Plant-Mediated Transformation of Perchlorate into Chloride

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The decontamination of perchlorate-contaminated water by woody plants was investigated in sand and hydroponic bioreactors. Willow trees were found to be the most favorable woody plants with phraetophytic characteristics in comparative screen tests with eastern cottonwoods and *Eucalyptus cineria*. Willows decontaminated aqueous solutions dosed with 10−100 mg/L of perchlorate to below the method detection limit of 2 μg/L. Two phytoprocesses were identified as important in the remediation of perchlorate-contaminated water: (a) uptake and phytodegradation of perchlorate in the tree branches and leaves and (b) rhizodegradation. Exposure of rooted willow trees to perchlorate-dosed media stimulated rhizodegradation. Homogeneous degradation studies using media from the root zone of dosed willow trees confirmed that rhizosphere-associated microorganisms mediated the degradation of perchlorate to chloride. Experiments conducted with varying ranges of nitrate concentrations clearly indicated that high nitrate concentrations interfered with rhizodegradation of perchlorate. This study provides evidence that the efficacy of phytoremediation of perchlorate-contaminated environments may depend on the concentration of competing terminal electron acceptors, such as nitrate, and the nitrogen source of the nutrient solution. Since perchlorate does not volatilize from water readily, a perchlorate remediation scheme may involve an intensively cultivated plantation of trees with phraetophytic characteristics and irrigation with the contaminated water.

Introduction

Perchloric acid and its salts have been used extensively in a number of commercial applications such as wet digestions, organic syntheses, electropolishing of metals, animal feed additives, explosives, pyrotechnics, missile propellants, and herbicides. In addition, it is found as a contaminant in certain fertilizers and bulk water treatment chemicals (*1, 2*). Perchlorate explosives and propellants are less sensitive to shock and environmental changes than dynamite. Accordingly, the United States military and National Aeronautical Space Administration (NASA) have extensively used ammonium perchlorate as solid rocket propellant.

The anion perchlorate poses potential environmental concerns because its ionic radius and charge are similar to that of iodine, which allows perchlorate to competitively block thyroid iodine uptake. At relatively high doses, perchlorate is known to interfere with the thyroid's ability to produce hormones and regulate metabolism (*3*). Iodine is an essential element for thyroid function. A severe deficiency can cause cretinism, which occurs in as many as 2−10% of the populations of isolated communities in many areas of the world. It is considered the world's most readily preventable cause of mental retardation (*4*). In iodine-deficient populations, a mild form of cognitive impairment occurs five times as frequent as cretinism, and the IQ curve of the entire population can be shifted to the left (*4*). The societal costs of such an IQ shift can be significant.

The first concerns about perchlorate in the environment surfaced in 1896 when sodium and potassium salts of perchlorate found in Chilean nitrate were noted to be harmful to certain agricultural crops (*5*). Recently, perchlorate has been found in groundwater at numerous hazardous waste sites in California, Nevada, Utah, and Texas (*1*). Water suppliers in both northern and southern California and the Las Vegas Water Authority have found perchlorate in their water supplies generally at levels less than 18 μg/L but ranging as high as 280 μg/L, with several in the 100−200 μg/L range. Perchlorate has also been detected at low levels (5−9 μg/L) in the Colorado River. The highest surface water concentrations of up to 1700 μg/L have been found in a Lake Mead inlet. The highest perchlorate concentration detected in an aquifer to date is about 8 mg/L (*6*).

Being a salt, ammonium perchlorate is readily soluble in water. Once dissolved in water, perchlorate is remarkably stable. Perchlorate is not air strippable and so is not a candidate for conventional pump-and-treat remediation. Various approaches have been investigated for the remediation of perchlorate contamination including adsorption by activated carbon, reverse osmosis, anion exchange, and bioremediation. Although there is no National Primary Drinking Water Regulation (NPDWR) or Health Advisory established for perchlorate at this time, the California Department of Health Services has established an action level for perchlorate in drinking water of 18 μg/L (*3, 6*).

Bioremediation has been suggested as a promising approach for the remediation of perchlorate-contaminated water. Anaerobic conditions are favored for microbial-mediated reduction of perchlorate. Anaerobic microbial oxidation of organic substrates (e.g., glucose or acetate) requires the use of an alternate electron acceptor, such as NO_3^-, Mn(IV), Fe(III), or SO_4^{2-}. Perchlorate has a high potential for utilization as an alternate electron acceptor because it is a highly oxidized compound with a +7 oxidation state. Rikken and co-workers (*7*) isolated the bacteria strain GR-1, which is capable of oxidizing acetate with oxygen, nitrate, Mn(IV), and perchlorate acting as terminal electron acceptors (TEAs). These authors observed that, in the presence of acetate, complete reduction of perchlorate to chloride by the cell suspensions occurred within a few hours. Both dissimilatory and assimilatory nitrate-reducing microorganisms are believed to be capable of destroying perchlorate and chlorate (refs *6−9* and references cited therein). From the latter studies, the enzymatic activity supporting the reduction of chloroxyanions has been linked to enzymes involved in nitrate reduction.

Phytoremediation is an emerging technology that is rapidly gaining interest and promises effective and inexpensive cleanup of certain hazardous waste sites. The use of plants to remediate contaminated environments is cost-effective and ecologically sound. Five mechanisms by which plants are believed to remove, degrade, or stabilize environmental contaminants have been identified in recent years.

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FIGURE 1. Photo of 6-month-old control and dosed rooted willow cuttings. The photo on the right shows the roots of willows in a dosed hydroponic bioreactor. The left port near the top of the reactor is for feeding and water replenishing, and on the right is the mininert sampling valve (modified from Burken and Schnoor (*17*)).

Plants may take up and assimilate contaminants (phytoaccumulation), volatilize the contaminants into the atmosphere (phytovolatilization), or degrade the contaminants within plant tissues using enzymes (phytodegradation). In the rhizosphere of some plants, released plant exudates and enzymes that stimulate biochemical activity may enhance the biodegradation of environmental contaminants (rhizodegradation). For metals, plants may be used to absorb and precipitate large quantities of toxic metals in soils, thus reducing their bioavailibility and preventing their entry into groundwater and food chains (phytostabilization) (*10*). However, no research in the current literature has focused on the use of green plants to decontaminate perchlorate-contaminated water.

