Phytofiltration of Arsenic from Drinking Water Using Arsenic-Hyperaccumulating Ferns

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Arsenic contamination of drinking water poses serious health risks to millions of people worldwide. Current technologies used to clean arsenic-contaminated water have significant drawbacks, such as high cost and generation of large volumes of toxic waste. In this study, we investigated the potential of using recently identified arsenic-hyperaccumulating ferns to remove arsenic from drinking water. Hydroponically cultivated, two arsenic-hyperaccumulating fern species (Pteris vittata and Pteris cretica cv. Mayii) and a nonaccumulating fern species (Nephrolepis exaltata) were suspended in water containing $^{73}$As-labeled arsenic with initial arsenic concentrations ranging from 20 to 500 $\mu$g L$^{-1}$. The efficiency of arsenic phytofiltration by these fern species was determined by continuously monitoring the depletion of $^{73}$As-labeled arsenic concentration in the water. With an initial water arsenic concentration of 200 $\mu$g L$^{-1}$, P. vittata reduced the arsenic concentration by 98.6% to 2.8 $\mu$g L$^{-1}$ in 24 h. When the initial water arsenic was 20 $\mu$g L$^{-1}$, P. vittata reduced the arsenic concentration to 7.2 $\mu$g L$^{-1}$ in 6 h and to 0.4 $\mu$g L$^{-1}$ in 24 h. At similar plant ages, both P. vittata and P. cretica had similar arsenic phytofiltration efficiency and were able to rapidly remove arsenic from water to achieve arsenic levels below the new drinking water limit of 10 $\mu$g L$^{-1}$. However, N. exaltata failed to reduce water arsenic to achieve the limit under the same experimental conditions. The significantly higher efficiency of arsenic phytofiltration by arsenic-hyperaccumulating fern species is associated with their ability to rapidly translocate absorbed arsenic from roots to shoots. The nonaccumulating fern N. exaltata was unable to translocate the absorbed arsenic to the shoots. Our results demonstrate that the arsenic-phytofiltration technique may provide the basis for a solar-powered hydroponic technique that enables small-scale cleanup of arsenic-contaminated drinking water.

Introduction

Arsenic contamination of drinking water poses significant health risks to millions of people worldwide. Arsenic is a known carcinogen and mutagen and is detrimental to the immune system (1–3). Ingestion of inorganic arsenic, the primary arsenic forms in drinking water, can increase the risk of cancers, such as skin, bladder, and prostate cancers. Recent research indicates that chronic exposure to 50 $\mu$g L$^{-1}$ arsenic in drinking water may result in human cancer risks as high as 13 in 1000 (4). Arsenic may also cause a number of other health disorders such as thickening of the skin, disturbances of the nervous system, and impairment of the hearing system (1, 2). Reducing arsenic exposure is the primary option for reducing the incidence of these severe health effects. Therefore, the United States Environmental Protection Agency (USEPA) has recently lowered the drinking water limit for arsenic to 10 $\mu$g L$^{-1}$, with a compliance deadline of January 2006.

The most common water treatment technologies for arsenic-contaminated water include the following: (1) Coagulation/Filtration, effective for removal of As(V) according to laboratory and pilot-plant tests. The main disadvantage of this method is the generation of large volumes of arsenic-contaminated coagulation sludge. The disposal of such contaminant wastes may be a concern, especially if nearby landfills are unwilling to accept such sludge. (2) Lime Softening, which requires an optimum pH range of greater than 10.5. (3) Activated Alumina, which is highly selective in removing As(V); however, the strong attraction to As(V) results in regeneration problems, possibly resulting in 5–10% loss of adsorptive capacity for each run (5–7).

Phytofiltration, the use of plants to remove contaminants from water, is an emerging technology (8). Dierberg et al. (9) introduced floating plant systems in which the contaminants could be removed in the harvested biomass of floating plants. However, these systems are not particularly efficient, especially in temperate zones (10). The initial research on phytofiltration of contaminants from water began with the use of wetlands for water purification (11). However, in these systems, the contaminants are often precipitated from water into the sediments, making the recovery of the contaminants difficult. Chandra et al. (12) investigated the ability of vascular aquatic plants to remove metals from contaminated water. These authors demonstrated promising results of Cr removal from water by Scirpus lacustris and Phragmites karka. In 1995, Dushenkov et al. (13) designed a hydroponic system using sunflower plants and Brassica juncea seedlings that were later used to remove uranium, lead, and cesium from contaminated waters (8, 14). However, sunflower and Brassica plants do not efficiently remove arsenic from water.

