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PHYTOREMEDIATION POTENTIAL OF WILLOW TREES FOR AQUIFERS CONTAMINATED WITH ETHANOL-BLENDED GASOLINE

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Abstract—Ethanol-blended gasoline has been used in Brazil for 20 years and, probably, is going to be more widely used in North America due to the MtBE environmental effects on groundwater. The potential impacts caused by the presence of ethanol in UST spills are related to the co-solvency effect and the preferential degradation of ethanol over the BTEX compounds. These interactions may increase the length of dissolved hydrocarbon plumes and the costs associated with site remediation. This study investigates the advantages of phytoremediation to overcome the problems associated with the presence of ethanol in groundwater contaminanted with gasoline–ethanol mixtures. Experiments were performed under lab conditions with cuttings of Willow tree (*Salix babylonica*) cultivated hydroponically. Results showed that the cuttings were able to reduce ethanol and benzene concentrations by more than 99% in less than a week. The uptake of both contaminants was confirmed by blank controls and was significantly related to cuttings transpiration capacity. Sorption onto roots biomass also markedly affected the behavior of contaminants in solution. Experiments to evaluate plants' toxicity to ethanol indicated that plants were only affected when aqueous ethanol concentration reached 2000 mg l^{-1}. Results suggest that phytoremediation can be a good complement to intrinsic remediation in shallow aquifer sites contaminated with ethanol-blended gasoline spills. © 2001 Elsevier Science Ltd. All rights reserved

Key words—*Salix babylonica*, ethanol, benzene, groundwater, phytoremediation

INTRODUCTION

Aquifer contamination by gasoline spills from underground storage tanks has been a constant concern worldwide. To further increase the difficulties to deal with the problem, a variety of different oxygenates, such as ethanol and methyl-tertiary-butyl-ether (MtBE) have been added to gasoline to reduce vehicular carbon monoxide and ozone precursor emissions into the atmosphere. Ethanol and ethanol-blended gasoline have been used as fuels in Brazil since the late 1970s. In that country, the National Alcohol Program was created to cope with the high oil prices in the 1970s and 1980s. The federal incentives, associated with the automobile industry and capital goods interests to boost the economy, and the strong environmental appeal, made the program a success. Today, approximately 75% of all automobiles run on gasoline containing 24% ethanol, and projects to add 3% ethanol on diesel are under revision by Brazilian authorities. On the other hand, MtBE is the most common oxygenate added to reformulated gasoline in North America, but this chemical is being banned in California due to its low biodegradability, taste and odor problems, and its persistence in subsurface systems (Keller *et al*., 1998). Ethanol, which is already used in reformulated gasoline in several Midwest states in the United States, is considered as a possible substitute for MtBE.

The main concern in dealing with aquifer contamination by ethanol-amended gasoline is focused on the complex physical, chemical and biological interactions between gasoline constituents and ethanol, which may limit the selection of proper environmental restoration technologies. Ethanol can increase the mass of hydrocarbons in groundwater due to co-solvency, and can also cause a preferential substrate utilization by soil microorganisms. In an ethanol-blended gasoline spill, the fuel will infiltrate through the unsatured zone spreading at the water table. Ethanol, being completely miscible in water, will then migrate to groundwater. In this case, a high aqueous ethanol concentration could not only enhance concentration of the most common contaminants, benzene, toluene, ethylbenzene and

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*Author to whom all correspondence should be addressed. Tel.: +55-48-331-7569; fax: +55-48-331-9823; e-mail: corseuil@ens.ufsc.br xylenes (BTEX) in groundwater, but also enhance the presence of the more hydrophobic and more toxic polycyclic aromatic hydrocarbons (Corseuil and Fernandes, 1999). Moreover, length and persistence of hydrocarbon plumes is directly related to the rate of contaminants biodegradation. Corseuil *et al.* (1998), have shown that ethanol is preferentially degraded under all common electron acceptor conditions found in subsurface systems. Therefore, ethanol can impede, or at least retard, natural hydrocarbon degradation in groundwater due to the depletion of the electron acceptors pool.

The presence of a large mass of petroleum hydrocarbons and ethanol in groundwater can make remediation of aquifers contaminated by ethanol-blended gasoline more difficult and costly than conventional gasoline (Corseuil *et al.*, 1996). One technology widely used for soil and groundwater decontamination that might be adequate to the ethanol problem is phytoremediation. Considering plants physiological features and that ethanol causes a high biological electron acceptors demand in groundwater, it is appropriate to think that phytoremediation can be a good alternative to clean-up sites contaminated with gasoline–ethanol spills. This technology could also be used as an intrinsic remediation complement, when it is found that natural attenuation processes are not sufficient to prevent plume migration from impacting the environment (Moreno, 1998).

