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BIODEGRADATION OF HAZARDOUS WASTE USING WHITE ROT FUNGUS: PROJECT PLANNING AND CONCEPT DEVELOPMENT DOCUMENT

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ABSTRACT

The white rot fungus *Phanerochaete chrysosporium* has been shown to effectively degrade pollutants such as trichlorophenol, polychlorinated biphenyls (PCBs), dioxins and other halogenated aromatic compounds. These refractory organic compounds and many others have been identified in the tank waste, groundwater and soil of various U.S. Department of Energy (DOE) sites. The treatment of these refractory organic compounds has been identified as a high priority for DOE's Research, Development, Demonstration, Testing, and Evaluation (RDDT&E) waste treatment programs. Unlike many bacteria, the white rot fungus *P. chrysosporium* is capable of degrading these types of refractory organics and may be valuable for the treatment of wastes containing multiple pollutants. The objectives of this project are to identify DOE waste problems amenable to white rot fungus treatment and to develop and demonstrate a white rot fungus treatment process for these hazardous organic compounds.

As a first step in this project, this paper describes the current state of technology using the white rot fungus for treatment of refractory organics, and describes the extent of waste problems amenable to white rot fungus treatment at various DOE sites. Based on this work, it is suggested that work in this field concentrate on remediation of DOE sites with high explosives-contaminated soils and aqueous wastes that have organic contamination from multiple refractory compounds.

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1.0 INTRODUCTION

Liquid wastes containing radioactive, hazardous, organic and regulated chemicals have been generated throughout the 40+ years of operations at various U.S. Department of Energy (DOE) sites. These wastes were released into the environment via trenches, landfills, pipes and accidental spills, contaminating large volumes of soil and groundwater. Elements of the discharged wastes include polychlorinated biphenyls (PCBs), trinitrotoluene (TNT) and other refractory organic compounds such as trichloroethylene (TCE), carbon tetrachloride, and chloroform. Current DOE policy prohibits the disposal of contaminated liquids directly to the environment, and remediation of existing contaminated groundwater, as well as soils, may be required. A treatment based on the biological mineralization of organic contaminants using a white rot fungus is a promising technology for the simultaneous removal of many organic compounds from contaminated systems.

Biological mineralization is the conversion of a toxic substrate to carbon dioxide and inorganic products through the use of microorganisms (i.e., bacteria, fungi, and algae). Bacterial consortiums are the most commonly used microorganisms for the treatment of a wide range of organic compounds found in wastewater streams; however, they have not been as effective on such refractory organic contaminants as 1,1-bis(4-chlorophenyl)-2,2,2 trichloro-ethane (DDT) (Bumpus and Aust 1987) and PCBs (Eaton 1985). Such compounds require organisms capable of degrading aromatic derivatives and/or halogenated aliphatic hydrocarbons. Examples of such organisms include bacterial strains of *Flavobacterium* (Crawford and Mohn 1985) and *Arthrobacter* (Edgehill and Finn 1983), which have demonstrated mineralization of pentachlorophenol (PCP) in soil, and the white rot fungus *Phanerochaete chrysosporium*, which has shown the potential to degrade a host of organic compounds including chloroanilines (Arjmand and Sandermann 1985), DDT (Bumpus and Aust 1987; Kohler et al. 1988; Rosiers 1987), anthracene oil (Bumpus 1989), Aroclor 1254 (a polychlorinated biphenyl) (Eaton 1985), PCP (Lamar, Glaser and Kirk 1990; Mileski et al. 1988) and TNT (Fernando, Bumpus and Aust 1990).

The lack of selectivity of the white rot fungus allows the use of a single organism for treating a mixture of organic compounds, as opposed to the standard use of bacterial consortiums for treating multicomponent contaminants. The nonspecific, aerobic degradative ability of the white rot fungus has been attributed to an extracellulary secreted ligninase enzyme system that is initiated when the fungus is nitrogen starved (Bumpus 1989; Hammel, Kalyanaraman and Kirk 1986; Kohler et al. 1988; Mileski et al. 1988; Rosiers 1987). The complex structure of lignin probably accounts for the flexibility of these enzymes, thus allowing the oxidation of a wide variety of structurally diverse compounds such as polycyclic aromatic hydrocarbons, dibenzo(p)dioxins and polychlorinated phenols and biphenyls.

Although it is not naturally found in the soil, the white rot fungus has nevertheless shown the ability to degrade contaminants in a soil medium (Arjmand and Sandermann 1985; Bumpus and Aust 1987; Bumpus 1989; Eaton 1985; Fernando, Bumpus and Aust 1990; Lamar, Glaser and Kirk 1990; Mileski et al. 1988; Rosiers 1987). Experimental designs usually involve placing fungus-impregnated wood chips in soil with an organic pollutant. Organic pollutants radiolabeled with carbon-14 are used to demonstrate the degree of mineralization, while degradation by-products are determined by analyzing compounds found in an organic solvent extract. In addition to wood chips and wood by-products, corncobs have also been shown to be effective as a support for the white rot fungus (Fernando, Bumpus and Aust 1990).

Factors affecting the ability of the white rot fungus to degrade organic pollutants in situ include the nitrogen content, water potential and manganese concentration of the soil (Kohler et al. 1988; Lamar et al. 1987; Lamar, Glaser and Kirk 1990). Access to the organic contaminant and the activity level of the white-rot ligninase system also affects the degradative ability of the fungi. Optimization of these factors will maximize the potential of any soil remediation technology involving the white rot fungus.

Aqueous phase treatment is also possible using the white rot fungus. Lewandowski, Armenante and Pak (1990) found that the best reactor design for treating aqueous streams involved the use of immobilized fungi. The primary factors limiting the degradative ability of the fungi in an aqueous phase are the fungi's access to oxygen and the mass transfer resistance, which prevents extensive contact between the organic contaminant and the growing fungi.

The primary remediation goal for soils, groundwaters and wastewaters at DOE sites is to reduce concentrations of organic contaminants to acceptable levels (i.e., levels that meet federal, state and local guidelines). On-site biological treatment processes that completely destroy the contaminants are of greatest benefit. On-site treatment of organic pollutants greatly reduces the risk to the public and workers by reducing the risk associated with transporting contaminated material. In situ treatment of soils and on-site groundwater treatment minimizes worker exposure by eliminating the need for excavation and transportation of the contaminants. By minimizing the need for excavation, the cost for treating contaminated soils is greatly reduced. Soils that can be treated on-site may require excavation; however, off-site transportation costs and hazards associated with disposal or incineration are eliminated. Biological treatment is a cost-effective method for destroying hazardous organic constituents with relatively low capital investment and operating costs.

