Phytoremediation of Volatile Pollutants through Genetic Engineering
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Improper chemical disposal and spills have resulted in widespread contamination of the environment. Some of these contaminated areas, termed Superfund sites, are polluted to dangerously high levels. In addition, there are over 500,000 contaminated industrial properties in the United States alone that have been abandoned due to the high cost of clean up. Billions of dollars are spent each year in attempts to remEDIATE polluted sites. Engineering methods for the remediation of contaminated sites include excavation, transport, soil washing, extraction, pumping and treating of contaminated water, addition of oxidants, or incineration. Another common clean up method, bioremediation, involves the use of specific microbial strains or communities known to degrade the pollutant. Phytoremediation is the use of plants to clean up contaminated sites (for recent reviews, see [1,2]). Phytoremediation is basically a solar driven pollutant extraction system to remove pollutants from water, soil, and air. It is considerably less expensive than the other methods; it is less intrusive and more aesthetically pleasing. By acting as soil stabilizers, plants minimize the amount of contaminated dust that leaves the site and could enter the surrounding neighborhoods. Unlike bioremediation done with microorganisms, phytoremediation is more easily monitored; the condition of the plants can be determined visually; and samples of plant tissue can be easily collected and tested for the presence of the pollutant over time. Phytoremediation is primarily an aerobic process, and its use can avoid the production of intermediates with increased toxicity that is characteristic of some bioremediation methods. Another advantage of phytoremediation over the engineering or bioremediation methods is the possibility of a producing a useful product, such as wood, pulp, or bioenergy, that could offset some of the overall cost of the remediation.

Phytoremediation of trichloroethylene and carbon tetrachloride
Phytodegradation refers to the process in which plants break down pollutants, either through internal or secreted enzymes. Phytodegradation of chlorinated hydrocarbons and explosives has been studied the most extensively. Trichloroethylene (TCE), one of the most common groundwater pollutants, and carbon tetrachloride (CT) are suspected human carcinogens. Hybrid poplars (Populus trichocarpa × P. deltoides) take up and degrade TCE, producing the same TCE metabolites as mammals[3,4], and also take up and degrade CT[5,6]. In controlled field studies with hybrid poplars, the trees removed over 99% of the added TCE[7] or CT[6]. Transpiration of TCE and CT from leaves and trunks was negligible. In order to determine if poplar cells have an inherent ability to degrade TCE and CT or if microorganisms are responsible for the degradation, studies were conducted with suspensions of pure poplar cell cultures. When these poplar cell cultures were dosed with TCE, the same metabolites that were found in the whole plant studies were also seen[4,8,9]. Experiments with poplar culture cells and whole plants demonstrated that the primary metabolite, trichloroethanol, is glycosylated as in mammalian systems[8]. Poplar culture experiments with CT showed that plant metabolism of CT was inhibited by the same chemicals that inhibit CT metabolism by P450 cytochromes in mammalian systems[8].

Disadvantages of phytoremediation
The primary disadvantage of phytoremediation when compared to engineering methods is that it is often too slow or only seasonally effective. Regulatory agencies often require significant progress in remediation to be made in only a few years, making most phytoremediation applications unsuitable. For some pollutants such as TCE and CT, the concentration of the pollutant is not reduced sufficiently to meet regulatory requirements. In some contaminated sites, the pollutants can be at phytotoxic concentrations or recalcitrant to degradation such that plants are not effective. For these reasons, attention recently has focused on ways to enhance the phytoremediation potential of plants using genetic engineering.

Genetic engineering of plants for enhanced metabolism of pollutants
A direct method for enhancing the effectiveness of phytoremediation is to overexpress in transgenic plants the genes involved in metabolism, uptake, or transport of specific pollutants (recently reviewed in [10,12]). This can be readily achieved for many plant species by using Agrobacterium tumefaciens-mediated plant transformation. Since phytoremediation is generally more effective when using large, fast-growing plants, the focus has been on poplar trees. Depending on the hybrid and particular clone, reasonable transformation frequencies can be achieved in poplar[13].

To increase the phytoremediation potential of the common pollutant TCE, we genetically engineered plants with a
mammalian cytochrome P450 enzyme known to metabolize it. The P450 2E1 enzyme controls the rate-limiting step in the metabolism of multiple environmental pollutants, including TCE, carbon tetrachloride, chloroform, benzene, vinyl chloride, and ethylene dibromide. When the cytochrome P450 2E1 gene (hCYP2E1) was overexpressed in tobacco plants, metabolism of TCE was substantially increased\(^1\). Furthermore, the transgenic tobacco removed 98% of the ethylene dibromide, compared with 63% removal by the null vector control plants. The P450 2E1 enzyme from rabbit was successfully expressed in hairy root cultures of *Atropa belladonna*\(^2\). These mammalian enzymes functioned well in plants without any need to modify the gene or to include the other enzymes, oxidoreductase and cytochrome b5, known to be required for full function of mammalian P450s. These common enzymes seem to be sufficiently similar in mammals and plants such that the P450s can function with either type.

In recently published work, clear enhancement of phytoremediation potential was obtained when the rabbit 2E1 gene was overexpressed in hybrid poplar (*P. tremula × P. alba*)\(^3\). TCE metabolism in two of the transgenic poplar lines was enhanced more than a hundred-fold, with an overall enhancement of over 40-fold in the transgenics compared to the control plants. The transgenic poplar clone with the highest expression of CYP2E1 removed TCE faster than other plant lines, taking up TCE at a rate 53-fold faster than the controls. The CYP2E1 transgenic poplar removed other P450 2E1 substrates, including chloroform, CT, and vinyl chloride from the hydroponic solution, at faster rates than did the control plants. When the transgenic plants were exposed to the volatile form of benzene and TCE, they removed these compounds from the air faster than the control plants. While the control plants barely removed any TCE from the air, the transgenic poplar removed 79% of the TCE during the one-week experiment. Therefore, overexpression of a single enzyme can lead to dramatic improvements in phytoremediation potential of a variety of pollutants from both water and air. By increasing the metabolism of TCE within the plant, lesser amounts of the unaltered compound would be released into the atmosphere via phytovolatilization.

**Safety concerns**

Due to the strict regulations governing the release of transgenic trees, it is likely that genetically engineered trees for phytoremediation will be used only on closely monitored sites such as SuperFund sites or military installations where the unintentional spread of the plant material would be unlikely. To prevent transgene flow, the trees would be cut down before they became sexually mature, after several years in the field. Careful selection of the species to be transformed can avoid routes of transgene release by using trees that will not resprout from wind-blown branches. If the transgene did “escape” into native populations, a gene involved in pollutant degradation would be unlikely to confer any selective advantage or negative environmental impact.

**Conclusions**

There are numerous reports in the literature supporting phytoremediation as an effective method in treating hazardous sites. Yet it is not used as widely as it could be. In the last several years, significant progress has been made to increase the efficiency of phytoremediation. Using genetic engineering, substantial increases in removal rates of hazardous pollutants, including nitroaromatics, solvents, and metals, has been achieved. With the increased effectiveness of phytoremediation, this “green technology” may be used to decrease the expense of clean-up and lead to an increased likelihood that sites will be restored rather than abandoned.

**Reference List**

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