Recently, an arsenic-hyperaccumulating fern species (Pteris vittata) has been identified by scientists from the University of Florida and shown to accumulate arsenic in the shoots to a concentration as high as 22000 mg kg$^{-1}$ (15, 16). Research has demonstrated that other species in the Pteris genus also hyperaccumulate arsenic in their shoots (17, 18). Greenhouse studies indicated that P. vittata accumulated an arsenic concentration in the above ground plant tissue more than 200-fold higher than most other plant species tested using arsenic-contaminated soil (19). This perennial fern species grows rapidly and generates substantial biomass (15); such properties make P. vittata a potential candidate to rapidly remove arsenic from drinking water. The development of a cost-effective and environmentally friendly method for removal of arsenic from water would facilitate the cleanup processes for arsenic-contaminated water, thus reducing the arsenic risk to human health. The objectives of this research were (i) to test whether arsenic hyperaccumulating ferns can rapidly extract arsenic from...
water with arsenic concentrations ranging from 20 to 500 μg L⁻¹ to less than 10 μg L⁻¹, the new U.S. drinking water limit for arsenic, (ii) to test whether the ferns can be used continuously to remove arsenic from drinking water, and how the phytotranslocation efficiency changes for each subsequent phytotranslocation, and (iii) to investigate the effects of competing ions (PO₄³⁻, SO₄²⁻, and NO₃⁻) on arsenic phytofiltration by arsenic-hyperaccumulating and nonaccumulating fern species.

Material and Methods

Fern Cultivation. Fern sporelings were initially grown in potting mixture (Metro mix 360, Scotts Sierra Horticultural Products Company, Marysville, OH). The sporelings were sprayed with water daily and weekly with a dilute nutrient solution (10 mL) were collected from each container over a 5 min. Following desorption, excess water in the seedlings was removed using paper towels, and then the roots and shoots were separated. After recording the fresh weight, the ⁷³As activity in the roots and shoots was determined by gamma counting.

Effect of Competing Anions on Arsenic Influx. To determine the initial effect of competing anions (PO₄³⁻, SO₄²⁻, and NO₃⁻) on arsenate (AsO₄³⁻) uptake by fern roots, short-term (60 min) arsenic influx into the roots of P. gregaria in the presence or absence of these anions was determined using ⁷³As-labeled arsenic. Hydroponically grown P. gregaria seedlings (root volume of 5 ± 2 mL per plant) were set in 0.1 mM CaCl₂ solution overnight. A few hours before the influx experiment, the seedlings were transferred to the ion uptake system with one plant per uptake well filled with 80 mL of 0.1 mM CaCl₂ solution. After the seedlings were set in the uptake wells for approximately 1 h, the arsenic influx was initiated by replacing the CaCl₂ solution with 75 mL of the experimental solution and with or without one of the following anions: PO₄³⁻, SO₄²⁻, NO₃⁻, 50 or 100 μM; and NO₃⁻, 100 μM. The volume ratio of plant roots to water for this experiment was 1:15. All anions used were prepared from potassium salt. Each treatment had three replicates.

The influx was terminated by vacuum withdrawal of the uptake solution. The roots were washed with ice-cold DI water for 30 s with the ice-cold desorption solution for 5 min. Following the desorption, excess water in the fern roots was removed using paper towels, and then the roots and shoots were separated. After recording the fresh weight, the ⁷³As radioactivity in the roots and shoots were determined by gamma counting. Data reported in this paper were analyzed using the Statistical Analysis System software (20). A probability of 0.05 or less was considered to be statistically significant.

Results

Time-Dependent Arsenic Removal. The arsenic-hyperaccumulating fern, P. vittata, rapidly removed ⁷³As-labeled arsenic from water (Figure 1). With an initial water arsenic concentration of 200 μg L⁻¹, a rapid reduction of the arsenic concentration was observed within 3 h after the arsenic was transferred into the water. The arsenic concentrations were determined through gamma counting (Wallac 1480 Wizard Automatic Gamma Counter, Perkin-Elmer, U.S.A.), which had a detection limit of 0.05 μg L⁻¹.

To test the efficiency of arsenic removal in successive phytofiltration experiments, the P. vittata plants used in the first phytofiltration experiment were given a recovery period of 12 h in 0.1 mM CaCl₂ solution, and then the second phytofiltration experiment was initiated. At the end of second experiment that lasted 35 h, the fern plants were given a second recovery period in nutrient solution for 24 h, followed by 12 h in 0.1 mM CaCl₂ solution before the third phytofiltration experiment started. The experimental procedure, initial water composition, and phytofiltration time (35 h) were the same in all the experiments.

