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Bioremediation of soil contaminated with munitions waste
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Q-351. Biodegradation of 2,4,6-Trinitrotoluene (TNT) by the White-Rot Basidiomycete Phlebia radiata

B. VAN AKEN,* H. NAVEAU, and S. N. AGATHOS. Unit of Bioengineering, Catholic Univ. of Louvain, Louvain-la-Neuve, Belgium.

Among the known ligninolytic microorganisms, the white-rot basidiomycete Phanerochaete chrysosporium is by far the most studied at the present time. Its ligninolytic capacities are mainly related to the possession of two extracellular and non-specific peroxidases : lignin peroxidase (LiP) and manganese dependent peroxidase (MnP). This non-specificity allows P. chrysosporium to degrade - and often to mineralize - a wide range of persistent and toxic xenobiotic pollutants among which the nitroaromatic explosive 2,4,6-trinitrotoluene (TNT). Other white-rot fungi could

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able to reduce TNT and to transform 4-AmDNT. On the other hand, ligninolytic extracellular fluid without mycelium was not able to attack either TNT or 4-AmDNT. However, the degradation of TNT by whole cultures involved a disappearance of 4-AmDNT while the latter accumulated in the presence of washed mycelium suggesting an intervention of ligninolytic peroxidases even though their exact role remains unclear. We concluded that P. radiata constitutes a good candidate for the degradation of TNT using mechanisms close to those of P. chrysosporium.

Q-352.

Biotransformation of Nitroaromatic Compounds by the White Rot Fungus *Phanerochaete chrysosporium* Under Non-Ligninolytic Conditions.

M.M. JACKSON, H. BANERJEE, R. SRIDHAR and S.K. DUTTA

Department of Biology, and Cancer Research Center, Howard University, Washington, D.C. 20059

Nitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (2,4-DNT) are extensively used in the manufacture of explosives. The wood-rotting fungus *P. chrysosporium* has been investigated as an useful organism for contaminated soil remediation. *P. chrysosporium* has a lignin degrading system composed of extracellular peroxidases and other enzymes which can degrade a variety of pollutants. We have used parameters such as HPLC analyses, ^{14}C-TNT mass-balance estimations and peroxidase assays to study biotransformation of these nitroaromatic compounds and its metabolites. HPLC analyses show that this fungus is able to biotransform these compounds under non-ligninolytic conditions. Mass-balance analyses using ^{14}C-TNT revealed that this organism degrades 15-20% TNT when grown in either low nitrogen medium (ligninolytic condition) or in malt extract medium (non-ligninolytic) as measured by $^{14}CO_2$ absorbed in NaOH. Since peroxidase is not produced by this fungus under non-ligninolytic conditions, there may be a novel gene and enzyme system responsible for biotransformation of nitroaromatic compounds.

Q-353. Bacterial Diversity of a Bench-Top Bioreactor Designed for the Degradation of 2,4,6-Trinitrotoluene (TNT)

M.M. EDERER,* B.K. MOBARRY, R. L. CRAWFORD. Univ. of Idaho, Moscow

We have been studying 2,4,6-trinitrotoluene (TNT) degradation by organisms isolated from a bench-top bioreactor established in 1989 from sewage sludge and fed exclusively with TNT since then. The anaerobic consortium present in this bioreactor grows on TNT as a sole source of carbon, nitrogen, and energy, completely mineralizing it to inorganic compounds. Three TNT-degrading Clostridium species were isolated from this bioreactor and subsequently identified as *Clostridium bifermentans*. This was confirmed by 16S rRNA gene analysis of one of the isolates, KMR-1. One other strain, tentatively identified as *Clostridium clostridiforme*, was isolated in an SRB (sulfate reducing bacterium) medium. We expected to find much greater diversity in the enrichable organisms, especially since we have not yet shown that the *Clostridium bifermentans* isolates can completely mineralize the explosive, as the bioreactor consortium can. To gain further insight into the composition of the bacterial population in the bioreactor, we isolated total DNA from a 10-ml bioreactor sample using a DNA isolation method designed to isolate nucleic acids from *Streptomyces sp.*, which are known to resist enzymatic lysis. We assumed that this method would be adequate to extract DNA from the majority of bacterial species in the bioreactor, even those resistant to lysis. The 16S rDNA primers used to amplify the 16S rRNA genes were designed with consensus sequences from 47 species of clostridia and pseudomonads and a single Flavobacterium strain. Four different regions of the gene were amplified and the products cloned at random. The nucleotide sequence of several of these clones revealed a much higher bacterial diversity than predicted from the enrichment studies. Phylogenetic analyses of the data indicate that the bioreactor consortium includes various obligately anaerobic genera, as well as several facultatively anaerobic genera.

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Biodegradation of Tetryl (2,4,6-trinitrophenylmethylnitramine) in a Soil Slurry Reactor with Molasses as Co-substrate

R. BOOPATHY AND J. MANNING

Environmental Research Division
Argonne National Laboratory, Argonne, IL 60439

Soil in some parts of the Joliet Army Ammunition Plant (Joliet, IL) is contaminated with tetryl. A laboratory study was conducted to determine whether tetryl can be biodegraded by native soil bacteria under soil slurry conditions with molasses as co-substrate. A 2-L laboratory [illegible] with a 20% (W/V) slurry of contaminated

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Transformation of 2,4-Dinitrotoluene by Anaerobic Bacteria.

I.A. DAVIDOVA* and J.M. SUFLITA.

The University of Oklahoma, Norman, OK.

Nitroaromatic compounds, such as 2,4-dinitrotoluene (DNT), are of high environmental concern because they are common toxic contaminants. Multiple electron-withdrawing nitro groups make DNT resistant to aerobic biodegradation, but it can be readily transformed under anaerobic conditions. We investigated the transformation of DNT by a variety of anaerobic bacteria including the sulfate reducer *Desulfomonilie tiedjei*, the methanogen *Methanobacterium thermoautotrophicum*, the homoacetogen *Eubacterium limosum* and the fermentative organism *Clostridium bifermentans*. All bacteria reduced DNT to 2,4-diaminotoluene (DAT). *D. tiedjei* and *M. thermoautotrophicum* accumulated DAT, but *C. bifermentans* and *E.limosum* were able to further metabolize the reduced product. In all cells, reduction of the first nitro group was nonspecific and occurred predominantly in the *p*-position. Apparently, DNT, which carries two electron-withdrawing nitro groups, acts like a strong nonspecific oxidizer. The reduction of the second nitro group was specific and required an appropriate electron donor. The organisms differed in their DAT-formation activity. *C. bifermentans* was found to be the most efficient in DNT transformation; *D. tiedjei* and *E. limosum* had comparable rates of DAT formation (21 to 25 μM/h/mg protein), while *M. thermoautotrophicum* was considerably slower in DAT formation (6.7± 0.6 μM/h/mg protein). A comparison of the efficiency of DNT transformation by bacteria of different physiological types is essential for evaluating various bioremedial approaches.

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