The goal of this project is to develop a bioremediation system that uses the organic degradative capabilities of the white rot fungus *Phanerochaete chrysosporium* for treating refractory organic contaminants found at various DOE sites. The approach to be used includes conducting bench-scale tests to collect engineering data, designing and installing a pilot-scale system based on these data, and operating the pilot-scale system to demonstrate the process on actual soils and groundwaters. The project duration is estimated to be five years. The project was initiated in fiscal year (FY) 1990.

This project planning document will be used as a planning tool for completing the work that has been initiated. It includes a description of the technology, waste information, permit requirements and cost/schedule information.

2.0 TECHNOLOGY DESCRIPTION

Conventional processes for the biological detoxification of hazardous organic wastes are based on the use of bacterial consortia. These waste treatment systems have generally been unable to rapidly metabolize aromatic compounds, along with their derivatives and other refractory organic contaminants (i.e., chlorinated aliphatic hydrocarbons). *Phanerochaete chrysosporium*, a white rot fungus in the class *Basidiomycetes*, has been shown to be capable of degrading many refractory organic compounds. The mechanism identified for this phenomenon is based on a nonselective extracellular enzyme that the white rot fungus uses to degrade lignin, a structural component of wood. Because of the complexities of the lignin structure, this enzyme system is very flexible in the types of organic compounds it reacts with and degrades.

Additional discussion on the conceptual methods for treating soil, both in situ and ex situ, and groundwater with the white rot fungus will be discussed in the following subsections.

2.1 Treatment of Soils

The white rot fungus is not naturally found in the soil and does not compete well when alone with the native microflora of nonsterile soils. To aid the fungus, wood chips are commonly used as both a support and growth media for the fungi. Wood by-products or other lignin-containing materials may also be used (Rosiers 1987). Impregnating lignin-containing materials with the white rot fungus is the first step for proven biological remediation of contaminated soils (Fernando, Bumpus and Aust 1990; Rosiers 1987).

Figure 2.1. illustrates the concept of in situ bioremediation of soils. This concept involves the use of standard land farming equipment to mix fungus-impregnated material into the contamination site. Mixing performs two very important functions: 1) it aerates the treatment site and provides oxygen for the aerobic microorganisms, and 2) it improves the contacting of the microorganisms with the contaminant they are to degrade. Nutrients may have to be added to the soil to maximize the performance of the fungi, especially in situations where the level of contamination is too low to sustain fungal growth. Additional batches of inoculum may also be added to improve the amount of contact between microorganisms and contaminants, as well as to

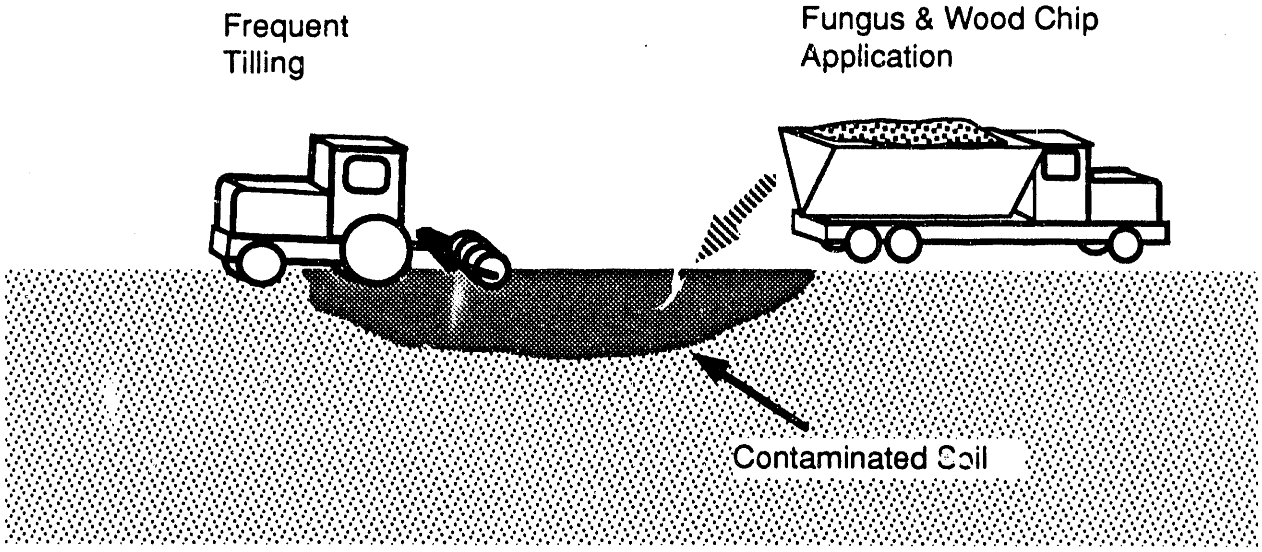

FIGURE 2.1. In Situ Soil Treatment Using the White Rot Fungus

improve the viability of the white rot fungus with native microflora. In some cases the depth of organic contamination in a soil column may be too great for effective in situ treatment. Figure 2.2 illustrates the ex situ treatment of a contaminated site, which first requires the excavation of the treatment site soil into an adjacent lined trench. The soil in the trench is then mixed with fungal inoculum and treated in the same way as sites that are treated in situ. An obvious drawback to ex situ treatment of contaminated soils is the added costs of excavation and preparation of a lined trench area. However, excavation followed by on-site treatment is a more attractive alternative than excavation and transportation to an approved disposal or incineration site.

Mixing of fungi-impregnated material with contaminated soils is the current state of technology for both in situ and ex situ bioremediation of contaminated soils. Development of an in situ treatment that does not require mixing is an attractive, cost-reducing alternative and may be required in some instances (e.g., situations involving explosives at levels of contamination that are close to their explosive limits, making the use of heavy equipment unsafe). Such a treatment should provide a delivery system for the oxygen, microorganisms, and nutrients that makes minimal use of machinery and provides an opportunity for good contact of the organic contaminants with the degrading organisms.

