

EPA Grant Number: R819165-01-0

Title: Remediation of TNT_Contaminated Soil by Cyanobacterial Mat

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Project Period: 1995-1997

Project Amount: \$63,000

Objectives/Hypothesis:

The objectives for this study are:

1. To develop bacterial consortia and Oscillatoria spp. for tolerance to TNT, to give “TNT-Oscillatoria”.
2. To determine rates of degradation and mineralization in TNT-spiked soil, using TNT-mat, i.e. mat containing TNT-tolerant bacteria.
3. To test mat for TNT degradation in actual field samples of soil contaminated with TNT.

Approach:

Bacteria were isolated from TNT-contaminated soil collected from Alabama Army Ammunition Plant (AAAP), Childersburg, AL. Enrichment cultures were started by placing 1 g soil in a flask of 50 ml nutrient broth (NB), then incubating on a rotary shaker. Subenrichment cultures were established with a 2% inoculum of the previous culture, along with TNT. Oscillatoria was cultured by placing mat onto Allen-Arnon (AA) agar (Allen and Arnon, 1955), then cutting out filaments of the algae growing from the mat, and adding the filaments to AA liquid medium. It was also grown from Oscillatoria which had previously been isolated from other mat. New “control” mat was prepared by blending previous mat with AA and Silage Medium (Judith Bender, personal communication), then adding silage. Both Oscillatoria and mat were maintained in an incubator set to a 12:12 LD cycle. This light regime was also used in all degradation and mineralization experiments.

To develop tolerance for TNT, bacterial consortia were incubated in increasing concentrations of TNT in NB. Consortia were cultured in mineral salts medium (MSM) to determine if TNT could be used as a sole carbon source and in a modified MSM supplemented with glucose and without any nitrogen compounds to determine if it could be used as a sole nitrogen source. Increases in growth were measured by increases in optical density at 600 nm on a Milton Roy Spectronic 21D Spectrophotometer. Mat which had not previously been exposed to TNT was incubated in 50 ppm and 100 ppm TNT. TNT-bacteria were integrated into mat. TNT-bacteria, TNT-mat and control mat were exposed to 100 ppm TNT in a NaOH plate assay to screen for degradative ability of TNT-bacteria and TNT-mat (Osmon and Klausmeier, 1972).

Results

Although these bacteria can not use TNT as a sole carbon source or nitrogen source, they do degrade it. Both TNT-mat and mar lacking TNT-bacteria are tolerant of TNT. Mineralization

was low. Using spiked soil, most degradation by mat occurred in the first four days. When “naturally” contaminated soil was used, soil treated with mat had a greater decrease in TNT concentration than did untreated soil. However, the mat did not appear to take up any TNT. This may be due to either the very high concentration of TNT in soil or due to TNT being bound to the humic fraction of the soil to the TNT: the degree of binding would increase over time. This binding may make it more difficult for the components of the mat to get to the TNT in order to degrade it. It may be desirable in future field studies using mat to periodically till the soil to the mat.

Supplemental Keywords

Bacterial consortia, degradation, and mineralization

Publications and Presentations

Mondecar, M., J. Bender, J. Ross, W. George and J. Preslan, “Removal of 2,4,6-Trinitrofluorene from Contaminated Water with Microbial Materials”, In Applied Biotechnology for Site Remediation , Lewis Publishers (1994).

Mondecar, M., “Remediation of TNT-Contained Soil by Cyanobacterial Material”, Final Project Submitted to the South and Southwest Hazardous Substance Research Center, September 1997.

For Further Information

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