# REPORT DOCUMENTATION PAGE

*Form 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 | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 23-26, 1995 | Technical Report, July 23-26, 1995 |

**4. TITLE AND SUBTITLE**
Phytoremediation of Hazardous Wastes

**5. FUNDING NUMBERS**
N/A

**6. AUTHOR(S)**
Steven C. McCutcheon, N. Lee Wolfe, Laura H. Carreria and Tse-Yuan Ou

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
US EPA Environmental Research Laboratory
960 College Station Road, Athens, GA 30605-2720

Dyn Corp., Inc.

**8. PERFORMING ORGANIZATION REPORT NUMBER**
N/A

**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**
Presented at the National Conference on Innovative Technologies for Site Remediation and Hazardous Waste Management, Pittsburgh, PA, pp.597-604, 23-26 July 1995. 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 | 12b. DISTRIBUTION CODE |
|---|---|
| Approved for public release: distribution is unlimited | A |

**13. ABSTRACT** ***(Maximum 200 Words)***

A new and innovative approach to phytoremediation (the use of plants to degrade hazardous contaminants) was developed. The new approach to phytoremediation involves rigorous pathway analyses, mass balance determinations, and identification of specific enzymes that break down trinitrotoluene (TNT), other explosives (RDX and HMX), nitrobenzene, and chlorinated solvents (e.g., TCE and PCE) (EPA 1994). As a good example, TNT is completely and rapidly degraded by nitroreductase and laccase enzymes. The aromatic ring is broken and the carbon in the ring fragments is incorporated into new plant fiber, as part of the natural liginification process. Half lives for TNT degradation approach 1 hr or less under ideal laboratory conditions. Continuous-flow pilot studies indicate that scale up residence times in created wetlands may be two to three times longer than in laboratory batch studies. The use of created wetlands and land farming techniques guided by rigorous field biochemistry and ecology promises to be a vital part of a newly evolving field, ecological engineering.

| 14. SUBJECT TERMS | 15. NUMBER OF PAGES |
|---|---|
| phytoremediation, nitroreductase, laccase enzymes, SERDP | 8 |
| | **16. PRICE CODE** N/A |

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| unclass | unclass | unclass | UL |

# Innovative Technologies for Site Remediation and Hazardous Waste Management

**Proceedings of the National Conference**

Sponsored by the
Environmental Engineering Division
of the
American Society of Civil Engineers

Co-sponsored by the
Canadian Society for Civil Engineering

in cooperation with the
American Academy of Environmental Engineers
Engineer's Society of Western Pennsylvania
Environmental & Engineering Geophysical Society
Ohio River Basin Consortium for Research and Education
Ohio River Valley Water Sanitation Commission
Pennsylvania Department of Environmental Resources
Pennsylvania Society of Professional Engineers
Pennsylvania Water Environment Association
Pittsburgh Section, American Institute of Chemical Engineers
Pittsburgh Section, American Society of Civil Engineers

Hosted by the
University of Pittsburgh

Pittsburgh, Pennsylvania
July 23-26, 1995

**Edited by Radisav D. Vidic and Frederick G. Pohland**

Published by the
American Society of Civil Engineers
345 East 47th Street
New York, New York 10017-2398

19980806 087

# Phytoremediation of Hazardous Wastes

Steven C. McCutcheon, Ph.D., P.E.[1]
N. Lee Wolfe, Ph.D.[2]
Laura H. Carreria, Ph.D.[3]
Tse-Yuan Ou, Ph.D.[4]

Abstract

A new and innovative approach to phytoremediation (the use of plants to degrade hazardous contaminants) was developed. The new approach to phytoremediation involves rigorous pathway analyses, mass balance determinations, and identification of specific enzymes that break down trinitrotoluene (TNT), other explosives (RDX and HMX), nitrobenzene, and chlorinated solvents (e.g., TCE and PCE) (EPA 1994). As a good example, TNT is completely and rapidly degraded by nitroreductase and laccase enzymes. The aromatic ring is broken and the carbon in the ring fragments is incorporated into new plant fiber, as part of the natural liginification process. Half lives for TNT degradation approach 1 hr or less under ideal laboratory conditions. Continuous-flow pilot studies indicate that scale up residence times in created wetlands may be two to three times longer than in laboratory batch studies. The use of created wetlands and land farming techniques guided by rigorous field biochemistry and ecology promises to be a vital part of a newly evolving field, ecological engineering.

