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Biocatalyst for 2,4-Dinitroanisole Biodegradation and Detection

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Background/Objectives. 2,4-Dinitroanisole (DNAN) serves as a replacement for 2,4,6-trinitrotoluene (TNT) in recently developed insensitive munitions. Numerous attempts to develop biocatalysts for destruction/degradation of TNT have met with limited success because the enzymes that attack TNT require substantial amounts of energy input in the form of external cofactors and electron donors. We previously isolated a *Nocardioides* strain, JS1661 able to mineralize DNAN under aerobic conditions. The initial reaction is catalyzed by a novel demethylase that hydrolyzes the ether bond and converts DNAN to 2,4-dinitrophenol (DNP) and methanol. The discovery of the enzyme involved in DNAN degradation will potentially provide the basis for the development of DNAN detection and destruction technologies that will be much simpler than current technologies used for TNT. Here we have explored the ability of both the isolate and the enzyme to destroy DNAN, the potential of the enzyme to serve as a biosensor and the isotopic fractionation during the initial reaction.

Approach/Activities. We evaluated the aerobic biodegradation of DNAN by JS1661 in non-sterile soil, aqueous media and in a fluidized bed bioreactor over a wide range of DNAN concentrations. Biogenic silica encapsulation was used to stabilize the enzyme and enable it to be packed into a model microcolumn for application as a biosensor or as a bioreactor for continuous destruction of DNAN. Finally, the isotopic fractionation during the initial reaction catalyzed by intact cells and by the enzyme was established.

Results/Lessons Learned. DNAN was completely degraded under all tested conditions with little or no accumulation of DNP and almost stoichiometric release of nitrite. The results revealed the robustness of the strain over a range of loading rates in various physical environments suggesting that it could provide the basis for waste treatment, bioremediation and bioaugmentation applications. Compound specific isotope analyses indicate that the action of the initial enzyme, DNAN demethylase, causes a substantial and characteristic carbon enrichment that can be used to estimate the impact of biodegradation.

Biogenic silica encapsulation was used to stabilize the enzyme and enable it to be packed into a model microcolumn for application as a biosensor or as a bioreactor for continuous destruction of DNAN. The immobilized enzyme was stable and not inhibited by other insensitive munitions constituents. An alternative method for DNAN detection involved coating the encapsulated enzyme on cellulose filter paper. The hydrolase- based biocatalyst could provide the basis for a wide spectrum of applications including detection, identification, destruction or inertion of explosives containing DNAN (demilitarization operations) and for environmental restorations.