The ability of Salicaceae family trees, particularly the *Populus* Genus, in the removal of a large array of organic contaminants by absorption onto plant roots is well documented (Paterson and Schnoor, 1992, 1993; Nair *et al.*, 1993; Schnoor *et al.*, 1995; Thompson *et al.*, 1998). Indeed, the fate of some volatile organic compounds (VOCs) after taken up by poplar roots has also been demonstrated. For instance, once translocated to above ground tissues, BTEX compounds can be volatilized from leaves to the surrounding atmosphere, a pathway that appeared to be intrinsically related to transpirative rates of poplar cuttings grown hidroponically (Burken and Schnoor, 1999). The present work has been carried out to evaluate the potential ability of *Salix babylonica* trees in the uptake of ethanol and benzene. Benzene was used as the target contaminant for the ethanol-blended gasoline because it is considered the most toxic of the BTEX and degrades slowly under anaerobic conditions or not at all (Alvarez and Vogel, 1995; Weiner and Lovley, 1998). Preliminary phytotoxicity tests were also done to assess the toxicity of ethanol prior to the establishment of groundwater phytoremediation systems.

METHODOLOGY

Plant stock1x(98)

Hardwood cuttings were excised from mature willow trees (*Salix babylonica*-Linnaeus) established at the campus of the Federal University of Santa Catarina, in Florianópolis, Santa Catarina, Southern Brazil. After collection, 30 cm long × 5 mm stem diameter cuttings were wrapped with 1 cm layer Teflon tape approximately 15 cm from the cutting base. Cuttings were then introduced through holes drilled in Neoprene stoppers, which were firmly fixed over the mouth of 500 ml Erlenmeyer flasks. Cuttings were allowed to root hydroponically in a 400 ml half-strength Hoagland's inorganic nutrient solution (Hoagland and Arnon, 1950) supplemented with Murashige and Skoog's formulation for nonessential elements (Murashige and Skoog, 1962). The pH of the solution was adjusted to 6.0 and the rooting period extended for 30 days under greenhouse conditions.

Ethanol and benzene uptake experiments

Prior to the beginning of the experiments, uniform cuttings were selected from the plant stock and nutrient solution was replaced by a new one autoclaved for 15 min at 121°C and 100 kPa. Experiments comprised three sets of treatments: reactors with plants, blank sterile controls and root/stem controls. Reactors with plants utilized the same configuration described for the plant stock reactors. Root/stem control reactors, on the other hand, had the aerial portion of the plant excised 1 day prior to the beginning of the experiments (Burken and Schnoor, 1999). Sodium azide ($1000\,\mathrm{mg\,l^{-1}}$) was used in the controls to inhibit microbial activity. Three replicates were prepared for each reactor type. Experiment with ethanol was carried out in 500 ml reactors placed in a greenhouse during a 5 day period. Ethanol mass was analytically calculated to reach an aqueous concentration of approximately $1500\,\mathrm{mg\,l^{-1}}$ in a 400 ml nutrient solution. The experiment with benzene, on the other hand, utilized a semi-continuous approach and was performed in 300 ml flasks with pre-drilled screw caps with Teflon-lined septum. Target benzene concentration ($20\,\mathrm{mg\,l^{-1}}$) was achieved using a benzene-saturated aqueous solution. About 5 ml of the benzene nutrient solution, lost by transpiration and sample collection, was replenished every day to maintain reactors volume at 250 ml level. Experiment was carried out in a closed room with temperature kept at 25 ± 2°C for a 1 week period. The photoperiod was set at $16\,\mathrm{h\,d^{-1}}$ and light was provided by 12 Phillips 40 W fluorescent tubes. To make the reactors airtight, the gap between the cuttings and the stopper/cap was sealed with Parafilm© (Nair *et al.*, 1992). Water transpiration was gravimetrically monitored daily in order to determine the mass balance in the reactors with plants. Every day, before nutrient solutions were replaced, 1 ml liquid samples were withdrawn from each reactor with gastight syringes. Samples were then transferred into Teflon-lined cap GC vials and stored at 4°C prior to the analyses.