Arsenic Translocation. To examine the efficiency of arsenic translocation in fern plants, uniformly grown seedlings of P. vittata and N. exaltata with average (±SD) root volumes of 5 ± 2 mL per plant were used. The selected seedlings were set in aerated 0.1 mM CaCl₂ solution for 12 h. Then the plants were transferred to an ion transport system having 20 uptake wells made from Plexiglas pipe with one plant per well. After the seedlings were set in the uptake wells for approximately 1 h, the experiment was initiated by replacing the CaCl₂ solution in each well with 75 mL of the experimental solution. The volume ratio of plant roots to water for this experiment was 1:15. Plant samples were collected at 0, 1, 6, 12, and 24 h following the initiation of the arsenic translocation experiment. For each sampling period, the uptake was terminated by replacing the uptake solution with ice-cold DI water for 30 s and ice-cold desorption solution (0.1 mM CaCl₂ and 1.0 mM KH₂PO₄, pH 6.5) for 5 min. Following desorption, excess water in the seedlings was removed using paper towels, and then the roots and shoots were separated.

The efficiency of arsenic removal by two arsenic hyperaccumulating ferns (P. vittata and P. gregaria cv. Mayii) and a nonaccumulating fern (Nephrolepis exaltata) was examined by monitoring the depletion of ⁷³As-labeled arsenic from the water after transferring the fern plants into the arsenic-containing solution. For the majority of experiments, P. vittata plants with root volumes of 40 ± 10 mL per plant were used. The selected fern plants were set in 8 L plastic containers filled with aerated 0.1 mM CaCl₂ solution for 12 h and then transferred into 1 L plastic containers (one plant per container) filled with 800 mL of aerated 0.1 mM CaCl₂ solution. Plants were suspended over the 1 L container, such that their roots were immersed in the solution, by placing the polyethylene cups described earlier through a hole bored into a plastic sheet (15 × 25 cm) that covered the plastic container. After the plants had been in the 1 L container for approximately 1 h, the CaCl₂ solution was replaced with 800 mL of the experimental solution containing 0.1 mM CaCl₂ and 200 μg L⁻¹ ⁷³As-labeled arsenic as sodium arsenite with ⁷³As radioactivity of 0.74 MBq L⁻¹. The volume ratio of plant roots to water was 1:2.0. For the experiment to test concentration-dependent arsenic removal, the experimental procedures were the same as described above except that there were four arsenic levels (20, 50, 200, and 500 μg L⁻¹) in the experimental solutions. Samples of solution (10 mL) were collected from each container over a 35-h period. The ⁷³As radioactivity in the water samples was determined through gamma counting (Wallac 1480 Wizard Automatic Gamma Counter, Perkin-Elmer, U.S.A.), which had a detection limit of 0.05 μg L⁻¹.
centrations ranging from 20 ìg L⁻¹ to 500 ìg L⁻¹. For initial arsenic concentrations of 20 and 50 ìg L⁻¹, the fern reduced water arsenic concentration to below 10 ìg L⁻¹ within 6 h and to 0.5 ìg L⁻¹ in 24 h (Figure 2). When the initial water arsenic concentration was 200 ìg L⁻¹, a rapid phase of arsenic reduction was observed in the first 18 h, with water arsenic concentration reduced from 200 ìg L⁻¹ to less than 20 ìg L⁻¹, and the arsenic was completely removed in 46 h (Figure 2). The results demonstrate that P. vittata was able to remove arsenic from contaminated water over a wide range of arsenic concentrations.

Repeated Arsenic Phytofiltration. To determine the sustainability of the arsenic hyperaccumulating fern to remove arsenic from drinking water, three experiments were conducted successively using the same P. vittata plants. The arsenic concentration in water was reduced from 50 ìg L⁻¹ to less than 10 ìg L⁻¹ within 24 h in the first and second experiment and in 30 h in the third experiment (Figure 3). In all three repeated experiments with the same fern plants, the water arsenic was reduced from 200 ìg L⁻¹ to less than 3 ìg L⁻¹ within 35 h. These results suggest that the arsenic-hyperaccumulating fern plants can be used repeatedly for continuous removal of arsenic from water.