We report here that woody plants are capable of decontaminating water polluted with perchlorate in sand bioreactors and hydroponic systems under laboratory conditions. The specific objective of this study was to determine the primary phytoprocesses involved in the decontamination of water polluted with perchlorate. The effect of NO_3^- (a likely competing TEA), used as N-source in the nutrient media was also investigated. Hydroponic systems rather than soil-based systems were used in order to minimize the abiotic and biochemical influences from the selected soil on the various phytoprocesses.

Materials and Methods

Sodium perchlorate, $NaClO_4 \cdot H_2O$ was obtained from Aldrich Chemical Co. (Milwaukee, WI). Full-strength Hoagland's solution (a nitrate nitrogen source) purchased from Carolina Biological Supply Company, Burlington, NC, and Stern's Miracle-Gro (6.8% ammonical nitrogen and 8.2% urea nitrogen) purchased from a local plant nursery were diluted with deionized water to make the desired strength of nutrient solution. A 50% NaOH (w/w) solution obtained from J. T. Baker, Phillipsburg, NJ, was used in preparing the IC eluent and plant extraction solutions. Willow (*Salix nigra*) and eastern cottonwood (*Populus deltoides* and hybrid *populus*) tree cuttings obtained from the Carswell Air Force Base (AFB) in Texas and 1-year old *Eucalyptus cineria* plants purchased from a local nursery in Athens, GA, were used in the screen tests. The *E. cineria* plants were washed with running tap water to remove all soil from the root zone before transferred to the bioreactors. The harvested cottonwood and willow cuttings [40 cm (length) by 4 mm (stem diameter)] were rooted hydroponically in half-strength Hoagland's solution (*16, 17*) for 3 months. A detailed description and schematic of the modified screw-cap 2-L culture flasks (bioreactors) used to conduct these experiments has been provided elsewhere (*17*). Because perchlorate is not a volatile compound, the aerial compartment shown in ref *17* was avoided in our design (Figure 1). The interface of the septum and the tree cuttings was sealed with Parafilm to minimize losses of water from the reactors by volatilization. Initial perchlorate degradation studies, including screen tests, were conducted with sand media (sand bioreactors). Because these preliminary experiments indicated that perchlorate degradation was mass-transfer limited in the sand bioreactors, subsequent studies were performed only in hydroponic bioreactors. The representative data of experiments conducted in sand bioreactors are clearly indicated.

Sand bioreactors consisted of pristine sand placed at the bottom of each 2-L screw-cap flask with the tree roots submerged in the diluted Hoagland's solution above. The sand occupied about half of the reactor volume. The total volume of the diluted nutrient solution was approximately 900 and 2 L in the sand and hydroponic (no sand) bioreactors, respectively. The growth solutions were 10%, 25%, and 50% full-strength Hoagland's solution. The concentration of diluted Hoagland's solution used in a given experiment was based on the targeted NO_3^- concentration in the bioreactor, as specified below. Three initial perchlorate concentrations, as ClO_4^-, of approximately 10, 20, and 100 mg/L were used in these experiments. Accompanying each set of experiments were two dosed blanks (no plant) and at least one undosed plant control.

The experiments were conducted in spring and summer months under natural environmental conditions. Each dosed reactor was wrapped in a sheet of Parafilm, and the solution portion of the reactor was shielded from light by aluminum foil. The water in the bioreactors was kept at a constant level by replenishing the growth solution lost by evapotranspiration with a known volume of deionized water or diluted nutrient solution. This necessitated adding deionized water and/or diluted Hoagland's solution two or three times per day to each bioreactor. A daily record of NO_3^- concentration in the bioreactors and the volume of water taken up by each tree were maintained over the duration of each study. The

decision on whether to add deionized water or diluted Hoagland's solution was based on the measured NO_3^- concentration taken each morning. A 1-mL aliquot of the solution was also withdrawn for perchlorate analysis. The sample was diluted with deionized water to the IC measurement range of the perchlorate ion. The total inorganic and organic carbon (TIC and TOC) in the growth media were measured at the beginning and end of the experiments.

The distribution of perchlorate in different plant fractions was determined by sacrificing the study trees for extraction and analysis at the termination of the experiments. The sacrificed trees were removed from the media, rinsed with deionized water, and sectioned into roots, lower stem, upper stem, branches, and leaves. The total wet weight and fraction weight of the sectioned tissues were measured before extraction. Each fraction was extracted several times by blending for 30−60 min with a solution of 1 mM NaOH (pH 11). The liquid phase was separated from the plant phase by centrifugation at 3000 rpm for 20 min. The number of extractions required for the complete removal of the extractable perchlorate and its analogues was dependent on the plant fraction. On average, three extractions were needed to completely remove the extractable fraction of perchlorate from the respective ground plant organs. The extract was analyzed for perchlorate, chlorate, chlorite, and chloride ions.

Sorption of Perchlorate to Sand. The sorption of perchlorate to sand used in the bioreactors was determined in separate sorption equilibrium studies. The same mass of sand (10 g) was weighed into 50-mL Pyrex glass centrifuge vials. Each vial was filled with half-strength Hoagland's solution containing the appropriate amount of perchlorate. The concentration range used in the experiment ranged from 0 to 100 mg/L. Corresponding controls containing dosed media without sand were prepared and handled in parallel with the samples. Duplicate vials were prepared for each control and sample concentration point.

