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Using Trees to Remediate Groundwaters Contaminated with Chlorinated Hydrocarbons

Stuart E. Strand
University of Washington
Anderson Hall
Box 352100
Seattle, Washington 98195
Phone: 206-543-5350
E-mail: sstrand@saul.u.washington.edu

Milton P. Gordon
University of Washington
Department of Biochemistry
Seattle, Washington 98195
Phone: 206-543-1769

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Stuart E. Strand, University of Washington

Milton P. Gordon, University of Washington

Research Objective

Industrial practices in the past have resulted in contamination of groundwater with chlorinated hydrocarbons (CHCs) at many DOE sites, such as Hanford and Savannah River. Such contamination is a major problem because existing groundwater remediation technologies are expensive and difficult. An inexpensive method for groundwater remediation is greatly needed. Trees could be used to remediate CHC polluted groundwater at minimal cost (phytoremediation). Before phytoremediation can be extensively applied, we must determine the range of compounds that are attacked, the effects of metabolic products on the plants and the environment, and the effect of transpiration and concentration of CHC on uptake and metabolism.

We will test the ability of hybrid poplar to take up and transform the chlorinated methanes, ethanes and ethylenes. The rate of uptake and transformation by poplar of TCE as a function of concentration in the soil, transpiration rate and illumination level will be determined. Methods will be developed to permit rapid testing of plants from contaminated sites for species able to oxidize and sequester chlorinated compounds. We will identify the nature of the bound residues of TCE metabolism in poplar. We will identify the mechanisms involved in CHC oxidation in poplar and use genetic manipulations to enhance that activity. We will introduce the genes for mammalian cytochrome P-450-IIE1, known to oxidize light CHCs such as TCE to attempt to increase the CHC metabolism capacity of poplar.

The results of this research will place phytoremediation of CHCs on a firm scientific footing, allowing a rational assessment of its application to groundwater contamination.

Research Progress and Implications

This report summarizes the results of the first 1.5 years of work on a three-year project.

Laboratory and field tests with poplar in tissue culture, bioreactors, and field sites have shown that, unlike bacteria, plants are able to carry out complete degradation of fully chlorinated alkanes and alkenes to carbon dioxide and chloride. Carbon dioxide was produced as a product of the degradation of trichloroethylene (TCE), carbon tetrachloride (CT), and perchloroethylene (PCE) when axenic tissue cultures of poplar cells were exposed to radiolabeled compounds. The apparent degradation of PCE and CT, fully chlorinated hydrocarbons, in these aerobic plants is remarkable when contrasted to the lack of comparable aerobic degradation by bacteria. Oxidized metabolites, such as trichloroethanol and di- and trichloroacetic acid, were detected in cell cultures exposed to TCE, suggesting the involvement of cytochrome P-450s or other monooxygenase activities. Mass balance experiments with small poplar plants in laboratory reactors showed that significant TCE and CT was volatilized from leaves, while a similar fraction of radiolabeled carbon from these chlorinated solvents was retained in the plant tissue.

Early in the project we discovered that large poplar growing in full sun took up TCE and CT without significant solvent transpiration. Pilot scale experiments in the field were carried out using cells containing approximately 12 m3 soil with an underlying send layer through which an artificially contaminated groundwater flowed. Hybrid poplar were planted in the cells (15 trees per cell). TCE or CT was added to the influent of the cells at concentrations ranging from one to 100 mg/L, but generally averaging 15 mg/L. Control cells consisted of poplar planted cells with no solvent exposure

and cells with TCE or CT exposure but no vegetation. Mass removal of TCE and CT from the groundwater in the cells exceeded 95 percent. Laboratory tests of soil from the cells showed that there was no enhancement of CT or TCE degradation in the rhizosphere soils. Transpiration of TCE and CT by the hybrid poplar at the test site was measured by two methods: long-path open FTIR and by bagging leaves and trapping solvent vapors. By both methods air emissions of the chlorinated solvents accounted for less than 6 percent of the total removal. All of the total chloride introduced to one of the planted cell as TCE during the 1997 season was recovered as chloride ion in the soil, suggesting that dechlorination of TCE was complete. Although some chlorinated metabolites (e.g., chloroacetic acids and trichloroethanol) were founded plant tissues, there was no significant increase in total organic chlorides in the tissue of trees exposed to solvent compared to unexposed controls. We conclude that poplar can destructively remove TCE and CT without harmful air emissions or accumulation of a hazardous solid waste.

The metabolism of TCE in poplars has been investigated in detail using axenic cultures of poplar cells or small plants grown in the greenhouse. The following metabolites were found: CO_2, trichloroethanol, chloral, trichloroacetaldehyde, and di- and trichloroacetic acid. In addition, carbon from the TCE was detected in the purified lignin fraction.

Many of the products observed in the plant experiments are identical to those which arise from the action of cytochrome P450 on TCE. We, accordingly, are carrying out the transformation of poplars with several heme containing cofactors and enzymes in attempts to increase the oxidizing capacity of the plant tissue. We prepared plasmids, containing mammalian cytochrome P450 2EI, cytochrome b5, and cytochrome oxidoreductase. This complex is stated to be responsible for the catabolism of CHCs in the mammalian liver. In addition, a plasmid containing mammalian beta-chain of hemoglobin was prepared. All of the plasmids were incorporated into the bacterium, Agrobacterium tumefaciens, and used to transform Nicotiana tabacum, Xanthi n.c. (the guinea pig of the plant world) and young shoots of Populus trichocarpa x P. deltoides. We now have nine tobacco plants growing with the enzyme complex containing the full cytochrome P450 2EI complex. We also have a number of tobacco plants with a tetrameric form of beta-chain hemoglobin. The poplar plants are still too small to be tested for foreign genes.

Planned Activities

In the next 1.5 years the following studies will be undertaken. Planned experiments in the field include tests of the of the TCE and CT degradative abilities of willow, various poplar, and black locust and of the ability of poplar to degrade PCE. Experiments to determine the biochemical mechanism of TCE and CT degradation in plants using axenic tissue culture are continuing. Metabolic studies with the transgenic plants, especially degradation of TCE, will be undertaken in the near future when the plants are sufficiently large. We are also in the process of preparing axenic suspension cultures of the transformed tissues for use in these studies.