Fern Species Variation in Arsenic Phytofiltration Efficiency. The efficiency of arsenic phytofiltration was simultaneously monitored for two arsenic-hyperaccumulating fern species (P. vittata and P. cretica) and a nonaccumulating fern (N. exaltata). The arsenic hyperaccumulating fern species reduced water arsenic concentration significantly faster (P < 0.01) than N. exaltata (Figure 4). The water arsenic concentration was reduced slightly by N. exaltata in the first 12 h and then was not significantly changed over the next 35 h. However, when the arsenic hyperaccumulating fern species were used, water arsenic concentration decreased rapidly during the first 18 h, and then the arsenic concentration was more slowly reduced further over the next 20 h (Figure 4). The fern plants used in this experiment had approximately half the root volume of those used for the time and concentration dependent arsenic removal experiments (Figures 1 and 2), which accounted for the longer phytofiltration time required.
Arsenic Uptake and Translocation. To further examine the significant differences in the efficiency of arsenic phytofiltration between P. cretica and N. exaltata, $^{79}$As-labeled arsenic uptake and translocation by these fern species were studied. Arsenic concentration in the roots of P. cretica increased in the first 6 h and then slightly decreased from 6 to 24 h (Figure 5). For N. exaltata, root arsenic concentration increased consistently over the entire experimental period. Shoot arsenic concentrations for P. cretica was significantly higher ($P < 0.01$) than that of N. exaltata at 6, 12, and 24 h following the $^{79}$As loading. After a lag period of 1 h following the $^{79}$As loading, shoot arsenic concentrations in P. cretica increased from 0.2 $\mu$g g$^{-1}$ to 1.7 $\mu$g g$^{-1}$ in 6 h and then increased steadily over the next 18 h. More than 65% of the absorbed arsenic was translocated to the shoots of P. cretica during the 24 h phytofiltration period. However, for the same loading period, arsenic concentrations in shoots of N. exaltata were negligible (Figure 5). The data indicate that arsenic absorbed by P. cretica was rapidly translocated to the shoots, which did not occur in N. exaltata.

Anion Effects on Arsenic Influx. For the three anions tested, PO$_4^{3-}$ (at both 50 and 100 $\mu$M) significantly ($P < 0.01$) inhibited arsenic influx into the roots of P. cretica (Figure 6). With an arsenic concentration of 200 $\mu$g L$^{-1}$ (2.67 $\mu$M), 50 $\mu$M PO$_4^{3-}$ inhibited the short-term arsenic influx by 88%, while 50 $\mu$M SO$_4^{2-}$ inhibited arsenic influx by only 9%. When the concentration of competing anions increased to 100 $\mu$M, the inhibition of arsenic influx was 98% by PO$_4^{3-}$, 28% by NO$_3^{-}$, and 24% by SO$_4^{2-}$ (Figure 6). The data suggest that the efficiency of arsenic phytofiltration by the arsenic hyperaccumulating fern could be significantly reduced where PO$_4^{3-}$ concentrations approach 50 $\mu$M in water; however, P. cretica could maintain selective arsenic uptake in the presence of SO$_4^{2-}$ and NO$_3^{-}$ ions at concentrations 18–37-fold higher than that of arsenic.

Discussion

The drinking water arsenic limit for the United States water treatment systems is currently 50 $\mu$g L$^{-1}$, as set by the USEPA based on a Public Health Service Standard established in 1942. Recent research indicates that chronic exposure to arsenic at this level may result in high human cancer risks (4). To reduce such health risks, the USEPA has set a new drinking water limit for arsenic at 10 $\mu$g L$^{-1}$ with a compliance deadline of January 2006. An estimated 6600 community water systems must take corrective action to comply with this new limit (5–7), and the annual cost for compliance with the new arsenic standard is estimated to range between $379 million and $445 million (6). The development of a cost-effective and environmentally friendly method for removal of arsenic from water would facilitate the cleanup of arsenic-contaminated drinking water, thus reducing the arsenic risk to human health.

Results from this research demonstrate that arsenic-hyperaccumulating fern species from the Pteris genus can rapidly remove arsenic from water with a wide range of initial arsenic concentrations to achieve the new drinking water limit for arsenic. The arsenic removal is rapid; for initial arsenic concentrations ranging from 20 to 200 $\mu$g L$^{-1}$, the fern can reduce the arsenic concentration to less than 10 $\mu$g L$^{-1}$ in 6–24 h (Figures 1, 2, and 4). The Pteris ferns continued to reduce the arsenic concentration to less than 0.5 $\mu$g L$^{-1}$ over the next few hours with no substantial arsenic efflux detected from the fern roots (when measured for an additional 10 h or longer). Also, the removal of arsenic was found to be robust through the repeated use of the fern plants without a significant difference in phytofiltration efficiency (Figure 3) and shown to be highly selective under high sulfate and nitrate concentrations ($\pm 50$ $\mu$M, Figure 6). The high arsenic phytofiltration efficiency of Pteris ferns is associated with their ability to rapidly translocate the absorbed arsenic from roots to shoots; however, the nonaccumulating fern species tested does not have such a characteristic and is unable to reduce water arsenic concentrations to achieve the new drinking water standard (Figure 5). The rapid translocation of arsenic from roots to shoots allows roots of...
the arsenic hyperaccumulating ferns to continuously absorb arsenic from water.