---

[1]Research Environmental Engineer, US EPA Environmental Research Laboratory, 960 College Station Road, Athens, GA 30605-2720, (706) 546-3301, fax:3340, EPASM@Athens.ath.epa.gov

[2]Research Chemist, U.S. EPA Environmental Res. Lab., Athens, GA 30605

[3]Research Biochemist, Dyn Corp., Inc., c/o US EPA, Athens, GA 30605

[4]Research Chemist, Dyn Corp., Inc., c/o US EPA, Athens, GA 30605

## Introduction

Estimates to clean up military bases, Department of Energy (DOE) facilities, plus Superfund and RCRA sites over the next 30 years are approximately $750 billion (range of estimates: $500 billion to $1 trillion or more). In investigating ways to reduce these overwhelming costs, we have discovered four natural processes occurring in sediments, soils, and aquifers that degrade: 1) aromatic nitrogen compounds (EPA 1994) that include important explosives (TNT, RDX, and HMX), their metabolites [dinitro,monoaminotoluene, mononitro,diaminotoluene, and triaminotoluene (TAT)], nitrobenzene, and several other nitroaromatics, and 2) chlorinated solvents like perchlorethylene (PCE) and trichloroethylene (TCE).

Approximately, 100 Army bases are contaminated from the manufacture, handling, and storage of TNT and other munitions. Many of these sites have contaminated ground waters, which in some cases have threatened and closed drinking water wells like those of the City of Milan, Tennessee. Over 1000 DOE sites and probably as many more military and private sites have chlorinated solvent plumes.

The proteins that reduce these compounds have been isolated, monoclonal antibodies derived, and immuno-specific assays developed to identify the specific enzymes involved. Using the assays, a number of plants have been found that break down munitions wastes and chlorinated solvents. The immuno-specific assays are developed by purifying the enzymes from reactive sediments and injecting the extract into the spleen of a laboratory mouse to develop antibodies. These antibodies are used in tests to identify the specific enzymes. All four sediment- derived proteins were traced back to plants. It has been a surprise so far that no microbial enzyme systems were among the natural processes discovered. At this time our isolation procedures have not been fully reviewed for a bias towards plants systems, but if there is no bias, this is an important early indication that innovative remediation research should begin to focus on plant systems. It would be premature to abandon bioremediation and other technologies, however. The most likely use of plants will be in a diverse ecosystem that may also include the right types of supplementary bacteria and fungi, if for no other reason than to assure appropriate nutrient cycling for plants.

Our lab tests have uncovered significant strengths of plant ecosystems. The aquatic plants identified so far can adapt to high metals concentrations, low pH, and high toxicity. Values of pH 4 are adjusted by plants to approximately pH 7, and metals sorbed or chelated to avoid poisoning of the plant's enzymes. Roots avoid highly contaminated sediments and plants remain vigorous in the presence of toxic soil concentrations, while continuously keeping dissolved concentrations of TNT and other toxic pollutants low enough for tadpoles and snails to thrive in overlying waters (Young 1995). Terrestrial plants slowly encroach upon sterile, bare spots high in TNT. These adaptive mechanisms evolved in plants to protect enzyme systems and survive under harsh conditions.