Determination of Nitrate Effects. The ability of NO_3^- in the diluted Hoagland's solution to influence the rate of perchlorate reduction in the rhizosphere of willows was investigated in separate experiments described in Figure 6 and Table 3. Initial experiments indicated that the rate of perchlorate removal was faster at NO_3^- concentrations of <100 mg/L than at higher levels. These experiments were performed in diluted Hoagland's solution supplemented with NO_3^- to achieve three desired concentration ranges: <100, 100−300, and >400 mg/L. The concentration range of NO_3^- generally found in groundwater is 0−20 mM [0−1240 mg/L] (6). In one set of experiments, the NO_3^- concentration was maintained at <100 mg/L, which is equivalent to the NO_3^- concentration in 10% full-strength Hoagland's solution. A second set of experiments was conducted at NO_3^- concentration range of 100−300 mg/L, which is equivalent to the NO_3^- concentration in 25% full-strength Hoagland's solution. In the third set of experiments, the NO_3^- concentration was >400 mg/L, which is equivalent to the NO_3^- concentration in 50% full-strength Hoagland's solution. The nitrate concentration in these experiments was maintained at the specified concentrations by adding sodium nitrate as needed to achieve the targeted NO_3^- levels. Each set of reactors was dosed multiple times with about 100 mg/L perchlorate. The chloride, nitrate, and acetate concentrations and the pH of the growth solution were measured during the course of each experiment. Controls consisted of willow trees grown in perchlorate-free media and dosed bioreactors with no plants.

Evidence that the nitrogen source of the nutrient solution may influence the phytoremediation of perchlorate was obtained by substituting Miracle-Gro, an ammonium and urea nitrogen source, for diluted Hoagland's solution in some experiments. The hydroponic experiments were conducted with willows grown on 2 and 0.5 g/L solutions of Miracle-
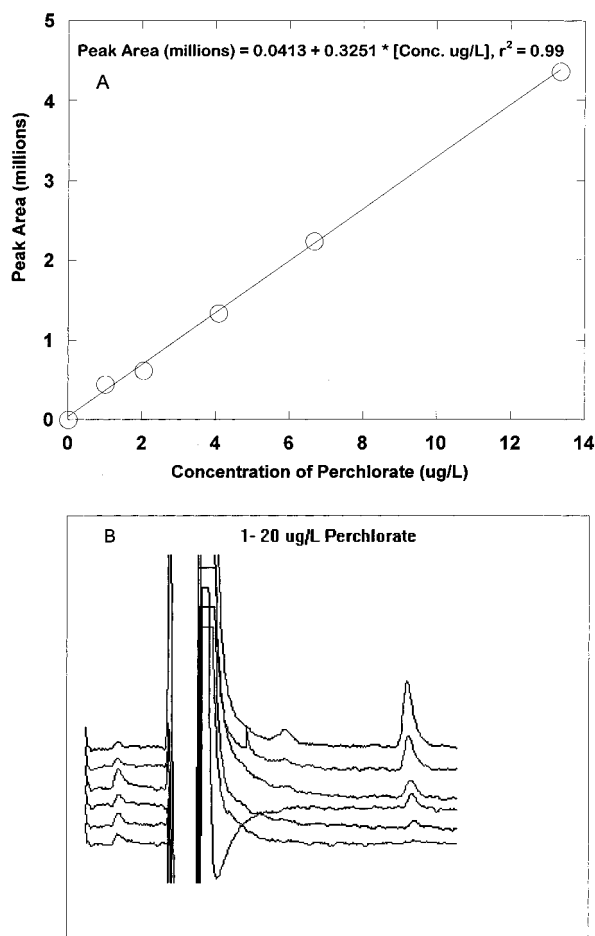

FIGURE 2. Calibration curve (A) and representative chromatograms (B) for low concentration of perchlorate in water (1−20 μg/L). Analysis was by ion chromatography using a 500-μL loop.

Gro. The nutrient solution was dosed with perchlorate to obtain an initial solution concentration of approximately 22 mg/L. For each nutrient concentration, two samples and one control were prepared and handled in parallel. The rate of removal of perchlorate from solution and uptake into the leaves was monitored as described above.

Homogeneous Studies. Four different sets of batch experiments described in Figure 7 were conducted to verify the effect of NO_3^- and acetate on rhizodegradation of perchlorate in the willow bioreactor. A total of 400 mL of growth solution was withdrawn from the rhizosphere of the willow reactor after perchlorate was completely degraded to the IC method detection limit of 2 μg/L. A total of 50 mL of the media was placed in eight serum bottles. Sodium nitrate was added to a pair of the vials to obtain a total NO_3^- concentration of 200 mg/L. Acetate was added to a second pair of vials to obtain an initial concentration of 400 mg/L. To the third pair of vials, both NO_3^- and acetate were added to obtain initial concentrations of 200 and 400 mg/L, respectively. One pair of vials contained the unamended media. Corresponding controls consisted of deionized water only or deionized water with the same concentrations of nitrate, acetate, or both compounds. The samples and controls were each dosed with perchlorate to obtain an initial solution concentration of 100 mg/L, sealed with serum wrap, and incubated at ambient temperature. One milliliter of rhizosphere media was withdrawn from sample and control bioreactors once every 24 h, diluted, and analyzed for perchlorate, nitrate, acetate, and chloride. The experiments were run in duplicate and replicated.
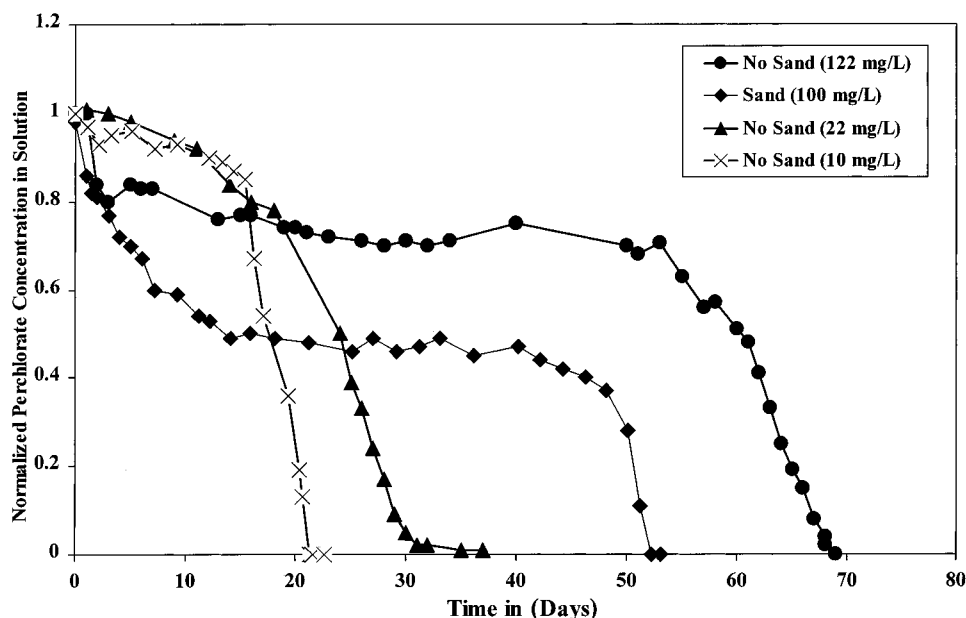
FIGURE 3. Representative plots of normalized concentrations of perchlorate remaining in solution as a function of time, pH 5.6, nitrate concentration <200 mg/L (<3.2 mM). Data are for first dose of perchlorate added to willow-planted bioreactors.