A Hewlett Packard (HP) 5890 Series II Gas Chromatograph coupled to a HP 7694 headspace autosampler was used for analysis of ethanol and benzene. Analyses were performed via flame ionization detector (FID) and separation achieved using a HP-1 capillary column (30 m length, 0.53 mm ID and 2.65 μm film thickness).

Ethanol phytotoxicity experiments

This experiment was conducted at three ethanol concentrations (500, 1000 and $2000\,\mathrm{mg\,l^{-1}}$) under greenhouse conditions. Toxicity was assessed through daily measurements of willow cuttings transpirative capacity that was gravimetrically monitored at 24 h intervals. Reactors (500 ml) with plants, but with no aqueous ethanol concentrations, were used as controls. Nutrient solution was totally replenished if nutrient solutions in both reactors changed by more than 10%. Prior to the beginning of the experiment, all cuttings were weighed and the achieved results for transpirative capacity were normalized. Three replicates were prepared for each set of treatments.

Statistical analysis

All the experiments had a completely randomized designed. One-way ANOVA was performed to compare treatments in the ethanol and benzene experiments. The Tukey honest significant difference (HSD) test, was used to distinguish treatments one from another. The *t*-test was performed for independent samples to compare the average values between plants dosed with ethanol and controls in the phytotoxicity experiment (Rothmans and Ericson, 1987).

RESULTS AND DISCUSSION

Ethanol uptake experiments

Although the release of ethanol as a result of the anaerobic respiratory pathway of root systems during flooded conditions has been documented, there are no reports about its absorption by plant roots. Analytical results for hydroponic willow tree cultures in the presence of ethanol showed that ethanol concentrations reduced more than 99% in a 5 day period (Fig. 1). Average concentrations in reactors with plants dropped from 1360 mg l^{-1} to about 9 mg l^{-1} of the initial concentration. In the controls with roots alone, ethanol reached a near steady-state level after declining up to 45% of its initial value. Plotting the data for ethanol on a mass basis yielded similar results. Both treatments (reactors with plants and with roots alone) had concentrations markedly lower than blank controls. According to the ANOVA model, ethanol average values were statistically different from blank controls. Mean comparison for the tested treatments using Tukey test suggested that the two main processes involved in the ethanol depletion from the aqueous solution were root uptake and sorption to plant biomass.

The transpiration stream concentration factor (TSCF), which has been utilized in phytoremediation studies to predict the uptake and translocation of organic compounds by plant species, can be calculated as function of the compound's log K_{ow} (Briggs *et al.*, 1982; Burken and Schnoor, 1999). According to the TSCF factor, hydrophilic compounds such as ethanol (log $K_{\mathrm{ow}}-0.31$), would have a limited movement across the organic membranes of the cell roots (endodermis). However, in our experiments, ethanol was readily taken up and removed from the hydroponic reactor at the same rate of the nutrient solution. Because of the reactor's high transpiration values (average daily rates of 43.2 ± 2.7 ml d^{-1}, $n = 3$), it is not an exaggeration to infer that the observed ethanol depletions, even on a concentration basis, can be related to the cuttings transpirative rates. Moreover, the excellent correlation found between ethanol uptake and water consumption (Fig. 2) also reinforces this conclusion. On the other hand, in the absence of a transpirative stream, as simulated in the stem/roots controls, ethanol losses are probably due to sorption onto the roots, since nutrient solutions of these controls contained sodium azide to prevent ethanol biodegradation.

Benzene uptake experiments

In these experiments, reactors were treated as semi-continuous chemostats to simulate a groundwater contamination, assuming that benzene plume, once dissolved in the aqueous phase, would move towards the root zone of an established tree population. In this way, every day after sampling, a small volume of the 20 mg l^{-1} benzene nutrient solution was added to the reactors with plants to refill the solution lost due to transpiration and sampling. Results for the reactors with plants showed that the benzene aqueous concentration was reduced by 70% after 1 day, and by more than 99% at experiment 7th day (Fig. 3). Blank controls, containing only the nutrient solutions and benzene, indicated that benzene losses were minimum, which shows that the experimental setup was adequately preventing benzene volatilization. On the other hand, aqueous benzene concentrations in the stem/roots control reactors stayed in the

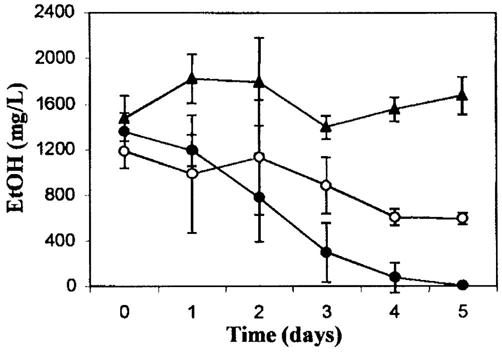

Fig. 1. Effect of willow cuttings and their isolated roots on ethanol depletion from solution. Error bars depict the standard deviation from the mean of triplicate microcosms. Error bars smaller than symbols are not depicted.