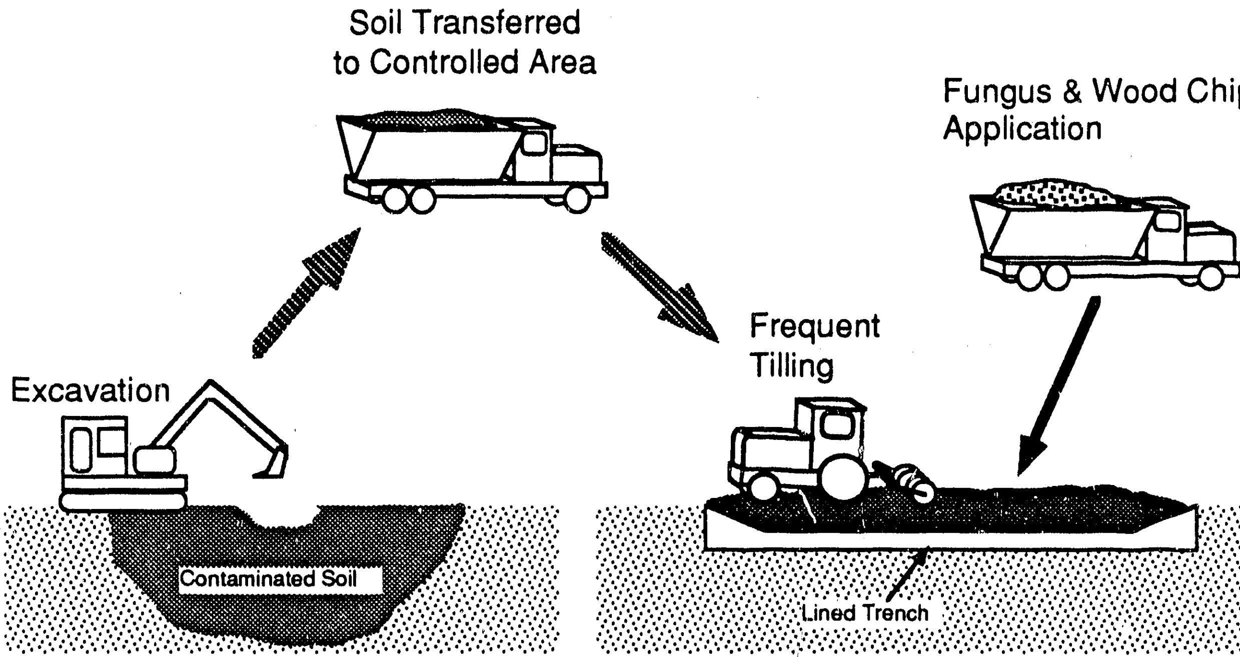

FIGURE 2.2. Ex Situ Soil Treatment Using the White Rot Fungus

Delivery of the white rot fungus, nutrients and oxygen through underground injection tubes holds promise, but limited mobility of the microorganisms through the soil creates a problem of either poor contact with organic contaminants or the need for many tubes. Use of excess liquid to mobilize the fungus is a possible solution, but this option also presents two potential problems: 1) excess liquid may mobilize the organic contaminants to the point where they can enter the groundwater, and 2) fungal, or bacterial, growth at the openings of the injection tubes could greatly increase the pumping requirements for the liquid system. The latter problem could be addressed by supplying nutrients at amounts that are toxic to the microorganisms at the concentrations at the tube openings, or by supplying nutrients in a time-released form to reduce the rate of microbial growth.

Possible alternatives to the exclusive use of the white rot fungus for treatment of contaminated soils include 1) the use of a consortium of fungus and bacteria to obtain a more complete mineralization of contaminating organic compounds; 2) use of anaerobic microorganisms that can degrade refractory organic compounds (eliminating the need for mixing the treatment site); and 3) combining in situ or ex situ soil washing with aqueous phase fungal treatment.

2.2 Treatment of Aqueous-phase Contaminants

The degradation and/or mineralization of refractory organics by the white rot fungus *Phanerochaete chrysosporium* has been shown to be more effective in an aqueous phase than in a soil column (Fernando, Bumpus and Aust 1990, Lamar, Glaser and Kirk 1990). An aqueous system removes two important limiting conditions that exist in a soil column: 1) aqueous phase treatment allows better contact between the contaminant and the degrading microorganism, and 2) nutrient limitations are overcome through better distribution in an aqueous phase. These two factors become very evident in the design of reactor vessels that are used to treat contaminated wastewater or groundwater.

Lewandowski, Armenante and Pak (1990) evaluated different reactor designs for the treatment of 2-chlorophenol by the white rot fungus *Phanerochaete chrysosporium* and found that the performance of reactors that used an immobilized fungus was far superior to reactors in which the fungus was freely suspended. A packed-bed reactor with a porous silica support and a well-mixed reactor with alginate beads as the supporting medium were the best-performing designs.

Figure 2.3 illustrates the use of a rotating biological contactor for the ex situ treatment of groundwater. The rotating biological contactor contains