TABLE 1. Results of Short-Term Experiments Showing the Distribution of Perchlorate in Roots, Stem, and Leaves of Willow Trees Grown on Diluted Hoagland's Solution in Sand Bioreactors for 26 Days[a]

portion	plant fraction (%)	perchlorate concn mean $\pm$ SD (mg/kg)	perchlorate mass mean $\pm$ SD (mg)	fraction of initial mass (%)
root	1.6	94.7 $\pm$ 1.1	0.04 $\pm$ 0.001	0.1
lower stem	22.6	28.1 $\pm$ 0.4	0.18 $\pm$ 0.03	0.2
upper stem	73.8	16.1 $\pm$ 0.5	0.34 $\pm$ 0.10	0.4
leaf	2.0	813.1 $\pm$ 11.2	0.48 $\pm$ 0.01	0.6
sand layer[b]		17.9 $\pm$ 0.7	25.82 $\pm$ 0.99	31.8
solution[c]		56.0 $\pm$ 0.7	50.36 $\pm$ 0.67	56.0

Perchlorate Mass Balance

initial	86.8 $\pm$ 1.0 (mg)
recovered	77.2 $\pm$ 1.7 (mg)
phytodegraded	11%

[a] The volume of aqueous phase was 900 mL, and the average initial perchlorate concentration was 96.41 $\pm$ 1.10 mg/L for two replicates. [b] Refers to perchlorate in the pore water in the sand layer since perchlorate did not sorb to the sand. [c] Refers to perchlorate remaining in diluted Hoagland's (nutrient) solution above the sand layer in which the tree roots were submerged.

Microbial contribution to perchlorate degradation in the rhizosphere was determined in two ways. Subsamples of the previously described rhizosphere solution were boiled for 2 h or filtered with a 0.45-μm membrane filter before being dosed with 10 mg/L perchlorate. Equal volumes (50 mL) of the autoclaved, filtered, and untreated media were each placed in three sterilized serum bottles dosed immediately with 10 mg/L perchlorate and sealed. The vials were continuously mixed on a shaker until sacrificed for analysis.

Ion Chromatography. A Dionex DX-100 ion chromatograph (IC) with SRS control was used for all analyses. The IC was equipped with a Dionex AI-450 chromatography automation system and the advanced computer interface module (ACI). An autosampler with a holding capacity of 60 5-mL vials was used. Sample injection volume was 25 μL for chloride, chlorite, chlorate, and nitrate analysis and was 500 μL for perchlorate analysis. Both an IONPAC AG11 guard column (2 $\times$ 50 mm) and an IONPAC AS 11 analytical column (4 $\times$ 250 mm) were used. The analytical conditions developed by Dionex Corporation for analysis of low concentrations of perchlorate in drinking water and groundwater by IC were followed. Flow rate of eluent was 1 mL/min. A 10 mM NaOH solution was used as the eluent for analysis of chloride,

chlorite, chlorate, nitrate, and acetate ions while a 100 mM NaOH solution was used for the perchlorate ion measurement. The working perchlorate concentration range for most analyses was 0–1000 μg/L, and the conductivity was less than 10 μS. The detection limit of perchlorate for the above method was 2 μg/L. The run time was 15 min. Deionized water (resistance of 18 MΩ-cm) was used as a system blank sample to establish the baseline and to confirm the presence or lack of contamination in the system. Low and/or high concentration calibration curves were determined each day of sample analysis to ensure accurate quantification of perchlorate (Figure 2).

