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SUPERFUND INNOVATIVE TECHNOLOGY EVALUATION

Demonstration Bulletin

Fungal Treatment Bulletin

USEPA-RREL/USDA-FPL

Technology Description: Fungal Treatment is a bioremediation process utilizing white-rot fungi as a means of destroying organic contaminants in soils. The Fungal Treatment technology was developed jointly by the Forest Products Laboratory (FPL) of the U.S. Department of Agriculture's Forest Services in Madison, WI, and the U.S. EPA Risk Reduction Engineering Laboratory's Biosystems Branch. The Fungal Treatment demonstration was conducted on the site of a former wood treating facility, the Brookhaven Wood Preserving (BWP) in Brookhaven, MS. The goal of this demonstration was to determine the use of Fungal Treatment to destroy pentachlorophenol (PCP) and select polynuclear aromatic hydrocarbons (PAHs: creosote constituents) in contaminated soil.

White-rot fungi are the major degraders of lignin, a polymeric component of wood which is resistant to biodegradation. Interest in these fungi for biotechnological applications has increased during the past decade, spurred by the ability of these organisms to degrade a wide variety of hazardous compounds (including PCP and PAHs). The fungal treatment process involves inoculation of the contaminated soil with selected fungal strain(s) followed by addition of nutrients (if needed), irrigation, and aeration of the soil through tilling/cultivating to provide optimal fungal growth conditions. Inoculation is accomplished by physically mixing the soil and the inoculum. Mixing can be performed in solids mixing equipment, or in-situ by placing the inoculum on the contaminated soil and tilling until the two are thoroughly mixed. In the case of ex-situ mixing, the soil-inoculum mix must then be spread over the ground. Land farming procedures such as irrigation, aeration and nutrient addition are then implemented periodically to sustain the fungal activity within the soil matrix. As a result of the fungal activity, the hazardous compounds are transformed and become irreversibly bound to soil organic matter, in which state they are not biologically active and thus do not present toxicity problems. The fungal treatment can take several weeks to several months to achieve the desired level of contaminant reductions.

Two species of white-rot fungi, *Phanerochaete chrysosporium* and *Phanerochaete sordida*, have exhibited the best growth potential in soil contaminated with wood preserving wastes. Studies conducted by the developer have indicated that lignin-degrading enzymes generated by these fungal species are capable of oxidizing and detoxifying some normally recalcitrant PAHs. In addition, these fungal species have been known to detoxify phenolic compounds (PCP is one of them) by methylation of the phenolic group. The methylated product is susceptible to further transformation by the fungi.

Prior to treatment, a treatability study either in the field or in a laboratory is performed using representative contaminated soil. Based on the treatability data, the developer will determine: (1) the applicable fungal species, (2) the inoculum loading level, (3) length of treatment time, and (4) the need for nutrients or other soil supplements.

Waste Applicability: This Fungal Treatment has been tested for treatment of soils contaminated with organic wood preserving compounds such as pentachlorophenol (PCP) and select polynuclear aromatic hydrocarbons (PAHs) found in creosote. Warm temperatures (greater than 80°F) and sufficient moisture (greater than 30%) in the target matrix are desirable for the optimal growth of the fungus and, thus, for the degradation of the contaminants.

Demonstration Results: Over a 20-wk period, from June 1992 through November 1992, the Fungal Treatment technology was tested under actual field conditions. The test matrix was produced from excavated wood preserving wastewater treatment sludges from unlined onsite surface impoundments. This soil-like material was screened to remove solid debris larger than three inches in size, and further mixed/diluted with clean soil to bring the PCP concentration down to an acceptable level for fungal growth and viability. This test soil was then used in the test and the two control plots as described below.

On the BWP site a Test Plot and two Control Plots, A and B, were constructed. In Control Plot B, the test soil mix only was homogenized and placed as a 10-in. bed on top of a sand layer. In Control Plot A, the test soil mix was homogenized with the sterile (i.e., non-inoculated or non-fungal) spawn material at a 10:1 w/w ratio and placed as a 10-in. bed on a sand layer. In the Test Plot, the test soil mix was homogenized with the fungal inoculum at a 10:1 w/w ratio and placed as a 10-in. bed on a sand layer. The Test and the two Control Plots were rototilled/cultivated about once a week over the duration of the study. Soil temperatures were recorded on a daily basis. Moisture content of the soil from each of the plots was determined on a weekly basis. If the moisture content in a plot dropped below the target level, it was irrigated with leachate collected from the same plot and/or with the municipal tap water. Soil samples from each of the plots were collected at the start of the study, and then at the end of the 5th, 9th, and 20th weeks of the study.

Data from the Demonstration are undergoing final review. Some key initial findings are as follows:

- Levels of PCP and the target PAHs found in the underlying sand layer and the leachate from each of the plots were insignificant, indicating low leachability and loss of these contaminants due to periodic irrigation of the soil and heavy rainfall.

- Levels of PCP, the target PAHs, and dioxins in the active air samples collected during the soil tilling events were insignificant, indicating a very low potential of air-borne contaminant transport as a result of Fungal Treatment activities.

An Applications Analysis Report and a Technology Evaluation Report describing the complete Fungal Treatment SITE demonstration will be available in Summer of 1993.

The developers collected data independently and will produce results via other publications.

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