

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 1994	3. REPORT TYPE AND DATES COVERED Article, June 1994	
4. TITLE AND SUBTITLE Biochemical Remediation Using Plant Enzymes			5. FUNDING NUMBERS N/A	
6. AUTHOR(S) N.L. Wolfe				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Environmental Research Laboratory Office of Research and Development U.S. EPA 960 College Station Road Athens, GA 30605-2720			8. PERFORMING ORGANIZATION REPORT NUMBER EPA/600/R-94/092	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) SERDP 901 North Stuart St. Suite 303 Arlington, VA 22203			10. SPONSORING / MONITORING AGENCY REPORT NUMBER N/A	
11. SUPPLEMENTARY NOTES Published in <u>1993 Highlights</u> by the Environmental Research Laboratory in Athens, GA, June 1994. No copyright is asserted in the United States under Title 17, U.S. code. The U.S. Government has a royalty-free license to exercise all rights under the copyright claimed herein for Government purposes. All other rights are reserved by the copyright owner.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release: distribution is unlimited			12b. DISTRIBUTION CODE A	
13. ABSTRACT (Maximum 200 Words) The transformation of trinitrotoluene (TNT) to environmentally acceptable compounds is achieved through a Lab-developed process that uses common aquatic weeds containing a nitroreductase enzyme. This research breakthrough provides an efficient and inexpensive technology for the cleanup of soils contaminated with munitions waste at military installations and other sites. 19980817 149				
14. SUBJECT TERMS biochemical remediation, TNT, nitroreductase, SERDP			15. NUMBER OF PAGES 3	
			16. PRICE CODE N/A	
17. SECURITY CLASSIFICATION OF REPORT unclass	18. SECURITY CLASSIFICATION OF THIS PAGE unclass	19. SECURITY CLASSIFICATION OF ABSTRACT unclass	20. LIMITATION OF ABSTRACT UL	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

DTIC QUALITY INSPECTED 1



Environmental Research Laboratory - Athens, GA

16 - 1994

1993 Highlights



PROGRAM HIGHLIGHTS

Biochemical Remediation Using Plant Enzymes

The transformation of trinitrotoluene (TNT) to environmentally acceptable compounds is achieved through a Lab-developed process that uses common aquatic weeds containing a nitroreductase enzyme. This research breakthrough provides an efficient and inexpensive technology for the cleanup of soils contaminated with munitions waste at military installations and other sites.

Dr. N. Lee Wolfe, research chemist, has a patent pending for the process, which is the first successful use of plant enzymes to remediate sites contaminated with chemicals. The remediation potential of the enzyme system was discovered through basic research at the Athens Lab on the degradation of pollutants in sediments.

Process development, which was partially funded by the Department of Defense's Strategic Environmental Research and Development Program, has occurred over the last 3 years and has involved the contributions of biochemists, analytical chemists, organic chemists, and engineers. The rapid development of the process was made possible by assembling a world class team of scientists and engineers at the Lab as soon as it became evident that the basic research had produced a viable, innovative technology.

Dr. Steven McCutcheon, lead engineer for the team, estimates that their interactive approach has reduced the time from lab bench studies to field demonstration by as much as 5 years. And, in just 3 years, the biochemical remediation process has caught up with other TNT-remediation technologies that have been in development for 10-12 years.

According to Dr. Wolfe, several plants, including stonewort, hornwort, and parrotfeather, possess the nitroreductase enzyme that mediates the cleanup process. In laboratory studies, the plants were successful in remediation of TNT-contaminated soil samples from seven munitions sites. A pilot-scale field study currently underway using parrotfeather shows predicted progress in TNT remediation. Several TNT-contaminated sites in the Southeastern United States are

**TNT remediation
technique can be
applied to solvents and
other organics**

being considered for an *in situ* remediation study in Spring 1994 to demonstrate the practical application of this remediation technique.

Conventional remediation of munitions waste sites usually involves the incineration of TNT-contaminated soils. Incineration costs about \$400 per ton and the resulting ash represents a continuing contamination and disposal problem. The use of composting to degrade these wastes has met with only limited success. Composting involves the use of bacteria to break down the TNT, but these bacteria are difficult to control. Moreover, they do not completely break the TNT down into environmentally benign products.

Biochemical site remediation using the aquatic plant process involves construction of an earthen berm around a TNT-contaminated site to create an artificial, shallow pond. Aquatic weeds would be grown in this pond, allowing the nitroreductase enzymes to attack the nitro group on the TNT molecule, producing triaminotoluene. The weeds also contain a laccase enzyme that rapidly oxidizes the triaminotoluene to harmless compounds.

This remediation process is based on using redox proteins (or the whole plant) to convert contaminants to environmentally acceptable compounds. In general, redox proteins observed to be effective for selected pollutants are isolated from soils or sediments, the proteins are purified and characterized, the monoclonal antibodies are produced, and a field immuno-specific assay is developed to identify the source of the redox protein in the environment. The use of an immunoassay to identify the plants that produce the required enzyme avoids the time-consuming study of potentially thousands of plants to determine which ones work.

In continuing work, the process is being applied to two other munitions, RDX and HMX, and to industrial nitroaromatics, chlorinated solvents, aromatic nitriles, anisoles, and other organic pollutants of soils, sediments, and aquifers. Successful application of the biochemical remediation process to chlorinated solvents would be particularly important. The thousands of sites across the United States that are contaminated with chlorinated solvents are one of the country's most pervasive cleanup problems. Chlorinated solvents are found at almost all problem sites at Department of Defense and Department of Energy installations and at a majority of hazardous waste sites that are being cleaned up under Superfund.

The EPA Lab work is already being extended by collaborating researchers at the University of Idaho, the University of Houston, and

the Georgia Institute of Technology. As articles by Dr. Wolfe and his Lab colleagues appear in the peer-reviewed literature, this exciting science approach to remediation will be quickly applied by other investigators in public and private laboratories, resulting in improved environmental cleanup. (*N.L. Wolfe, 706-546-3429*)

Pesticides in On-Farm Wells

First results are in from the Lab's groundwater quality research project in the Little Coharie Watershed in North Carolina. Relatively high levels of nitrate-N were found in the sampling of 21 research wells and 78 on-farm drinking wells. Only two of the wells had pesticide concentrations (atrazine and metolachlor) that were higher than EPA's Maximum Contaminant Level.

The Little Coharie Watershed is one of the fastest-growing, highest-production agricultural areas in the eastern United States. Agrichemicals applied in these fields can move rapidly into the underlying shallow aquifers.

The continuing study provides data concerning potential exposure to agricultural chemicals and information about the advantages and disadvantages of using existing wells versus new stainless-steel-cased "research" wells designed specifically for monitoring nitrates and pesticides in shallow groundwater. The database developed in the study also will be used to conduct watershed-scale performance testing of exposure assessment and nonpoint source models.

The study is being conducted in cooperation with the North Carolina Cooperative Extension Service, North Carolina State University, the U.S. Department of Agriculture, and the landowners within the Little Coharie Watershed. The monitoring program has received strong cooperation from the landowners. (*C.N. Smith, 706-546-3175*)

Study compares pesticides data from research wells and on-farm wells

Assessing Soil Carbon Policies

The goal of EPA's BIOME Agroecosystems Assessment Project, which is part of the Global Climate Change Research Program, is to evaluate the degree to which agroecosystems can be technically managed, on a sustainable basis, to conserve and sequester carbon, reduce accumulation of atmospheric carbon dioxide, and provide