Results and Discussion

Three woody plants [eastern cottonwoods (*Populus deltoides* and hybrid *populus*), *Eucalyptus cineria,* and willow (*Salix nigra*)] were screened to determine the plant(s) most favorable for degrading perchlorate. The fastest kinetics was obtained with *E. cineria,* which had the largest fraction root and leaf of 6.6% and 46%, respectively. However, this species of *Eucalyptus* had low survivability in the hydroponic system. Cottonwoods were the least effective plants for removing perchlorate from solution and did not thrive well in the
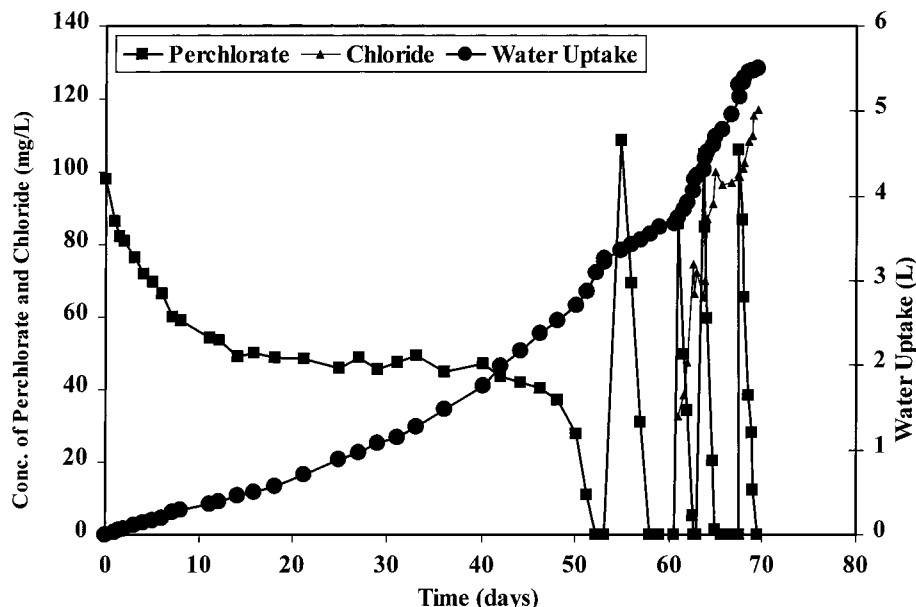
FIGURE 4. Perchlorate loss and cumulative water uptake as a function of time in sand bioreactors dosed five times with approximately 100 mg/L of perchlorate. Also shown is the increasing chloride concentration in the root zone from rhizodegradation of multiple doses of perchlorate.
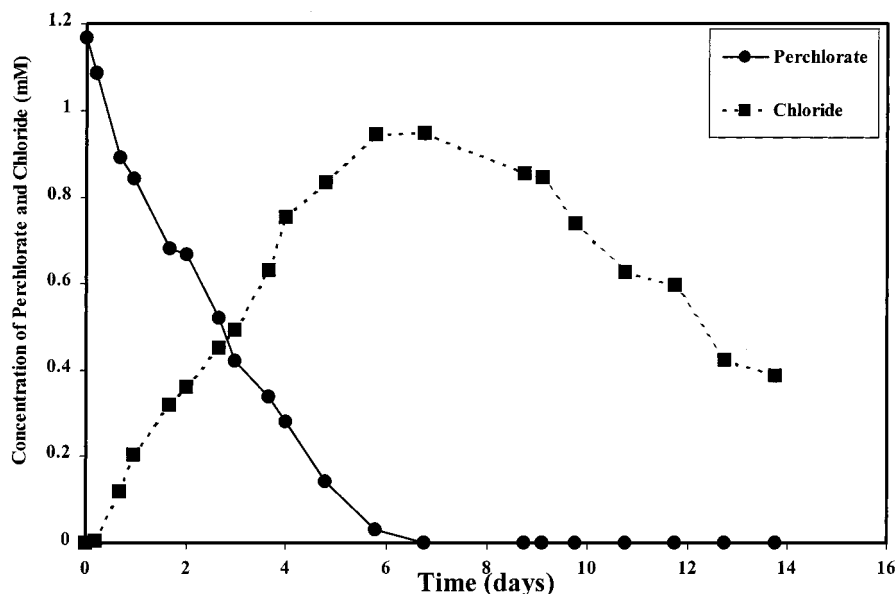

FIGURE 5. Representative plot of chloride released from the degradation of perchlorate in the rhizosphere of a willow tree. Data were corrected for background chloride, and a chloride mass balance of 86% was estimated for this experiment. An average chloride mass balance of 92.1 ± 6.7 was estimated for four experiments (Table 2).

hydroponic systems. Willow trees were the best overall candidates for the detailed studies because they thrived well and progressively performed better than the other trees under the conditions of our experiments. Conger and Portier (11) recently reported similar observations.

The rooted cuttings of willow grown in bioreactors and dosed with 10, 20, and 100 mg/L of perchlorate ions decontaminated the aqueous phase to below the IC method detection limit of 2 μg/L (Figure 3). A slow initial rate of perchlorate removal was followed by faster kinetics in experiments performed with an initial perchlorate concentration of 10 and 20 mg/L. The disappearance rate at higher concentrations of perchlorate (100 mg/L) was initially described by pseudo-first-order kinetics, which changed to zero order toward the end of the experiment. Three distinct reaction phases were observed when the sand and hydro-

ponic bioreactors were first dosed with the high concentrations of perchlorate. Phase 1 was described by a rapid decrease in perchlorate concentration, which increased linearly with the volume of water evapotranspired, by each tree. This phase was attributed to perchlorate uptake into the plant with the transpiration stream. In phase 2, the progressive increase in water uptake was not accompanied by any significant loss of perchlorate from solution. We speculate that as the perchlorate fraction taken up by the tree and transported to the leaves approached toxic levels, the willow developed defense mechanisms to resist further perchlorate uptake. Phase 2 persisted for twice as long in the bioreactors containing no sand than in the sand bioreactors. The limited data collected in this phase of the study do not suffice to explain the plateau in the kinetic plots in Figure 3. However, Doddema and co-workers (12) have suggested

TABLE 2. Kinetics and Chloride Mass Balance Results for One of Three Willow Trees Dosed Multiple Times with 100 mg/L of Perchlorate

spike	water uptake rate (mL/day)	initial perchlorate concn (mg/L)	zero-order rate constant (mg L^{-1} h^{-1})	Cl$^-$ mass balance[a] (%)
1	64.5	98	NA[b]	ND[c]
2	129	109	1.52 ± 0.42	84
3	93	86	2.04 ± 0.07	110.8
4	95	105	3.24 ± 0.22	92.2
5	80	106	2.58 ± 0.18	81.3
av	92.3 ± 10.7	100.8 ± 4.1	2.35 ± 0.37	92.1 ± 6.7

[a] Background chloride concentrations were subtracted before calculating individual and average percentages. [b] NA, not applicable to the kinetic data. [c] ND, not determined due to significant uptake into plant.

that the uptake and assimilation of nitrate could result in a shift in the metabolic state of some plants and inhibit the uptake of chlorate ions.