Although it is not a panacea for cleanup, phytoremediation has great strengths: 1) lightly managed ecosystems that are more highly evolved than bacterial cultures to control pH and metals concentrations are used, 2) the complete break down and incorporation of contaminant molecules into new plant biomass is achieved, 3) fast and efficient degradation of contaminants can be achieved for some contamination problems, 4) aesthetic ecosystems can be maintained in place of the sterile soils produced by incineration, 5) current risk based cleanup standards and drinking water standards can be achieved in many cases, 6) overall costs are expected to be an order of magnitude lower in many cases, and 7) reduced costs of disposal of treated residual soils (i.e., compared to bioslurries and other ex situ treatments) are possible. Disadvantages include: 1) longer times may be required to achieve strict cleanup standards compared to incineration, 2) mass transport limitations many slow treatments and make degradation of bound residuals very difficult (same for other biotreatments), and 3) any in situ treatment but especially a lightly managed ecosystem may involve limited control and release of intermediates if the process does not work as expected.

## Pathway Analyses and Mass Balances

Extensive pathway analyses of metabolites, mass balances, and radiolabeled studies show that TNT is completely broken down and incorporated into new plant lignin, effectively ending any toxicity. These pathways are different from bioremediation processes that rarely break TNT down into TAT. DNT buildup can result from some bioremediation processes. Bioslurry treatments typically require highly moderated anaerobic and aerobic sequences to mineralize TNT.

## Plants

Several submerged and emergent aquatic plants and a few terrestrial plants containing the nitroreductase and the other important enzymes have been identified so far (Schnoor et al. 1995). The best candidate plant now is parrot feather that has evolved into both emergent and submergent varieties.

Approximately 10 to 20 percent of the plants tested, contain nitroreductase. The plants containing nitroreductase span many genotypes, hinting that this non-specific enzyme is an ancient one. Algae spirogyra, Algae nitella (stonewort), blue-green algae, and Anthrocerotae (hornwort) break down TNT, as does duckweed, hydrilla, and other emergent plants. Trees like poplar (populus) and sycamore contain the nitroreductase and readily break down TNT.

## Kinetics

The kinetics of the plant enzymatic reactions are remarkably fast compared to other biotreatments (see Figure 1). Reaction half-lives on the order of one hour have been observed for the degradation of TNT. This is at least one or two orders of magnitude faster than rates reported in TNT bioremediation research supported at this laboratory. However, the remediation of TNT-contaminated soils and sediments by either plants or bacteria is most likely mass-transport limited by the rate of dissolution or desorption of TNT.

## Costs

The cost of TNT remediation by either plants or bacteria may be similar if hardy, adaptable strains of natural bacteria can be found. Compared to incineration costs of $400 to $1200 per ton, phytoremediation of TNT may be a highly competitive technology worthy of further investigation. Many states now hesitate to issue permits for incineration of TNT-contaminated soils because of the co-mingling of lead and other heavy metals at many munitions facilities. The capability of plants to adapt to metal poisoning may be an important answer to concern about air pollution from incineration.

## Other Munitions - RDX and HMX

The nitroreductase seems to be an ancient enzyme prevalent in many simple aquatic plants. Its lack of specificity in rapidly breaking down nitrobenzene, TNT, DNT, HMX, and RDX -- all aromatic molecules with nitrogen groups in different configurations -- may be due to its ancient nature. Tests indicate that nitrate is the preferred form of nitrogen. TNT degradation may be slowed when nitrate is present. Nevertheless, the versatility of the nitroreductase seems unparalleled at the moment.

Pathway analyses, mass balances and kinetics studies are underway for RDX and HMX. Early results suggest that degradation rates are up to 20 times slower than TNT degradation.

Preliminary Conclusions

An important engineering break at this stage is that the reduction of TNT by nitroreductase is not sensitive to the presence of oxygen. Thus, anaerobic-aerobic cycling required in some bioslurry reactors is not necessary. Unfortunately, the dehalogenase that breaks down chlorinated solvents like TCE and PCE is not so hardy in typical engineered systems. The dehalogenase requires either encapsulation or sorption to a medium like sand to preserve activity. However, several species of algae produce dehalogenase, making it feasible to consider the use of anaerobic lagoons as a treatment scheme for pumped ground waters. Dehalogenase activity has also been discovered in cypress trees, ferns and other wetland vegetation, making it possible to identify natural wetlands that will treat contaminated ground water plumes that intersect the surface. Avoiding disruption of fragile wetlands during remediation by using lightly engineered ecosystems is an intriguing possibility.

