Project ID: 54889

USING TREES TO REMEDIATE GROUNDWATERS CONTAMINATED WITH CHLORINATED HYDROCARBONS

April 20, 2000

PI: Stuart E. Strand, University of Washington, Box 352100, Seattle WA 98195, 206-543-5350, sstrand@u.washington.edu
Co-PI: Milton P. Gordon, University of Washington, Box 357350, Seattle WA 98195, 206-543-1769, miltong@u.washington.edu

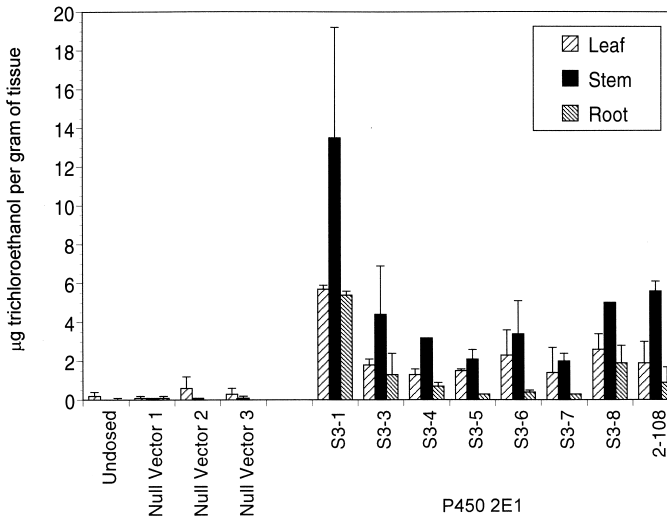
Two graduate students and two post-doctorates have been actively involved.

This project addresses the need for more economical methods for treatment of groundwaters contaminated with chlorinated hydrocarbons (CHCs) as requested in needs statements from Hanford and Savannah River, among other DOE sites. Product from this project will lead to improved knowledge of phytoremediation mechanisms for treatment of CHCs and to varieties of plants capable of greater uptake and degradation of CHCs.

Research objectives: (1) increase the metabolic degradation of chlorinated hydrocarbons in plants and (2) understand the mechanism of CHC degradation in plant tissue

Research progress and implications: This report summarizes work after 3.5 years of a three-year project with a one-year no cost extension.

Although plants have been shown to take up and degrade important groundwater contaminants such as trichloroethylene (TCE), the land area and time required for phytoremediation are often excessive. Our goal was to solve these problems by introducing into plants an enzyme known to metabolize many halogenated hydrocarbons. As a proof-of-concept study, we introduced into tobacco a gene encoding the human P450 IIE1 (CYP2E1) enzyme known to oxidize TCE, ethylene dibromide (EDB), and other hazardous compounds. Metabolism of TCE in the transgenic plants was dramatically increased, by up to 642-fold. The plants also showed an increased uptake and debromination of EDB. The increased metabolism correlated with the level of CYP2E1 protein. These experiments demonstrate that the metabolism of



Enhanced Metabolism of TCE by Transgenic Plants

Production of TCE metabolite, trichloroethanol, in tissues of transgenic tobacco plants after exposure to TCE for five days. Three transgenic plants containing the null vector (controls, left) and eight transgenic plants containing cytochrome P450 2E1 (right).

common halogenated pollutants can be enhanced in plants through the introduction of cytochrome P450.

The biochemical mechanism of carbon tetrachloride (CT) transformation by poplar cells was investigated using both radiolabelled and non-radiolabelled cell culture experiments. Results indicated that poplar cells were able to transform CT to CO2 and chloroform (CF) with some binding of CT intermediates to poplar cells. The production of CO2 was increased under aerobic condition while the formations of CF and cell binding of CT intermediates were enhanced under anaerobic condition. Both CO_2 production and cell binding of CT intermediates were significantly inhibited by both P-450 general inhibitor (CO) and specific P-450 2E1 inhibitors (chlorzoxazone, isoniazid, 4-methylpyrazole and 1-phenylimidazole), suggesting an involvement of a P450-2E1-like enzyme in the CT metabolism in poplar cells. This study demonstrates a potentially useful biodegradative process for CT that operates under aerobic conditions.

Applications to DOE Technology Needs:

Transgenic trees and phytoremediation of PCE, TCE and CT can be used to sub-irrigate plantations of trees at Hanford and Savannah River with water pumped from the contaminated aquifers. Nitrate in the Hanford groundwater would also be removed by the trees. Comparison to pump and treat with activated carbon adsorption and regeneration suggests that 30 year costs for pump and irrigate phytoremediation will be 20% or less of conventional system cost. Applied to the estimated $50-70M costs for expanded pump and treat at Hanford, a cost savings of $40-65M can be anticipated. Costs can be further reduced by using transgenic trees, requiring less land area and allowing more intensive application of contaminated water.

Phytoremediation using transgenic trees can be used to treat soil extraction vapors from the 200W area at Hanford for a fraction of existing equipment. CT-contaminated gases from vapor extraction would be diffused into the root zones of the plants. Phytoremediation of CHC contaminated vapors is practical only if plants used are able to reduce concentration of CHCs. Thus, this application requires transgenic plants

Advantages of using transgenic plants:

- Less land area required -- less irrigation equipment
- Water can be treated, rather than consumed

Summary:

- First report of a potentially useful biodegradative process for CT that operates under aerobic conditions
- Transgenic plants that can have 5X or more increased rate of metabolism of halogenated hydrocarbons
- Transgenic plants that can reduce concentrations of halogenated hydrocarbons in water

Planned activities: We are developing transgenic poplar using molecular methods to increase activity. We expect transgenic poplar to be ready for testing in August 2000.

Information Access: A publication has been accepted by Proc. Nat. Acad. Science. A second publication has been submitted and is in review.