The fastest rate of perchlorate removal was observed in phase 3. The very fast rate of decrease in perchlorate concentration observed in this phase was attributed to rhizodegradation since the removal of perchlorate from solution greatly surpassed the rate of water uptake by the tree. For rhizodegradation to predominate in phase 3, we believe that the biostimulation of perchlorate-reducing microorganisms occurred during phase 2. In contrast to experiments conducted at higher perchlorate concentrations, this very rapid kinetics phase was initiated in a shorter time (16−20 days) in experiments conducted with a lower perchlorate concentration (Figure 3). The perchlorate concentration in the unplanted reactors did not change over the course of this study. The pH in the rhizosphere of dosed bioreactors was 5.5 ± 0.1 throughout the duration of each experiment. Because the control and sample plants continued to grow at the same rate under similar experimental conditions, it was not clear if the perchlorate concentration used in this study was toxic to the willow trees. Early agronomists found that the phytotoxicity of perchlorate varied with the concentration, type of plant, soil conditions, and lighting in which the plants were grown. Cook (13) found that the lethal dose of perchlorate in solution to four different plants was approximately 2500 mg/L, which is about 2 orders of magnitude higher than the concentrations used in this study. Lauffs in 1902 found that, in small concentrations, perchlorate had a stimulating influence on plant growth and that the deleterious effects of perchlorate at higher concentrations were lowered by nitrate (14).

The distribution of perchlorate in the pore water in the sand layer, in the diluted Hoagland's solution above the sand, and in various organs of the willow (sacrificed for analysis at the termination of the short-term kinetic studies) is presented in Table 1. The larger perchlorate concentration measured in the bioreactor was due to the higher concentration recovered from the sand layer, which was not directly accessible to the tree roots. Sorption studies confirmed that perchlorate was not sorbed by the sand, indicating that the higher perchlorate concentration measured in the sand layer was due to dissolved perchlorate trapped in the pore water. The diffusion of perchlorate ions from the porous sand media to the root zone should be rate limiting since uptake and phytodegradation was the predominant perchlorate removal mechanism within the first month of the experiment.

Comparing the perchlorate concentration (mass/mass) extracted from the different plant organs (Table 1), it is evident that the phytoextracted fraction of perchlorate was mostly accumulated in leaves and branches rather than in the roots and lower stem. The 11% of unaccounted perchlorate was
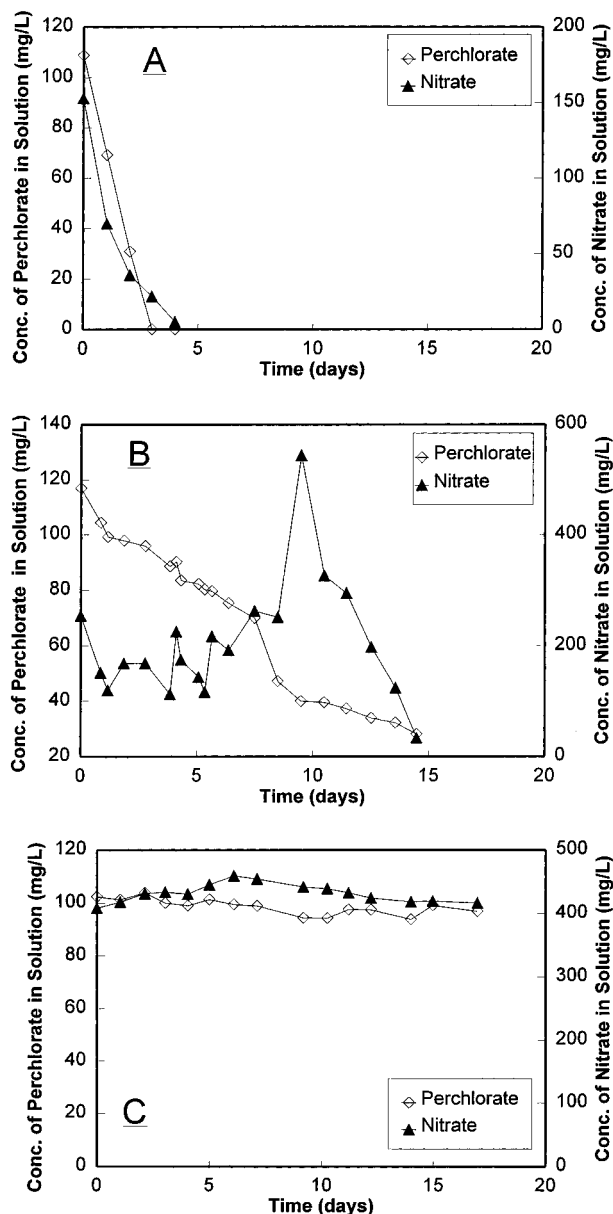

FIGURE 6. Effect of nitrate concentration on the rate of removal of perchlorate from dosed nutrient (diluted Hoagland's) solution. The experiments were conducted with willows growing on three ranges of nitrate concentrations: (A) <100 mg/L, (B) 100−300 mg/L, and (C) >400 mg/L.

assumed to be phytodegraded to chloride. Evidence of phytodegradation of the phytoextracted perchlorate in the tree leaves was obtained by comparing the perchlorate concentration extracted from the leaves of willow trees used in short-term (<30 days) and long-term experiments (>60 days). The initial perchlorate concentration used in the compared experiments was 100 mg/L, and the nutrient solution was 25% of full-strength Hoagland's solution. For short-term experiments, the concentration of perchlorate extracted from leaves of willow trees after 26 days (Table 1) was 813 mg/kg. For the long-term experiments, the perchlorate concentration in extracted fresh leaves of willow trees dosed five times (Figure 4) was 261 and 755 mg/kg in leaves showing senile properties, whereas the chloride concentrations in the same leaves were 226 and 803 mg/kg, respectively. The relatively lower concentration of perchlorate extracted from willow leaves of trees used in long-term experiments suggests that perchlorate ions did not just