Another engineering break is the recent discovery that poplar trees and other terrestrial plants contain nitroreductase and dehalogenase. Poplar trees are just beginning to be used to remove nitrate and pesticides from runoff in mid-western farming areas, and to control and treat landfill leachates (Schnoor et al. 1995). This opens the possibilities to not only ecological engineering of created wetlands, but may lead to the use of other great American strengths -- farming and silviculture -- to remediate hazardous wastes. Husbandry of crops and trees to clean up hazardous waste sites could be an answer to more than one problem facing American society.

## Ongoing Studies

Young (1995) has conducted batch pilot studies at the demobilized Alabama Army Ammunition Plant in Childersburg, and at Auburn University. These pilots studies show rapid removal of TNT and all metabolites from water, and reasonable removal of TNT from soil. TNT concentrations in flooded basins with soils starting at approximately 5000 mg/L quickly reach saturation, and within two weeks of the introduction of plants, TNT levels drop below detection. Tadpoles and snails grow among the introduced parrot feather. Within several weeks, parrot feather begins to root in previously sterile soil.

Continuous-flow pilot tests are underway in our laboratories and at the Georgia Institute of Technology (ES&T 1995). Scale up residence times may be approximately three to five times longer than times derived from laboratory batch studies.

This summer we plan to conduct field investigations at Volunteer Army Ammunition Plant in Chattanooga, Tennessee. In 1997, a field demonstration using a created wetland is planned together with the Army Environmental Center, the Waterways Experiment Station, and the Tennessee Valley Authority, to clean up ground waters to the TNT drinking water standard of 2 parts per billion.

## Acknowledgments

Much of the funding for these investigations came from the Strategic Environmental Research and Development Program (SERDP) of the Department of Defense, Department of Energy, and EPA. The Army Waterways Experiment Station through Douglas Gunnison and more recently Mark Zappi and the SERDP Biotreatment Consortium have provided funding and collaboration. David Burris and Mark Smith of the Air Force Armstrong Laboratory at Tyndall AFB have provided funding and collaboration. Ron Wynn of the Alabama Army Ammunition Plant has provided access to soils and contaminated sites. Randy Cerar and Mark Hampton of the Army Environmental Center have provided advice on field conditions and applications. David Young and his advisor at Auburn, Rod Jenkins, have provided early data on field batch studies that we appreciate. Mike Saunders, of Georgia Tech, Joe Hughes and Jackie Shanks of Rice University and other investigators with the EPA Hazardous Substance Research Center/South and Southwest have

provided early confirmation of pathways and new techniques involving tissue cultures. Karl-Erik Erikson and Jeff Dean of the University of Georgia have provided monoclonal antibodies for laccase enzymes, tissue cultures, and other collaboration that is very much appreciated. Jerry Schnoor at the University of Iowa and colleagues at Iowa State University provided poplar tissue cultures and cuttings, and exudate from the rhizosphere of poplar trees.

APPENDIX I. - References

ES&T Science News, 1995, Enzymes set for bioremediation field study, 29(1), 18(A).

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L., and Carreria, L.H., 1995, Phytoremediation: an emerging technology for contaminated soils, ES&T, feature article, accepted for publication July issue.

US EPA Office of Research and Development, 1994, Water weeds: experimental cleanup tool, Science Notes No. 7, EPA/600/N-94/012.

Wolfe, N.L., McCutcheon, S.C., Carreria, L.H., Ou, T.Y., and Gunnison, D., 1994, Biochemical remediation of 2,4,6 trinitrotoluene (TNT), invited presentation, EPA Region 8, U.S. Army Environmental Center, Kansas City, KS, December.

Young, D., 1995, Ph.D. dissertation, Auburn University, AL.