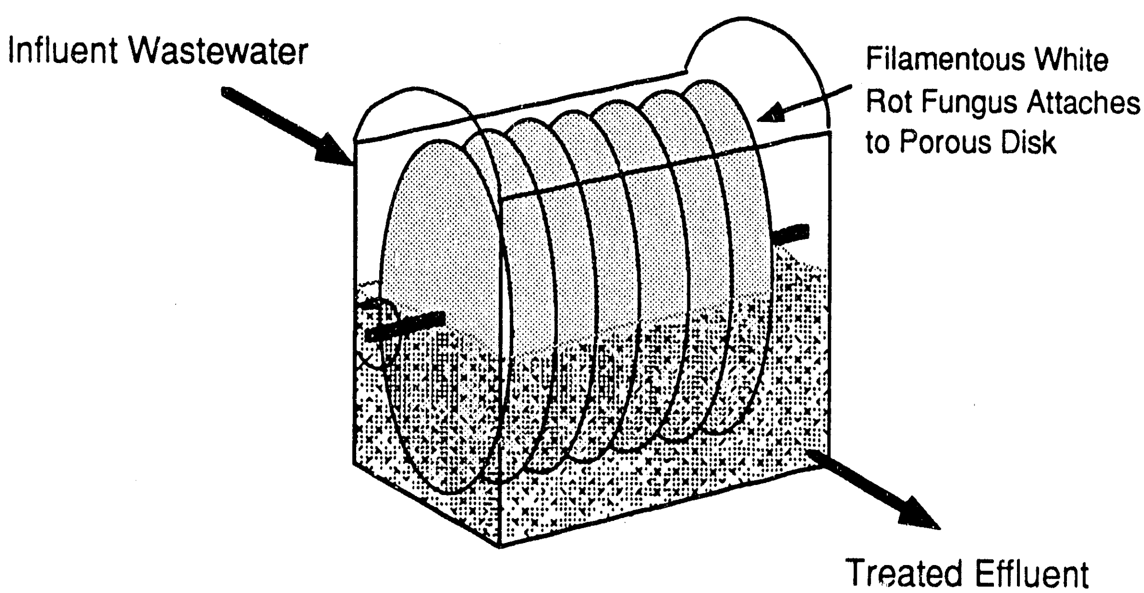

FIGURE 2.3. Rotating Biological Contactor with the White Rot Fungus

many of the features of Lewandowski's well-mixed reactor. With this design, filamentous white rot fungus attaches to a porous disk that rotates through a contaminated stream. Influent waste water enters the contactor, contacts the white rot fungus for a period of time and is then pumped from the contactor. The contactor can operate in either a continuous or semi-batch mode. Batch operation is not efficient unless nutrients can be added to sustain the growth of fungi, thus making the operation semi-batch. Because the white rot fungus has been shown to be more effective in degrading aqueous contaminants than soil contaminants, the treatment of soils may involve leaching the soil contaminants into an aqueous phase. Current pump-and-treat methods exist that will allow the addition and removal of an aqueous stream to the soil column. However, since natural flows of water through the soil column remove the refractory organics slowly, it is unlikely that pure water will sufficiently leach the organics from the soil. Choi and Aomine (1974a and 1974b) found that pH was a major factor controlling the adsorption of pentachlorophenol (PCP) to soil, suggesting that adjusting the pH may improve leaching of refractory organics from a soil column.

3.0 WASTE SITE INFORMATION

Comprehensive information on the levels of organic contaminants at various DOE sites was not available at the time of this writing. This section provides a general overview of selected contamination problems in the DOE system that may be amenable to treatment by the white rot fungus *Phanerochaete chrysosporium*.

Tables 3.1-3.3[1] list the refractory organic compounds on the Hanford, Savannah River and Oak Ridge sites that have been shown, or are believed to be, amenable to treatment by the white rot fungus. The criteria for inclusion of these organic compounds falls into two categories: 1) the compound was selected if it was present at levels above the federal guidelines, if it is not presently regulated, or if a regulatory limit is not currently specified; and 2) the contaminant is found in an environmental medium that is likely to be conducive to treatment by the white rot fungus. Hanford in-tank waste is included since it is believed that the organic compounds found in some in-tank mixed waste can be destroyed to produce a low-level waste.

Table 3.4 highlights those DOE sites that contain high explosives identified as potential health hazards, along with the identified compounds. High explosives contamination is present at both DOE and DOD facilities. Current treatment at DOD facilities involves costly incineration of HE-contaminated soil. Comrosting is a potential alternative to incineration, but has not yet been found to be cost effective (Remediation Technologies, Inc., 1990). A 1989 U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) workshop on composting identified fungal treatment as a possible composting alternative, a conclusion supported by the recent observation of TNT degradation by the white rot fungus (Fernando, Bumpus and Aust, 1990). The primary advantage of fungal treatment is the potential for complete mineralization, which is generally not attained with conventional composting (Roy F. Weston, Inc. 1988).

Table 3.5 lists the various organic compounds found at those DOE sites listed in Table 3.6. Table 3.5 does not include all organic compounds found on DOE sites, but lists those compounds believed to be amenable to treatment by the white rot fungus.

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(1) G. R. Bilyard, R. J. Bischof, J. Joseph, J. F. Keller, and M. V. Norton, March 1990, Draft Report, "Chemicals and Radioactive Substances Important to Operations and Activities at Department of Energy Sites."

TABLE 3.1. Selected Organic Compounds at the Hanford Site

Compound	Environmental Medium[a]	Selection Criteria	Criterion Basis	Reference
Benzene	TW	NS	NS	Klemm 1988
2,4 D	TW	NS	NS	Klemm 1988
Dibutylphthalate	TW	NS	NS	U. S. DOE 1987
Endrin	TW	NS	NS	Klemm 1988
Lindane	TW	NS	NS	Klemm 1988
Naphthylamine	TW	NS	NS	Klemm 1988
	GW	NS	NS	PNL 1987
Phthalic acid	TW	NS	NS	U. S. DOE 1987
2,4,5-TP Silvex	TW	NS	NS	Klemm 1988
Toluene	TW	NS	NS	Klemm 1988
Toxaphene	TW	NS	NS	Klemm 1988
Xylene	TW	NS	NS	Klemm 1988

(a) TW - Contaminants associated with in-tank waste onsite
GW - Groundwater
NS - Not Specified

TABLE 3.2. Selected Organic Compounds at the Oak Ridge Reservation

Compound	Environmental Medium[a]	Selection Criteria	Criterion Basis	Reference[b]
Benzene	GW	0.005 mg/L	NS	Martin Marietta 1989
Benzo[a]anthracene	GW	NS	NS	Martin Marietta 1989
Bis(2-ethylhexyl) Phthalate	GW	NS	NS	Martin Marietta 1989
Chrysene	GW	NS	NS	Martin Marietta 1989
Diethylphthalate	GW	NS	NS	Martin Marietta 1989
Naphthalene	GW	NS	NS	Martin Marietta 1989
Polychlorinated Biphenyls	SO	22,700 Kg	NS	SARA 1986
Tetrachlorobenzene	GW	NS	NS	Martin Marietta 1989
Toluene	GW	NS	NS	Martin Marietta 1989

(a) GW - Groundwater
SO - Soil
NS - Not Specified
(b) SARA citation provided the Selection Criteria.