TABLE 3. Summary Results of Perchlorate-Treated and Control Willow Trees under Different Experimental Conditions[a]

bioreactor	type of nutrient medium[b]	ammonium or nitrate concn in solution (mg/L)	initial perchlorate concn (mg/L)	max perchlorate detected in leaf[d] (mg/kg)	estd zero-order rate constant (mg L^{-1} h^{-1})
hydroponic	Miracle-Gro (2 g/L)	≤136[c]	22–23	15.0 ± 0.4	0.389 ± 0.005
hydroponic	Miracle-Gro (0.5 g/L)	≤34[c]	22–23	8.5 ± 0.9	0.284 ± 0.014
control	Miracle-Gro (2 g/L)	≤136[c]	0	0	NA
hydroponic	water only	<10	13	144.5 ± 5.4	0.007 ± 0.001
hydroponic	Hoagland's	<200	10	100.0 ± 3.6	0.003 ± 0.000[e]
					0.053 ± 0.004[e]
hydroponic	Hoagland's	<200	23	132.0 ± 2.9	0.025 ± 0.001
hydroponic	Hoagland's	<100	41	110.5 ± 2.8	0.311 ± 0.011
hydoponic	Hoagland's	≥400	102	2219.5 ± 7.0	0.019 ± 0.001
sand	Hoagland's	≤100	101	626.7 ± 7.4	2.35 ± 0.37[f]
sand	Hoagland's	≤200	116	ND	0.802 ± 0.039[f]
sand	Hoagland's	100–300	117	ND	0.232 ± 0.015[f]
control	Hoagland's	≤200	0	0	NA
control	Hoagland's	≥400	0	0	NA

[a] These results are averages for multiple doses of perchlorate applied to each bioreactor when rhizodegradation was the dominant phytoprocess. ND, not determined. NA, analytical method does not apply to the data. [b] Stern's Miracle-Gro consists of 15% total nitrogen with 6.8% as ammonical nitrogen and 8.2% urea nitrogen. Hoagland's solution is a medium with nitrate as the nitrogen source. [c] Refers to NH_4^+ concentration. [d] Perchlorate concentrations in the leaves increased to a maximum during the experiment before decreasing to undetectable levels after perchlorate was completely removed from solution. [e] Removal of perchlorate from solution was described by two zero-order equations (slow followed by fast kinetics). [f] Applies only to the diluted Hoagland's (nutrient) solution phase above the sand layer in which the tree roots were submerged because perchlorate remained in the pore water of the sand layer after perchlorate was completely removed from the solution phase above.

accumulate in the leaves but were very slowly transformed by the plant deoxygenase or reducing enzymes. Generally, the perchlorate concentration taken up into the leaves increased to a maximum before decreasing to undetectable levels after perchlorate was completely removed from solution.

Figure 4 shows that initial exposure of willows to perchlorate-dosed nutrient (Hoagland's) solution caused the trees to become more effective in removing perchlorate from the solution. Similar observations were made in experiments conducted at lower perchlorate concentrations of 10 and 20 mg/L. The NO_3^- levels in the latter experiments were <200 mg/L. The very fast kinetics observed after the initial spike shown in Figure 4 suggested that the predominant reaction mechanism was degradation in the root zone (i.e., rhizodegradation). Each dose of perchlorate was degraded to a point below the method detection limit of 2 μg/L before the reactors were dosed again. The rate of reduction of subsequent doses of perchlorate added to the media increased by several orders of magnitude and was described by zero-order kinetics. For example, the rate of degradation of the second dose of perchlorate added to the willow bioreactor was much faster than that of the initial spike. Specifically, 109 mg/L of perchlorate was degraded to below the method detection limit of 2 μg/L in fewer than 72 h. When this reactor was dosed for a third time with an initial concentration of 86 mg/L, the perchlorate was degraded to a point below the method detection limit in 43 h. Figure 4 shows that after the dosing of each bioreactor for a total of five times, the reaction rate did not decrease. The estimated zero-order rate constants, average water uptake rate, and chloride mass balances for the experiments in Figure 4 are presented in Table 2.

Analyses for possible degradation products of perchlorate such as chlorate, chlorite, hypochlorite, and dichlorooxides were performed, but these compounds were not detected in the media or plant extract. Evidence for complete degradation of perchlorate to chloride was obtained by monitoring the change in chloride concentration in the rhizosphere of dosed trees (Figures 4 and 5 and Table 2). The chloride concentration in the full-strength Hoagland's solution was below the background level (0.5 mg/L) in the diluted nutrient solution. The chloride concentration in the media increased as each dose of perchlorate was completely degraded to below the IC detection limit. A chloride mass balance of 86% was calculated for the representative study in Figure 5 and 92.1

± 6.7% was calculated for four replicate experiments (Table 2). Because willows assimilate chloride, the 14% chloride fraction not accounted for in this experiment (Figure 5) was assumed taken up into the tree as chloride and/or perchlorate. The nearly stoichiometric degradation of perchlorate to chloride in the rhizosphere suggests that there was no accumulation of potential intermediates.

The TIC and TOC measurements taken at the beginning and end of experiments showed an increase in TIC and a small decrease in TOC. For example, in one experiment, the TIC increased from 2.3 mg/L at the beginning of the experiment to 79.5 mg/L while TOC decreased from 87.3 to 61.8 mg/L. This suggests that organic carbon was utilized to produce inorganic carbon as perchlorate was degraded to chloride in the rhizosphere. If acetate is the source of reducing equivalent for the reduction of perchlorate to chloride, the following reaction can be written:

$$CH_3COO^- + ClO_4^- \rightarrow 2HCO_3^- + H^+ + Cl^-$$
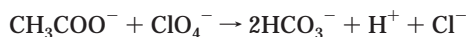
Rikken et al. (7) proposed a three-step mechanism of perchlorate reduction by anaerobic bacteria in which chlorate and chlorite are intermediate products and bicarbonate and chloride are end products.