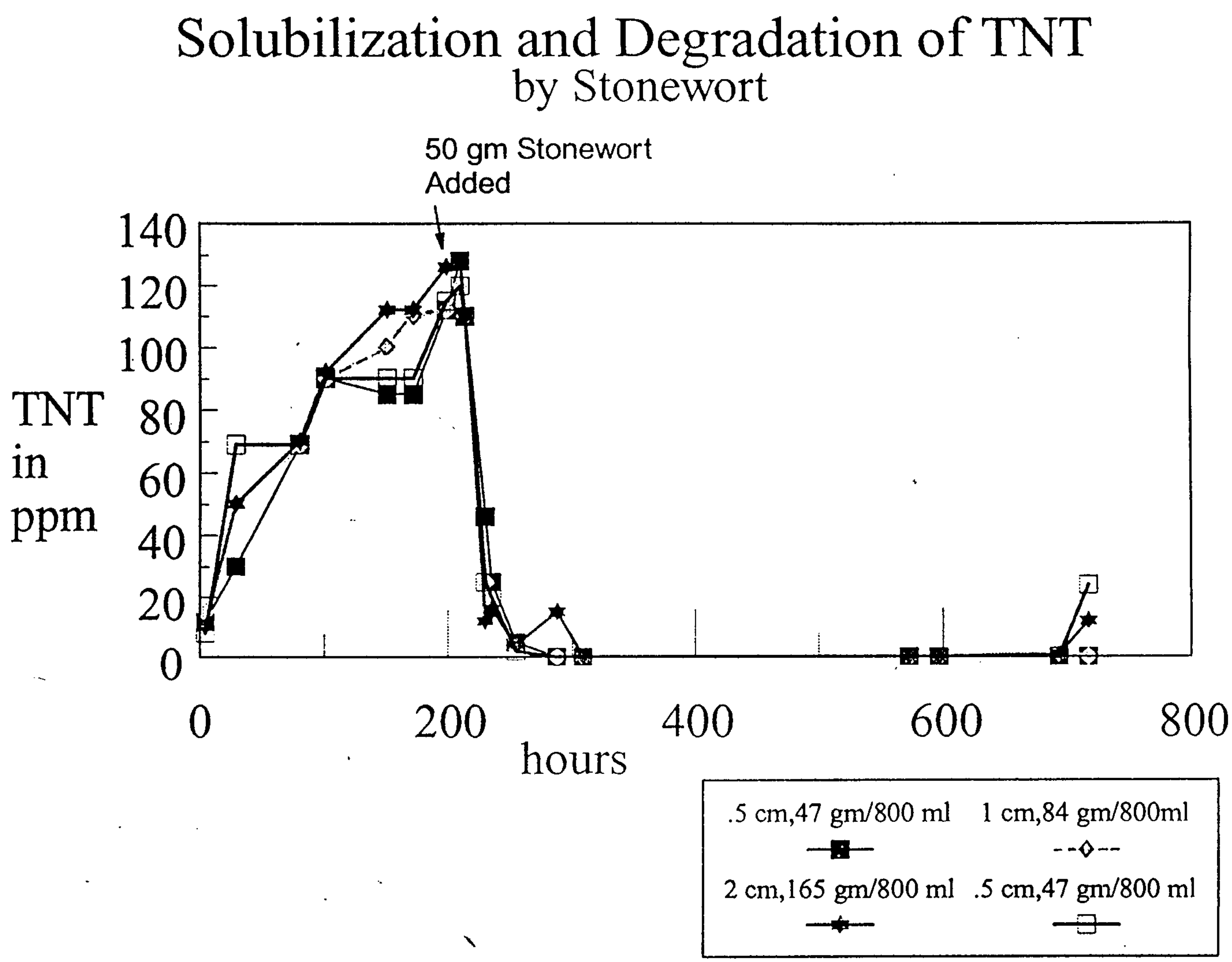


Figure 1. Batch study of TNT dissolution and degradation when plants are introduced.