TABLE 3.3. Selected Organic Compounds at the Savannah River Site

Compound	Environmental Medium[a]	Selection Criteria	Criterion Basis[b]	Reference[c]
Bis(2-ethylhexyl)-Phthalate	SO	4000.0 ppm	NS	Looney et al. 1987
Benzene	GW	2.5 μg/L	RB/TB	50 FR 46902
2,4 D	GW	100.0 μg/L	RB/TB	40 CFR 141
	SO	20.0 ppm	NS	Looney et al. 1987
Endrin	GW	0.2 μg/L	RB/TB	40 CFR 141
	SO	0.04 ppm	NS	Looney et al. 1987
Hexachlorobiphenyl	SO	1.0 ppm	NS	Looney et al. 1987
Lindane	GW	4.0 μg/L	RB/TB	40 CFR 141
Pentachlorobiphenyl	SO	1.0 ppm	NS	Looney et al. 1987
Polychlorinated Biphenyls	SO	NS	NS	Looney et al. 1987
2,4,5-TP Silvex	GW	10.0 μg/L	NS	U. S. EPA 1976
	SO	2.0 ppm	NS	Looney et al. 1987
Tetrachlorobiphenyl	SO	1.0 ppm	NS	Looney et al. 1987
Toxaphene	GW	5.0 μg/L	RB/TB	40 CFR 141
	SO	1.0 ppm	NS	Looney et al. 1987

(a) GW - Groundwater
SO - Soil
(b) RB - Risk Based
TB - Technology Based
NS - Not Specified
(c) FR and CFR citations provide the Selection Criteria.

TABLE 3.4. DOE-DP Sites with High Explosives

DOE-DP Site

Los Alamos National Laboratory (LANL)
Lawrence Livermore National Laboratory (LLNL)
Pantex Facility

High Explosives

di-N-butyl phthalate
HMX - 1,3,5,7-Tetrazocine, Octagen
TATB - $3HN_2$-$3NO_2$-Benzene
PETN - Pentaerythrite tetranitrate
RDX - Hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT - Trinitrotoluene

TABLE 3.5. Organic Compounds Found at DOE Sites (Strenge 1989)

RDX, Hexahydro-1,3,4-Trinitro-1,3,5-Triazine
Endrin, Hexadrin
4-Aminobiphenyl, 4-Biphenylamine
Gamma-hexachlorocyclohexane, Lindane, Gamma-BHC, Gamma-HCH, BHC
2-Naphthlamine, USAF CB-22
Benz(a)anthracene, 1,2-benzanthracene
Anthracene, Paranaphthalene
Phenanthrene
Aniline, Aminobenzene, Phenylamine
2-Methylphenol, O-cresylic acid
P-nitroaniline, Paranitroaniline (solid)
1,2,4,5-Tetrachlorobenzene
1,2 Dichlorobenzene, Dowtherm E
1,4-Dichlorobenzene, Paracide, PDB
1,3-Dichlorobenzene
Dimethyl phthalate, Solvarone
2,3,4,6,-Tetrachlorophenol, Dowicide 6
TNT, Trinitrotoluene (dry)
Aroclor 1016, PCB 1016
Pentachloronitrobenzene, Tritisan
2,4-D, Amidox, Estone, Fernesta
2,4,6-Trichlorophenol, Omal
Chrysene, Benz(a)phenanthrene
Initiating explosive pentaerythrite tetranitrate, PETN
1,3,5,7-Tetrazocine, Octahydro-1,3,5,7-tetranitro, Octagen
Xylene, Benzene (dimethyl)
DDT, Kopsol, Dicophane
Toluene, Toluol
Chlorobenzene, Phenyl chloride
Dibenzol(a,h)anthracene, 1,2,5,6-DBA
Hexachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzo-p-Dioxin, TCDD
Di-n-octylphthalate
2,4-Dichlorophenol, DCP, NCI-C55345
Pyrene, Benzo(def)phenanthrene
Hexachlorobenzene
P-chloroaniline, 1-amino-4-chlorobenzene
O-Nitroaniline, Devol orange B
2,4-Dinitrotoluene, DNT
2-Chloronaphthalene
Silvex, Propon, Aqua-vex
Pentachlorophenol, Permite, EP 30
Aroclor, PCB (General Classification), Therminal
Dicofol, DTMC, Carbax, Kelthane
2-Chlorophenol, Phenol
4-Chlorophenyl phenyl ether
TATB 3NH2-3NO2-BNZNE
DDD, Rhothane
2,4,5-Trichlorophenol, Nurelle
Pentachlorobenzene, QCB
1,2,4-Trichlorobenzene, unsym-Trichlorobenzene
Naphthalene, Naphthalin
Toxaphene, Phenacide, Motox

TABLE 3.6. DOE Sites with Organic Contamination. DOE sites included in the DOE Environmental Survey (Droppo et al. 1990)

Ames Laboratory
Argonne National Laboratory (ANL)
Brookhaven National Laboratory (BNL)
Component Development and Integration Facility (CDIF)
Feed Materials Production Center (Fernald Facility)
Fermi National Accelerator Laboratory
Hanford Site
Idaho National Engineering Laboratory (INEL)
Kansas City Plant (KCP)
Laboratory for Energy-related Health Research (LEHR)
Lawrence Berkeley Laboratory (LBL)
Lawrence Livermore National Laboratory (LLNL)
Los Alamos National Laboratory (LANL)
Morgantown Energy Technology Center (METC)
Mound Plant
National Institute for Petroleum and Energy Research (NIPER)
Naval Petroleum and Oil Shale Reserves in Colorado (NPRC)
Naval Petroleum Reserves in California (NPRC)
Nevada Test Site (NTS)
Oak Ridge Gas Diffusion Plant (ORGDP)
Oak Ridge National Laboratory (ORNL)
Paducah Gaseous Diffusion Plant
Pantex Facility
Pinellas Plant
Pittsburgh Energy Technology Center (PETC)
Portsmouth Uranium Enrichment Complex (PUEC)
Princeton Plasma Physics Laboratory (PPPL)
Rocky Flats Plant (RFP)
Sandia National Laboratories (Albuquerque)
Sandia National Laboratory Livermore (SNLL)
Santa Susana Field Laboratories (SSFL)
Savannah River Site (SRS)
Solar Energy Research Institute (SERI)
Stanford Linear Accelerator Center (SLAC)
Strategic Petroleum Reserve (SPR)
Y-12 Plant (part of Oak Ridge Reservation)

The concentrations of organic compounds identified in Tables 3.1-3.5 and the presence of other co-contaminants were not available at the time of this writing. Evaluation of specific waste problems for their susceptibility to fungal treatment requires more specific information on contaminant concentrations, co-contaminants, and the current remediation strategy for the waste problem. In addition, treatability studies must be performed with specific waste forms. From the limited DOE waste information available, it is recommended that current Research, Development, Demonstration, Testing and Evaluation (RDDT&E) for white rot fungus treatment focus on the two primary areas of waste treatment with the potential for the greatest benefit. Fungal treatment of high-explosives-contaminated soils could provide an effective alternative to incineration and advancement to the current composting technology, could be rapidly implemented, and has a high likelihood of success based on recent laboratory experiments. Fungal treatment of aqueous wastes with organic contamination from multiple refractory compounds also has a high likelihood for success. Treatment of DOE tank wastes or by-product wastes from treatment of tank wastes would require more extensive laboratory testing and scale-up; however, biological treatment of refractory organics in mixed wastes could provide a cost effective technology with widespread applicability.

