNT removal did not vary between treatment conditions. Irreversible sorption of TNT to organic amendment on

chemical reaction may explain the variation in initial (*i.e.*, day zero) TNT concentrations. Biotreatment of soils containing high concentrations of TNT may have been moisture limited. Independent analysis of soil moisture content as a function of soil pressure potential indicated that the field moisture capacity of soil I was zero. A field capacity measurement of zero would suggest that biological activity in the soil compost would have been insignificant.

In both soil I and soil II, there were no traces of the intermediates 2-amino 4,6 Dinitrotoluene, 4-amino 2,6 dinitrotoluene or dinitrobenzene. The absence of these intermediate compounds suggest that the soil compost system maintained aerobic conditions throughout the incubation period.

RECOMMENDATIONS

Over the twenty five day study period, it appeared that bioaugmentation with the white rot fungus, *Phanerochaete chrysosporium* did not enhance the TNT removal rate above that which was observed with the addition of organic amendment alone. However, previous work has shown that corn cobs may, in fact, have significant numbers of white rot fungus naturally associated with them. Since no attempt was made in this study to enumerate the numbers of white rot fungi in compost units receiving only organic amendment, microbial enumeration would be essential if bioaugmentation is to be properly evaluated.

A serious flaw in the present study is clarification of the removal mechanism during fungal compost treatment. Irreversible sorption of TNT to organic amendments or chemical reaction may be significant during compost treatment. Understanding the mechanism of removal would be important from the standpoint of managing and optimizing the treatment system. It is recommended that a similar study as to the one described here be conducted in which radiolabeled ^{14}C TNT is used to perform a mass balance during treatment. Obtaining a mass would be essential in determining the mechanisms of removal and estimating the essential removal rates such as mineralization, sorption, and biodegradation of TNT during compost treatment. In addition to

obtaining a mass balance, a set of biologically poisoned control reactors should be evaluated during the experimental program. Poisoned control reactors would assist in identifying whether TNT removal is biologically or chemically mediated.

Finally, it is recommended that the chemical composition of the highly contaminated soil be reviewed in detail in order to clarify the reasons for the field moisture capacity to be reduced to zero. Although, the moisture infiltration behavior of soil I was characteristic of a sodic soil, the sodium concentration of this soil is not high (Table 1). This suggests that TNT or other materials associated with explosive manufacturing contained in the soil may be limiting moisture penetration and retention. It will be impossible to biotreat these soils unless the water holding capacity is improved considerably.

REFERENCES

Berry, D.F. and S.A. Boyd. (1985) "Decontamination of Soil Through Enhanced Formation of Bound Residues". *Environ. Sci. Technol.* Vol. 19, pp. 1132-1133.

Dosoretz, C.G., S.B. Dass, C.A. Bedd, and H.E. Grethlein. (1990). "Protease-Mediated Degradation of Lignin Peroxidase in Liquid Cultures by *Phanerochaete chrysosporium*". *Appl. and Environ. Microb.* Vol. 56, no. 11, pp. 3429-3434.

Haemmerli, S.D., M.S.A. Leisola, D. Sanglard, and A. Fiechter. (1986). "Oxidation of Benzo(a)pyrene by Extracellular Ligninases of *Phanerochaete chrysosporium*". *J. Biol. Chem.* Vol. 261, pp. 6900-6903.

Haug, R.T. 1980. Compost Engineering, Principles and Practice. Ann Arbor Science Publishers, Inc., Woburn, Massachusetts.

Qiu, X.J. and M.J. McFarland. (1991). "Bound Residue Formation in PAH Contaminated Soil Composting Using *Phanerochaete chrysosporium*". *Journal of Hazardous Wastes and Hazardous Materials.* Vol. 8, no. 2, pp. 115-126.

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.* Vol. 8, pp. 209-212.

Spiker, J.K., D.L. Crawford, and R.L. Crawford. (1992). "Influence of 2,4,6 Trinitrotoluene(TNT) Concentration on the Degradation of TNT in Explosive Contaminated Soils by the White Rot Fungus *Phanerochaete chrysosporium*". *Applied and Environmental Microbiology.* Vol. 58, no. 9, pp. 3199-3202.

Tsai, T.S. (1991). "Biotreatment of Red Water - A Hazardous Waste Stream from Explosive Manufacture with Fungal Systems". *Journal of Hazardous Wastes and Hazardous Materials.* Vol. 8, no. 2, pp. 231-244.

U.S. Toxic and Hazardous Materials Agency (USATHAMA) Final Report No: DAAL03-86-D-0001.

Williams, R.T. and C. A. Myler. (1990). "Bioremediation Using Composting". *Biocycle,* November.