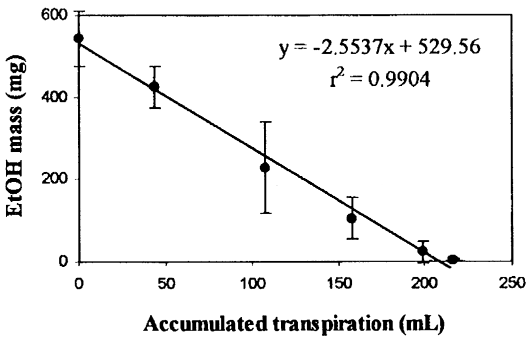

Fig. 2. Relationship between ethanol depletion from solution and accumulated transpiration for reactors with plants. Error bars depict the standard deviation from the mean of triplicate microcosms. Error bars smaller than symbols are not depicted.

range of 5 and 9 mg l^{-1} throughout the experiment. When data were statistically compared, it was found that there were significant differences among treatments with a probability degree of 99.9% ($P \leq 0.001$). These results suggest that initially benzene was being sorbed onto root biomass, but plant uptake and biological degradation became the predominant mechanisms afterwards, which indicates that uptake and microbial transformation are responsible for benzene depletion from the reactors nutrient solutions.

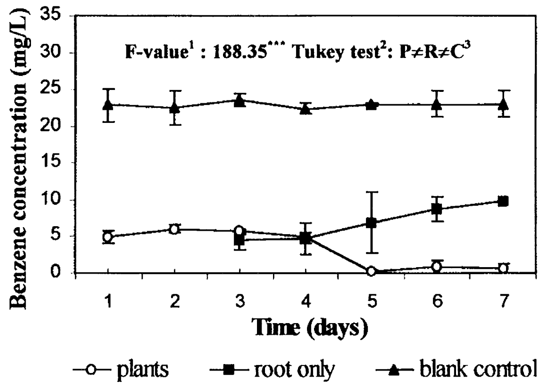

Fig. 3. Effect of willow cuttings and their isolated roots on benzene aqueous concentration for semi-continuous hydroponic reactors. Initial aqueous benzene concentration in the start of the experiment was approximately 20 mg l^{-1} for all treatments. Each data point represents the mean ± standard deviation of three replicates. (^{1}F-value is presented on the effect of treatments on benzene concentration; ***Significance at the $P \leq 0.001$ level; ^{2}Tukey Honest significant difference (HSD) test for mean comparison at an alpha level of 0.05.)

Ethanol phytotoxicity

Due to its high solubility in water, ethanol could be present in high concentrations in groundwater near source zones of ethanol-blended gasoline spills. Furthermore, this compound is highly toxic not only for many plants but also for microorganisms. Hunt *et al.* (1997), for instance, reported that ethanol concentrations in microcosm experiments higher than 40,000 mg l^{-1} were toxic to the microorganisms, as shown by complete lack of oxygen consumption. In order to accomplish a successful dose–response relationship between each set of treatment, transpiration measurements for individual cuttings were normalized to their respective biomass. Results obtained for transpiration measurements for dosed reactors and controls were practically similar when ethanol concentration ranged between 500 and 1000 mg l^{-1} (Fig. 4). Cuttings appeared not to be affected by this concentration range, once visible symptoms of phytotoxicity, such as chlorosis or leaf abscission, were absent. However, transpiration was markedly reduced when ethanol concentration was increased to 2000 mg l^{-1}. In fact, differences in transpiration measurements between dosed reactors and controls were around 0.08 and 0.05 ml g^{-1} for the 500 and 1000 mg l^{-1} experiments, respectively. On the other hand, at 2000 mg l^{-1} ethanol concentration, this difference increased by one order of magnitude, reaching an average value of 0.57 ml g^{-1}. This difference was statistically confirmed by the *t*-test.