4.0 BY-PRODUCT INFORMATION

An important consideration in evaluating different technologies for the degradation of refractory organic compounds in the environment is the biochemical pathway through which the compounds degrade. Degrading organic contaminants is undesirable if the products formed from degradation pose a greater problem (i.e., the by-products and/or intermediates are more toxic and/or more refractory). A literature search showed that very little work has been done to identify the biodegradation by-products and intermediates of refractory organic compounds found on DOE sites. Figure 4.1 presents the

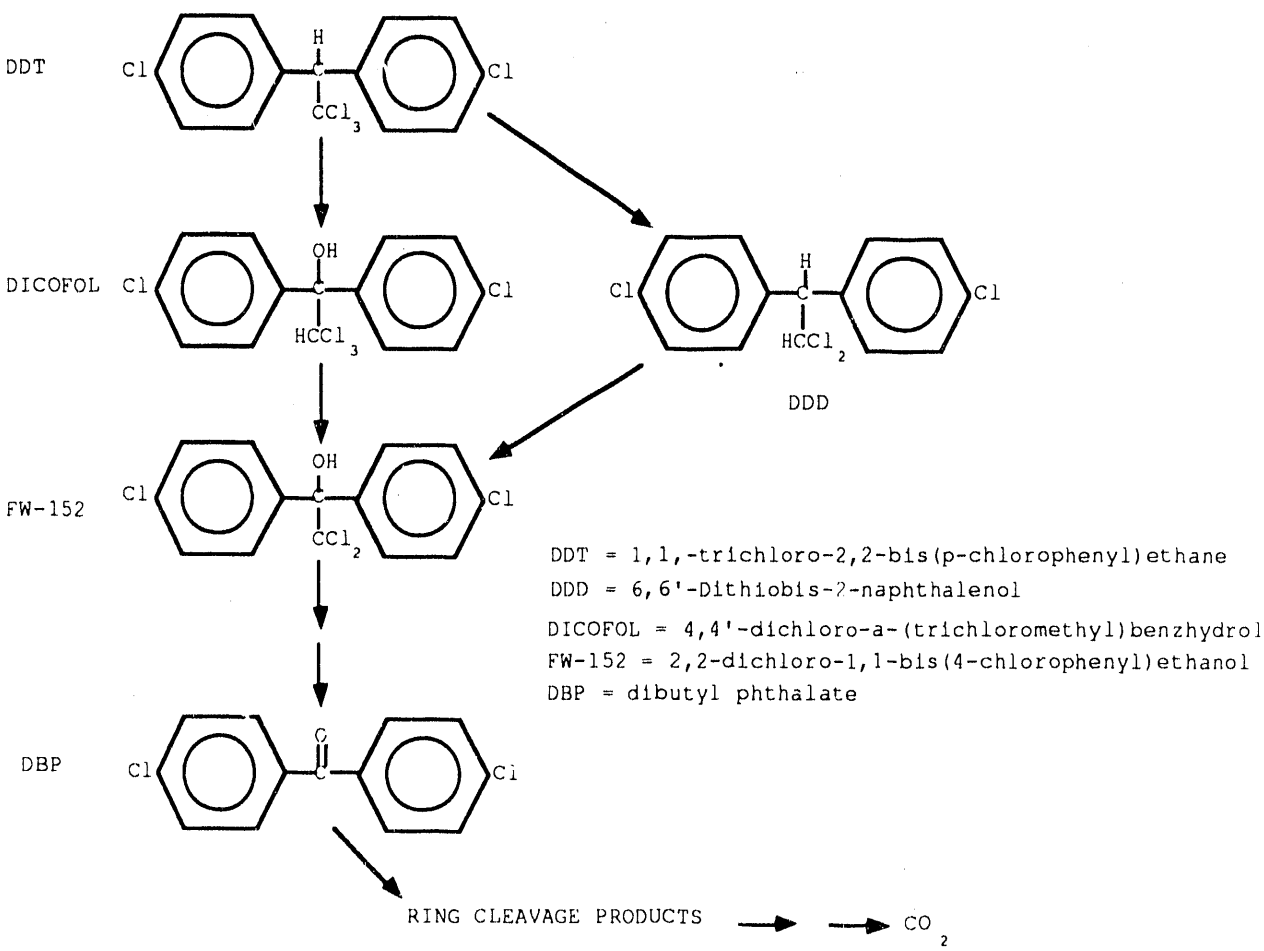


FIGURE 4.1. Proposed Degradation Pathway for DDT by the White Rot Fungus *Phanerochaete chrysosporium* (Adapted from Bumpus and Aust 1987)

proposed biochemical pathway for the degradation of DDT by the white rot fungus *Phanerochaete chrysosporium* (Bumpus and Aust 1987). Ensuring that the end products, possibly ring cleavage products in the case of DDT degradation, are non-hazardous is of primary importance when evaluating the feasibility and benefits of any treatment system. Figure 4.2 presents the proposed biotransformation scheme for TNT in compost using naturally occurring bacteria (Roy F. Weston, Inc. 1988). Bacterial degradation of TNT appears to produce aromatic end products rather than alkanes or carbon dioxide. By comparison, recent fungal degradation studies with TNT have demonstrated mineralization to carbon dioxide (Fernando, Bumpus, and Aust 1990).