These results demonstrate that this phytofiltration technology may provide the basis for a solar-powered hydroponic technique that enables cleanup of arsenic-contaminated drinking water and which has several potential advantages over existing water treatment technologies. First, other forms of drinking water arsenic treatment generally produce chemical sludges for which disposal is increasingly expensive (6). In particular, coagulation/filtration systems produce large amounts of sludge, as can activated alumina systems if disposable beds are used. By contrast, arsenic recovered through phytofiltration may easily and safely be disposed of, and possibly recycled for industrial uses, eliminating the costs and liability of landfill disposal. Our recent study demonstrated that using a specific-designed instrument to press arsenic-loaded fresh fern biomass, approximately 75% of the arsenic in the fern could be removed as plant sap (Edenspace Systems Corporation, unpublished data). The arsenic-rich sap may be further processed to recycle the arsenic or may be simply evaporated to significantly reduce the volume of arsenic waste. Second, phytofiltration does not require hazardous chemicals, such as strong acids or bases used to regenerate alumina beds or exchange resins. Third, phytofiltration is not affected by certain anions (e.g. sulfate) that reduce the efficiency of other systems (Figure 6). The arsenic phytofiltration efficiency was reduced by either 50 or 100 μM P. Such P levels are normal in solution culture but are much higher than that in drinking water systems. Based on an extensive survey of water quality in the United States from 1992 to 1995, Nolan and Stoner (21) reported that the median concentration of P was 0.32 ± μM in major aquifer and 0.64 ± μM in water from urban areas. Because the P concentration in drinking water is 0.6–1.2% of the P concentrations tested in this study, we expect the effect of such low P on arsenic phytofiltration to be limited although further study is needed to confirm this speculation. Furthermore, the ferns will deplete the P in the drinking water during phytofiltration, which will further reduce the P impact on arsenic phytofiltration. Finally, because plant cultivation and harvesting are relatively inexpensive processes, arsenic phytofiltration could have a significant cost saving advantage compared to current available technologies for treatment of arsenic-contaminated drinking water. Because of the ferns’ selective uptake of arsenic, and the high concentrations that they can store in their biomass (15, 17), it is expected that the mean residence time (time between recharge or replacement of the ferns) would be high, which would keep operating costs down. These fern species are perennial; therefore, the arsenic accumulated in the shoots can be removed by cutting the mature fronds, and the fern can generate new fronds within a few weeks. The fern plants can thereby be used repeatedly in phytofiltration of arsenic from water.

Technical details still need to be finalized prior to field application of the arsenic phytofiltration technology for drinking water treatment. However, this research has provided an initial guideline for arsenic phytofiltration that can be operationally divided into three stages: fern growth stage, water treatment stage, and fern recovery stage. For the growth stage, fern seedlings can be generated from spores in potting mix. After fern seedlings reach 10 cm high, the plants can be transferred to a nutrient solution. Once the ferns have approximately 30–50 mL of root volume per plant, the water treatment can be initiated. A possible scenario for water treatment could be a flow through system that has several connected treatment cells. The amount of water needed for a small community will determine the number of treatment cells needed. For water containing 20–50 μg L⁻¹ of arsenic, two to five times above the new drinking limit, one Pteris fern with 40 mL of roots can reduce the arsenic in 800 mL of water to less than 10 μg L⁻¹ within 6 h (Figure 2). For every 1000 fern plants of this size to be used for water treatment, a total of 3200 L of water can be treated in 24 h. The amount of water purified can serve approximately 400 people assuming each person consumes 8 L of drinking water daily.

Near-term targets for the technology would include small-scale applications for communities that rely on untreated water or point-of-use treatment methods. The majority of the 6600 water treatment systems who must take corrective action to comply with the new drinking water arsenic limit are small treatment systems, for whom the per capita cost of corrective action is predicted to be particularly high. There is also significant potential to use arsenic phytofiltration as a new remediation technology in developing countries where there is serious arsenic contamination of drinking water, such as Bangladesh and India (22). Long-term targets for this technology include removal of arsenic from dredge spoils, sludge, and liquid waste from various industry facilities.

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