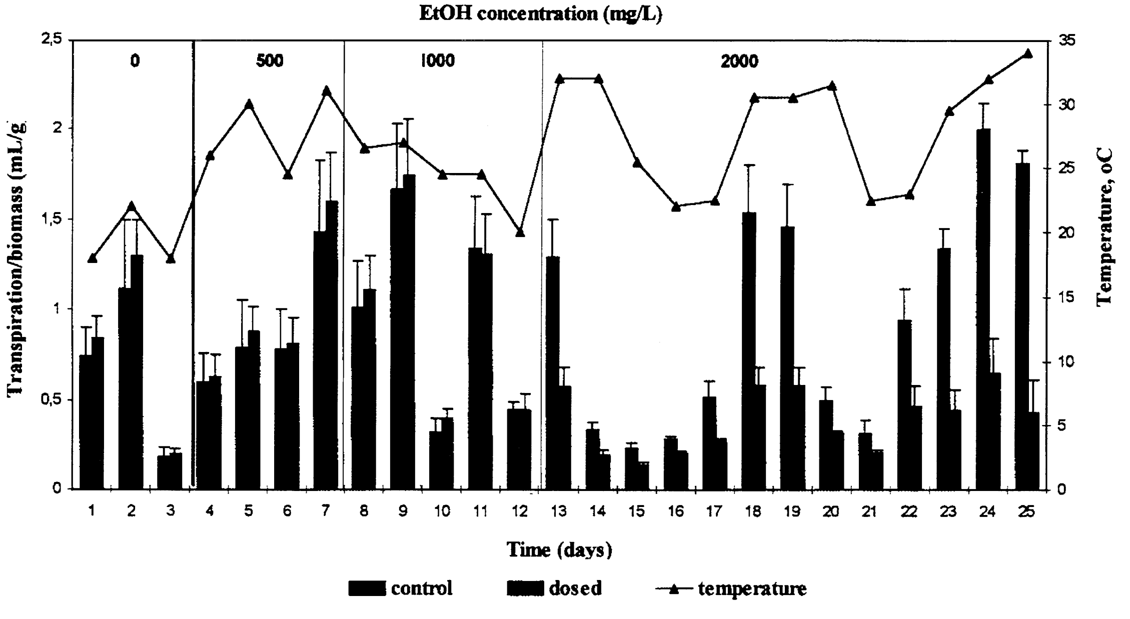

Fig. 4. Effect of increasing ethanol concentrations on willow cuttings transpiration rates. Values were normalized to total plant biomass. Controls are reactors with plants and nutrient solution. Dosed are reactors with plants and nutrient solutions containing ethanol. Daily average temperatures under greenhouse conditions are also presented. Error bars represent ± one standard deviation from the mean of three replicates.

Leaf drying and chlorosis were also observed as visible symptoms of phytotoxicity, but cuttings, although keeping reduced rates of transpiration, were able to survive after exposure to 2000 mg l^{-1} ethanol. Besides, transpiration measurements during the high-dose period followed a similar pattern of the control plants and were conducive to temperature variations recorded in the greenhouse, which is a sign of tolerance of this species to ethanol.

CONCLUSIONS

The complex physical, chemical and biological interactions between ethanol and BTEX may jeopardize the efficiency of natural attenuation as a cost-effective approach towards clean-up of aquifers contaminated with ethanol-blended gasoline. Even though experiments were performed in lab-scale, results shown in this study suggest that deep-rooted willow trees may significantly remove ethanol and benzene from shallow aquifers contaminated with ethanol and gasoline mixtures. The plant efficiency to uptake ethanol indicates that the phytoremediation process could easily overcome the possible negative impacts of ethanol in ethanol-blended gasoline spills. Additionally, these trees may retard the transport of ethanol and other soluble gasoline constituents by sorption to root biomass. In this context, the presence of willow trees might play a fundamental role, because organic carbon in aquifer material can be built up as a consequence of roots decay and roots exudates release. When coupled to the ability of willow trees to form roots under low-oxygenation conditions (as low as 1 ppm) (Hartmann and Kester, 1975) creating optimized conditions for microbial growth, both plant uptake and sorption processes could be potentially advantageous to in situ natural attenuation at oxygen-limited aquifers, where the possible lack of electron acceptors caused by ethanol, does not favor BTEX biotransformations. Based on these assumptions, future field-scale research aiming to combining and integrating phytoremediation to natural attenuation approaches towards mitigating the impacts of ethanol-blended gasoline spills should be encouraged.

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