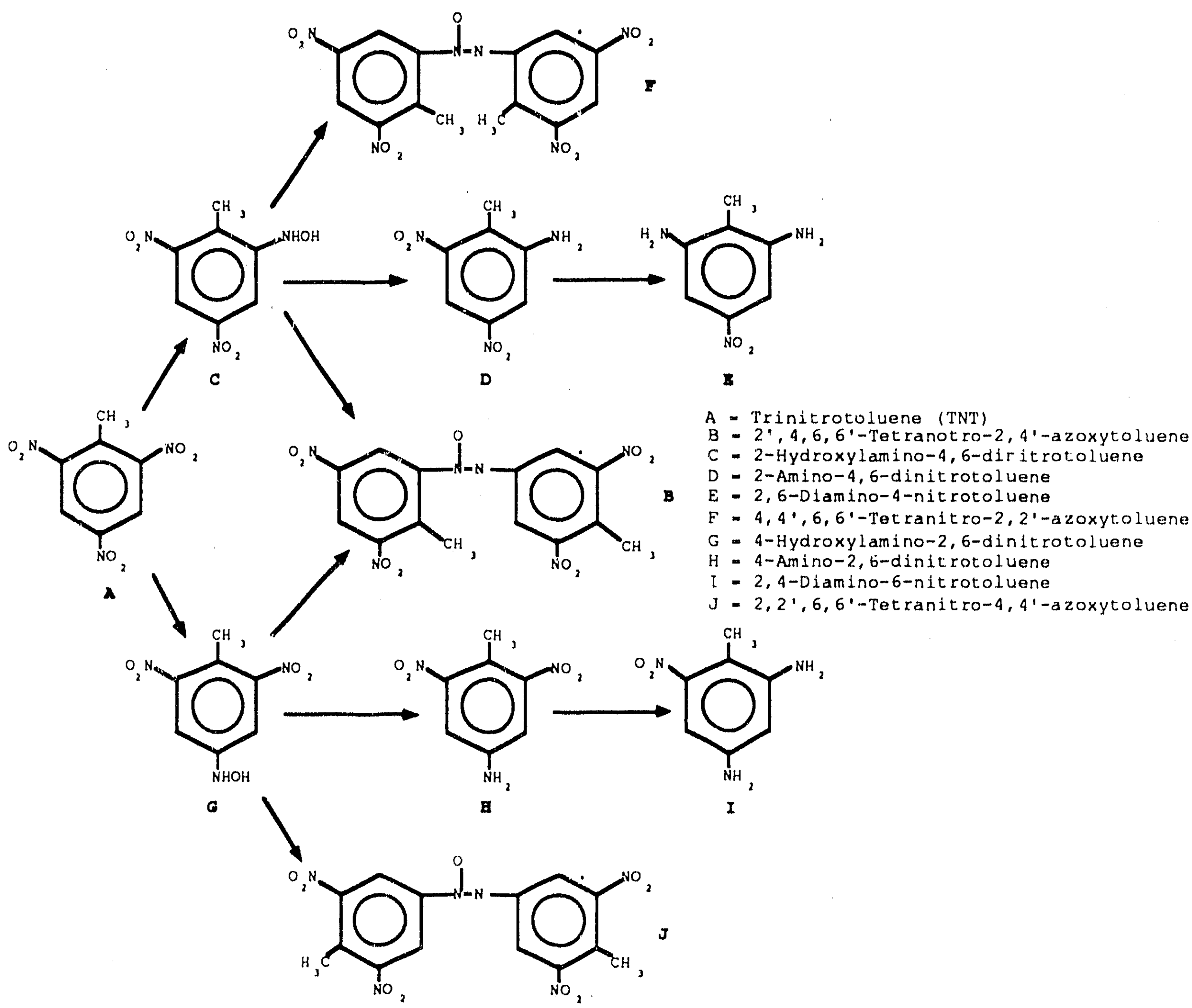


FIGURE 4.2. Proposed Degradation Pathway for TNT. Degradation through naturally occurring microorganisms (Adapted from Roy F. Weston, Inc. 1988)

The scope of this project includes identification of the by-products and intermediates produced by treatment with the white rot fungus. Refractory organic compounds to be studied will be selected based on the following criteria: 1) the organic compound is at a sufficient concentration to warrant bioremediation, 2) the organic compound is a problem at a number of DOE sites, and 3) by-products produced from the degradation of the organic compound are non-hazardous. Successful implementation of a fungal treatment process must ensure that the contaminant is degraded to non-hazardous products and that the overall toxicity of the waste is reduced.

5.0 PERMITTING AND REGULATORY REQUIREMENTS

Laboratory-, bench-, and pilot-scale testing with actual waste (i.e. contaminated soils, groundwaters, or wastewaters) will be conducted as Small Quantity Treatability Tests. NEPA documentation may be required for some of these tests. Field demonstration activities would require NEPA documentation (e.g., an Environmental Assessment) before initiating the tests. If the field demonstration is conducted at Hanford, the Washington State Department of Ecology may require that field testing be conducted under Hanford's existing Part A Interim Status Treatment, Storage, and Disposal Facility permit for Biological Treatment Test Facilities, or under a new Research, Development, and Demonstration Permit. All testing with simulated and actual waste materials will be conducted in compliance with applicable state and federal regulations and DOE orders.

6.0 COST/SCHEDULE INFORMATION

The white rot fungus technology will be developed for application to DOE waste problems over a 5-year period, depending on availability of funding. Research will be conducted to identify effective methods and conditions for remediating contaminated soils, wastewaters, and groundwaters using the white rot fungus. The technology will be developed from bench-scale evaluation through field-scale demonstration. The research and development phase of the project will focus on the capability of white rot fungus to degrade DOE pollutants that are present at concentrations requiring remediation, are present at more than one DOE site, and could be destroyed more economically and effectively than with conventional treatment technologies. As identified in the waste information section, the pollutants of interest will include TNT and other high explosives, and aqueous wastes containing multiple refractory organics. Laboratory studies will be designed to determine optimum culture conditions to maximize the rate of degradation. Collaboration with other researchers and industry will be emphasized to avoid the use of unsuccessful methods and to reduce the time required to reach a field demonstration.

Information obtained from laboratory- and bench-scale testing will be used to design and operate pilot-scale processes for in situ or ex situ soil treatment and ex situ groundwater treatment of refractory organics. The task will conclude with a field demonstration of the white rot fungus wastewater, groundwater, or soil treatment processes at a selected DOE waste site. Equipment used in the pilot-scale testing will be designed for use at a field location. White rot fungus treatment processes would provide a safe and economical means of treating hazardous organic wastes. The technology offers a means of permanently destroying the contaminants on site; therefore, the cost of treatment and potential risk to operating personnel and the public is reduced.

