Microbiological Aspects of Bioremediation

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Abstract: In August 1995, the Institute for the Ecology of Industrial Areas (IETU) in Katowice, and the U.S. Department of Energy (DOE) signed a long-term agreement for common co-operation in development and implementation of innovative environmental remediation technologies. In this co-operation, major attempts have been focused on the demonstration of bioremediation techniques to clean up soil heavily contaminated with petroleum hydrocarbons in an aerobic biopile at the Czechowice Oil Refinery (CZOR). The microbiological studies in the Petroleum Refinery Biopile Demonstration Project were to determine the composition, activities and role of microbes during bioremediation of clayey soil mixture with acidic petroleum waste slurry under highly modified biopile conditions. Generally, microbiological studies were divided into two parts: (1) monitoring of microbiological changes in the column experiment (under laboratory conditions) and in the refinery biopile (under field conditions), and (2) isolation and characterization of microorganisms (bacterial and fungal strains) capable of degrading petroleum hydrocarbons. This summarizes the microbiological work for the bioremediation project at the Department of Environmental Microbiology (IETU). Some novel microbiological methods and the role of microbes in the bioremediation process are presented.

In recent years, there has been increasing interest in developing *in situ* techniques for remediation of oil-contaminated soil. Bioremediation is popular and publicly acceptable due to its low costs and environmental safety. Biodegradation by naturally occurring populations (consortia) of microorganisms is a major mechanism for the degradation of petroleum contaminants from the environment. Microorganisms degrade these organic pollutants using their own enzymatic pathways; the end products of aerobic hydrocarbon mineralization are microbial biomass, CO_2 and water.

The US Department of Energy and the Institute for the Ecology of Industrial Areas, Katowice, Poland have been cooperating in the development and implementation of innovative environmental remediation technologies since 1995. In this co-operation, major attempts have been focused on the demonstration of bioremediation techniques to clean up soil heavily contaminated with petroleum hydrocarbons in an aerobic biopile at the Czechowice Oil Refinery (CZOR).

Microbiological studies in the Petroleum Refinery Sludge Lagoon Biopile Demonstration project were conducted to determine the composition and role of a microbial community during the bioremediation of clayey soil mixture with petroleum waste slurry under highly modified biopile conditions [in relation to the "natural" acidic slurry lagoon conditions]. The original microbial community from the refinery's environment consisted of several sorts of microorganisms such as yeasts, filamentous fungi and some bacteria that are able to degrade both chain and aromatic (PAHs) hydrocarbons at extremely low pH (2.5 or

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even lower). The combination of physical and chemical properties (temperature changes, low pH, and limited nutrient availability) of the refinery's environment served as selective pressure factors directing the evolution of this community. Due to its extreme environmental flexibility, this community worked well under highly modified biopile conditions. Microbiological studies yielded information on the composition and activities of the community during biodegradation of petroleum contaminants in the biopile soil.

Microbiological studies were generally divided into two parts: (**1**) monitoring of microbiological changes in the column experiment (under laboratory conditions) and in the refinery biopile (under field conditions), and (**2**) isolation and characterization of microorganisms (bacterial and fungal strains) capable of degrading petroleum hydrocarbons. Monitoring of microbiological changes in both columns and the biopile was conducted using the following methods:

⇒ bacterial and fungal numbers were measured with epifluorescence DAPI and Calcafluor white methods, and with the enrichment method in Biolog boxes; and

⇒ microbiological activity was measured by dehydrogenase activity using the TTC method.

Members of the microbial community from the lagoons including the biopile at the refinery, also were characterized from molecular, physiological and taxonomic points of view. The culture-dependent (traditional plating methods) and -independent methods from biochemistry and molecular biology were used. Molecular techniques such as RFLP, PCR, DNA-DNA, DNA-RNA hybridization, etc. have been developed as sensitive methods to assessing the microbial diversity of environmental samples (called molecular microbial ecology). Some of these methods were used in the microbiological work to characterize bacterial populations. Culture-dependent techniques such as Biolog-generated community-level physiological profiles (CLPP) have been used to estimate the *ex situ* metabolic potential of members of the microbial community. CLPP provides an indication of the metabolic diversity present in soil samples with respect to the number of substrates and conditions. In this study, the approach combining culture-dependent and -independent techniques was used to evaluate the microbial population in the soil that was during remediated. Results allowed selection of highly efficient bacterial strains for production of inoculum. The inoculum was a combination of selected indigenous bacterial strains (bioaugmentation) and mineral salts + microelements (biostimulation). Subsequently, the inoculum was tested under laboratory (columns) and field (biopile) conditions. Enrichment of soil and leachate with the inoculum increased petroleum hydrocarbon uptake and improved bioremediation effects.

Microbiological studies that have been performed at the CZOR biopile are designed, managed and implemented under the direction of the Savannah River Technology Center/IETU team in cooperation with the CZOR and Florida State University, for the US Department of Energy.

Table 1. List of species and strains isolated from waste and soil of the Czechowice Oil Refinery, Poland

Species	Taxonomic group	Number of strains
Chryseomonas chlororaphis	Bacteria	2
Chryseomonas luteola	Bacteria	8
Pseudomonas aureofaciens	Bacteria	8
Pseudomonas cepacia	Bacteria	2
Pseudomonas fluorescens	Bacteria	20
Pseudomonas mendocina	Bacteria	2
Pseudomonas sp.	Bacteria	8
Sphingomonas paucimobilis	Bacteria	2
Stenotrophomonas maltophilia	Bacteria	2
Aphanoascus reticulisporus	Fungi	3
Aphanoascus keratinophilum	Fungi	6
Candida famata	Fungi	14
Exophiala sp.	Fungi	6
Fusarium sp.	Fungi	11
Geomyces pannorum	Fungi	3
Geotrichum candidum	Fungi	3
Microsporum gypseum	Fungi	5
Paecilomyces lilacinus	Fungi	3
Penicillium sp.	Fungi	4
Phialophora sp.	Fungi	2
Phoma sp.	Fungi	3
Pseudallescheria boydii	Fungi	4
Scopulariopsis brevicaulis	Fungi	1
Trichophyton ajelloi	Fungi	15
Trichophyton terrestre	Fungi	4
Total: 26 species	**-**	**Total: 141 strains**

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