Bioremediation Advances: Discovery, Development and Deployment of Biocatalysts

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Sludge samples obtained from a 100 year old waste site at the Czechowice Oil Refinery near Czechowice-Dziedzice, Poland were analyzed for high-potential microorganisms for bioremediation applications. The aged sludge was acidic (pH 2) and contained high concentrations of asphaltics and polycyclic aromatic hydrocarbons (PAHs). Additionally, the waste lagoon contained spent catalysts, diatomaceous earth, silica gel, and coal fly ash. Approximately 120,000 tons of this petrogenic waste was deposited in unlined lagoons 3 meters deep covering a total of 3.8 hectares. A total of 60 bacteria, 50 fungi, and several yeast spp. Have been isolated from the sludge on acidic minimum salts medium exposed to naphthalene vapor. The isolates were characterized by classical taxonomic criteria, BIOLOG©, and analysis of the SSU rRNA genes. A number of the bacterial isolates were commonly encountered group possessed high nucleotide sequence similarity (99-100%) to *Ralstonia* sp. KN1 (eight isolates). In addition, isolates belonging to the Actinobacteria (high G+C gram positives), and Firmicutes (low G+C gram positives) were found. Several of the *Ralstonia* isolates and a *Bacillus* isolate grew with catechol, naphthalene, or fluorene as the sole carbon source indicating a high bioremediation potential. In addition three of the *Ralstonia* spp. Appear to produce biosurfactants in exceptional quantities. The fungal isolates that grew on naphthalene vapor appear to represent taxa that have not been previously reported to degrade PAHs. The bacteria are being screened for scale-up and potential application in a bioreactor at the Institute for Ecology of Industrial Areas (IETU), our Polish international partner. A similar bioreactor at SRS will be deployed for bioremediation of mixed waste. This international project is part of the joint ongoing effort between the U.S. Department of Energy, SRS, Florida State University and the IETU to develop efficient bioremediation strategies for Central and Eastern Europe and domestic U.S. Department of Energy applications.