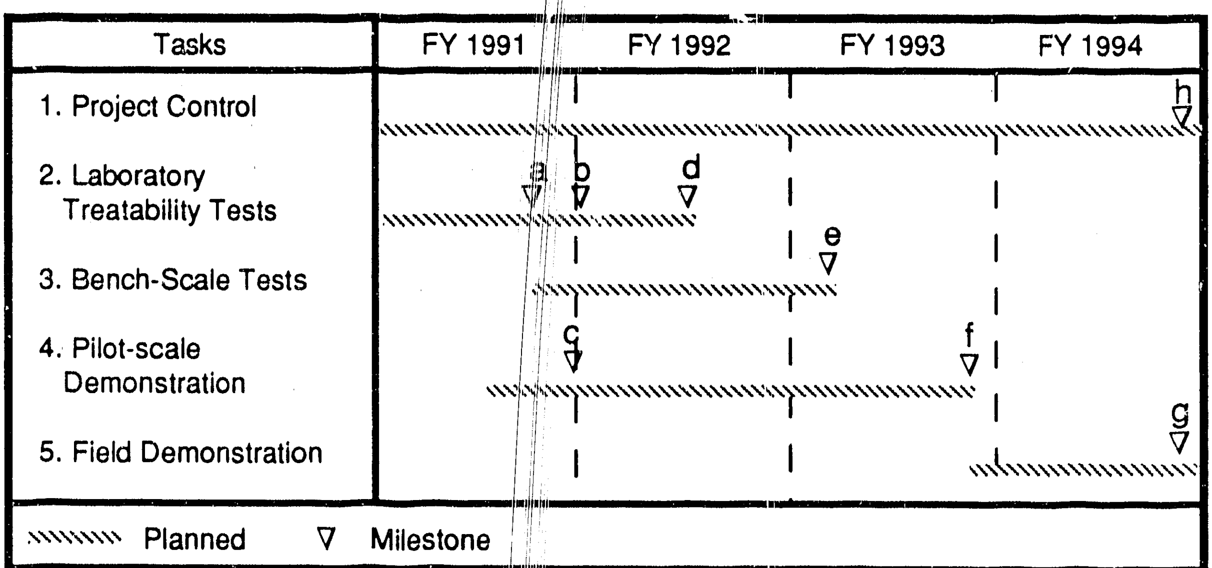
Five subtasks, to be completed in FY-1990 through FY-1994, are required to meet the task objectives:

- Project Management
- Laboratory-scale Treatability Studies
- Bench-scale Kinetics Testing
- Pilot-scale Demonstration
- Field Demonstration

A description of the subtasks is provided below. The overall project schedule is shown in Figure 6.1. Project costs are summarized in Table 6.1.

<u>Subtask 1</u> - Project Management - The function of the project management task is to provide project planning, schedule control, and overall administrative and fiscal control for the project. Report preparation, milestone completion, and technology transfer activities are included in the project management task.

<u>Subtask 2</u> - Laboratory-scale Treatability Studies - The research and development phase of the project will focus on the capability of white rot fungus to degrade pollutants which are present at several DOE sites. These pollutants include TNT and other high explosives, and aqueous wastes with multiple refractory organics. Laboratory studies will be designed to determine optimum culture conditions to maximize the rate of degradation.



Milestone Explanations:

a. Complete aqueous phase simulated tank waste tests (6/30/91-KEY)
b. Complete side-by-side TNT soil destruction test (9/30/91-KEY)
c. Complete design and procurement of demonstration test equipment (9/30/91)
d. Complete laboratory-scale soil studies (3/30/92)
e. Complete bench-scale kinetics tests (11/30/92)
f. Complete pilot-scale demonstration (7/30/93 - KEY)
g. Complete soil treatment field demonstration (9/30/94 - KEY)
h. Issue DT&E Operational Readiness evaluation report (9/30/94)

<u>FIGURE 6.1</u>. Project Task and Milestone Schedule

TABLE 6.1. Funding Levels by Fiscal Year

Subtask Activities	Funding Levels ($K) FY 1990	FY 1991	FY 1992	FY 1993	FY 1994
Subtask 1 - Project Management	48	60	60	60	60
Subtask 2 - Laboratory-scale Treatability Studies	65	90	60	0	0
Subtask 3 - Bench-scale Kinetics Testing	0	60	40	60	0
Subtask 4 - Pilot-scale Demonstration	0	100[a]	150	150	0
Subtask 5 - Field Demonstration	0	0	0	150	300
TOTAL	113	310	310	420	360

(a) Capital Equipment funding required for pilot-scale equipment.

Tests have been conducted to confirm TNT destruction, and a subcontract with Dr. Ron Crawford of the University of Idaho has been initiated. Dr. Crawford will conduct side-by-side comparisons of bacterial and fungal composting processes for TNT destruction in soils. Additional studies will be conducted in FY-1991 using aqueous systems with the fungus in either a suspension culture or attached to a support matrix. Data providing concentrations for the contaminants identified in Tables 3.1-3.5 will be obtained if available to identify several contaminants of interest to be studied for susceptibility to degradation. Based on the results of these tests, and the environmental medium of the contaminants, soil studies will be initiated in FY-1991 to determine the technical feasibility of both in situ and ex situ soil treatment. Factors such as soil water potential, nitrogen concentration, and temperature have been shown to affect the growth of white rot fungus in soils. Soil studies will focus on the effect of these factors as well as those identified in aqueous laboratory studies. Tests will be conducted using both shallow soil beds where composting or tilling is feasible, and deeper soil columns where injection methods may be required to induce fungal growth. Tests to evaluate the effect of radionuclides on the biodegradation will be conducted if applicable for the selected contaminants.

Subtask 3 - Bench-scale Kinetics Testing - Results of the laboratory studies will be used as a basis for bench-scale tests using a proven bioreactor system for the treatment of groundwaters or wastewaters, and simulated soil beds or columns for the treatment of contaminated soils. Depending on the parameters identified in the laboratory tests, a series of bench-scale tests will be conducted to collect kinetic data essential for scale-up to a pilot-scale treatment process. The primary concern will be uniformity of fungal growth and pollutant degradation as a function of soil depth or bioreactor configuration. These tests will use various methods for initiating and maintaining fungal growth to determine the most efficient procedures and processing techniques.

Subtask 4 - Pilot-scale Demonstration - Kinetic data and operating experience from the bench-scale tests will be used to evaluate the technical and economic feasibility of biodegradation using white rot fungus. In addition, a pilot-scale process or processing equipment will be procured. Based on the cost effectiveness of other bioremediation technologies, it is anticipated that pilot-scale testing will be warranted. The pilot-scale system would be initially tested using a simulated waste stream, and tested later using contaminated groundwater, wastewater, soil, or sludge.

Subtask 5 - Field Demonstration - Following pilot-scale testing, either wastewater, groundwater, or soil treatment processes will be demonstrated on a selected DOE waste problem. Equipment used in the pilot-scale testing will be designed for use at a field location.

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