Effect of Nitrate Concentration and Nitrogen Source. The results of perchlorate degradation at three concentration ranges of NO_3^- (<100, 100–300, and >400 mg/L) are presented in Figure 6a–c. The fastest perchlorate degradation kinetics were observed at NO_3^- concentrations of <100 mg/L (Figure 6a) and decreased with increasing NO_3^- concentration (Figure 6b,c). The utilization rates of NO_3^- and perchlorate were described by first- and zero-order kinetics, respectively, at the lowest NO_3^- concentration range. Average perchlorate utilization rates of 23.6 ± 3.7 and 2.3 ± 0.2 μM/h (2.35 ± 0.37 and 0.23 ± 0.02 mg L^{-1} h^{-1}) were obtained for willows grown in sand bioreactors with the NO_3^- concentration maintained at <100 and 100–300 mg/L, respectively. The slow reduction rates of perchlorate at high NO_3^- concentrations were attributed to competing reactions in which both anions were utilized as electron acceptors. This was independently confirmed in similar hydroponic studies in which willows were grown on a nutrient solution with ammonium and urea as the nitrogen sources. The results in Table 3 show that under similar experimental conditions willows grown on
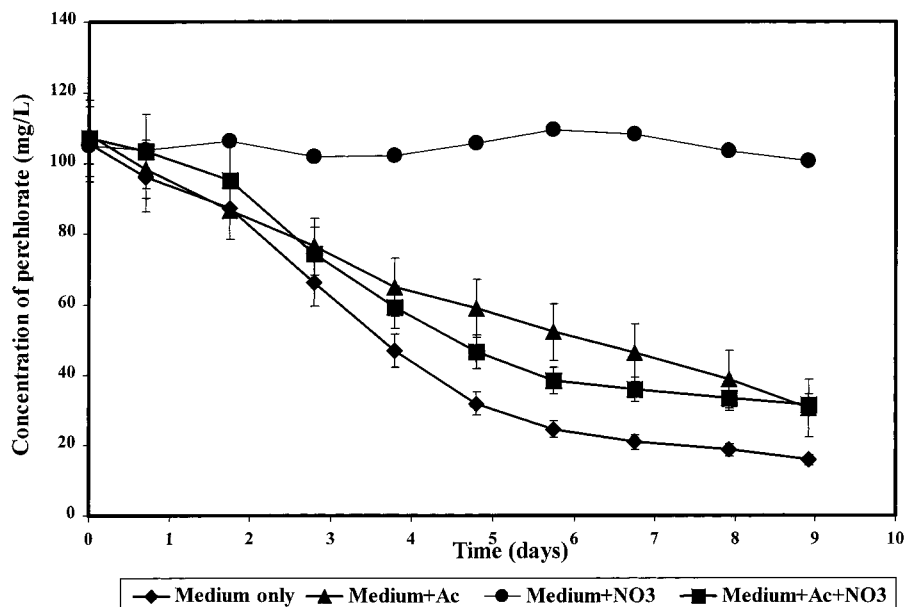
FIGURE 7. Homogeneous degradation of perchlorate using growth medium withdrawn from the root zone of willow trees. Equal volumes (50 mL) of the growth medium were each amended with either 400 mg/L acetate (▲) or 200 mg/L nitrate (●) or a mixture 400 and 200 mg/L acetate and nitrate (■). The nitrate concentration in the raw solution (◆) was undetectable, and acetate was 83 mg/L.

latter removed perchlorate from solution at a faster rate and the uptake of perchlorate into the leaves was less as compared to trees grown on nitrate. These results provide direct evidence that the type of nitrogen source may have an important influence on phytoremediation of perchlorate.

Figure 7 shows that the growth media withdrawn from the rhizosphere of willow trees used in previous experiments also degraded perchlorate to chloride. However, no perchlorate loss or chloride formation was observed in portions of the same growth media supplemented with NaNO₃ to achieve 200 mg/L NO₃⁻ in solution. These results offer further confirmation that high NO₃⁻ activity in the rhizosphere may hinder the reduction of perchlorate as a result of competition for electrons between NO₃⁻ and perchlorate and/or the inhibition of bacterial growth at high NO₃⁻ concentration.

It was also observed that the same growth media amended with acetate or both acetate and NO₃⁻ did not inhibit the reduction of perchlorate to chloride. This suggests that a high concentration of the carbon (electron) source is not inhibitory and may reverse the inhibitory effects observed at high NO₃⁻ activity in the root zone. The concentration of acetate and NO₃⁻ in the serum bottles was 400 and 200 mg/L, respectively. Acetate is one of many plant exudates (*15*) that could have been used by the rhizosphere bacteria as a reducing agent for perchlorate reduction. The acetate concentration measured in the willow rhizosphere ranged between 124 and 254 mg/L. We believe that acetate and other organic compounds exuded into the reactor by the willow trees apparently stimulated the growth of bacteria consortia that utilized NO₃⁻ and perchlorate as terminal electron acceptors. Because perchlorate was degraded in the un-amended media in the absence of willow roots and not in the filtered, boiled, and autoclaved samples, we concluded that microbes associated with the root zone mediated the rhizodegradation reactions.

Overall, the results of this study show that selected plants can be used to detoxify environments contaminated with perchlorate. Our data suggest that the effectiveness of phytoremediation of perchlorate-contaminated environments may depend on the concentration of competing TEAs, such as NO₃⁻. We have shown that the decontamination of perchlorate-contaminated water by woody plants depends

on the N-source of the nutrient solution. Woody plants grown on ammonium and urea removed perchlorate from solution at a higher rate and had lower perchlorate concentrations in the leaves than plants grown on a nutrient solution with nitrate as the nitrogen source (Table 3). The initial or prolonged exposure of the rooted willow trees to perchlorate-dosed media stimulated the growth of perchlorate-degrading microorganisms in the rhizosphere. Two phytoprocesses were identified as important in the remediation of perchlorate-contaminated water: (a) uptake and phytodegradation of perchlorate in the tree branches and leafs and (b) rhizo-degradation. Pilot testing of the approach should provide data needed to validate the feasibility of phytoremediation of perchlorate-contaminated sites. Since perchlorate does not volatilize from water readily, a perchlorate remediation scheme may involve an intensively cultivated plantation of trees with phraetophytic characteristics and irrigation with the contaminated water.

Acknowledgments

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