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Activity of *Desulfitobacterium* sp. Strain Viet1 Demonstrates Bioavailability of 2,4-Dichlorophenol Previously Sequestered by the Aquatic Plant *Lemna minor*

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Aquatic plants take up and sequester organic contaminants such as chlorophenols through incorporation in cell wall materials and storage in vacuoles. The ultimate fate of plant-sequestered chlorophenols, however, remains unclear. This research investigated 2,4-dichlorophenol (2,4-DCP) sequestration by the aquatic plant *Lemna minor* and evaluated contaminant release and bioavailability after plant death and cellular disruption. ^{14}C-labeled 2,4-DCP was used to establish that contaminant removed from the aqueous phase was retained internal to *L. minor*. An assay with *Desulfitobacterium* sp. strain Viet1 was used to assess the readily bioavailable fraction of plant-sequestered 2,4-DCP and plant metabolites of 2,4-DCP. In plant-free systems, strain Viet1 dechlorinated 2,4-DCP to stoichiometric amounts of 4-chlorophenol (4-CP) as a stable and quantifiable end product. Anaerobic microcosms containing inactivated *L. minor*, which had accumulated 3.8 μmol of 2,4-DCP equivalents/g of plant material (fresh weight) during a preceding aerobic exposure, were inoculated with strain Viet1. After 118 d of incubation with strain Viet1, 43.5% (±1.4%) of the contaminant was recovered as 4-CP, indicating a large portion of plant-sequestered 2,4-DCP was bioavailable for dechlorination by strain Viet1. In contrast, 4-CP formation was not observed in autoclaved microcosms, and only 26.1% (±1.0%) of plant-sequestered 2,4-DCP was recovered in the aqueous phase. These findings demonstrate contaminant cycling between plants and microorganisms, and emphasize that understanding the mechanisms and pathways of contaminant sequestration by plants is critical for predicting long-term contaminant fate.

Introduction

Organic contaminants are frequently taken up by plants, metabolized by plant enzymes, and incorporated into plant tissues (*1*−*6*). Limited research has been conducted regarding the bioavailability and long-term fate of plant-sequestered contaminants and plant-produced metabolites of recalcitrant compounds. Predicting the long-term fate and bioavailability of plant-sequestered contaminants and metabolites in natural and engineered (e.g., phytoremediation) systems requires not only an understanding of the contaminant transformation pathways in plants but also an understanding of contaminant and contaminant metabolite cycling between plants and other components of the biosphere (e.g., microorganisms). Metabolism of organic contaminants by plants can proceed through introduction or modification of functional groups (i.e., introduction of a hydroxyl group or reduction of a nitro group) followed by conjugation of a functional group with a sugar or peptide (*4*, *7*). The conjugated contaminant is then incorporated into cellular materials or transported into vacuoles for storage and reduction of toxicity (*4*, *7*). A diagram detailing the uptake, conjugation, and complexation of 2,4-dichlorophenol (2,4-DCP) by the aquatic plant *Lemna minor* is provided in the Supporting Information, Figure SI-1 (modified from Day and Saunders (*7*)). 2,4-DCP partitions into *L. minor* and is subsequently metabolized primarily through glucosidation, where a hydroxyl group is conjugated with a glucose moiety, forming 2,4-dichlorophenyl-β-D-glucopyranoside (DCP-G; *7*). The glucosylated chlorophenol is then conjugated with a malonyl moiety (2,4-dichlorophenyl•β-D-(6-*O*-malonyl)-glucopyranoside, DCP-MG) or an apiose moiety (2,4-dichlorophenyl-β-D-glucopuranosyl-(6→1)-β-D-apiofuranoside, DCP-AG) for sequestration in vacuoles or cell wall material, respectively (*7*). Unmetabolized, plant-sequestered 2,4-DCP (subsequently referred to as "parent 2,4-DCP") and conjugated metabolites of 2,4-DCP that exist in the plant cytosol and vacuoles can be analyzed using freezing and chemical extraction techniques (*7*, *8*). Skidmore et al. (*9*) discussed covalent binding of contaminants to plant macromolecules and provided evidence for incorporation of organic contaminants into cell walls. Contaminants incorporated into cellular components are not expected to be extractable, even with rigorous procedures, nor are they readily bioavailable. In contrast, it is reasonable to conjecture that parent 2,4-DCP and conjugated metabolites in the plant cytosol or vacuoles become bioavailable after plant death and cellular decay.

The ultimate fate of contaminants may, however, depend on processes that occur external to plants. Microorganisms have been shown to have the ability to degrade plant- and fungi-produced metabolites, including metabolites of organic pollutants (*10*, *11*). Francova et al. (*10*) established that dioxygenase enzyme systems of *Comamonas testosteroni* and *Burkholderia* sp. strain LB400 attack hydroxylated polychlorinated biphenyls typical of those generated through plant metabolism from polychlorinated biphenyls. Transformation of synthetic conjugated chlorinated hydroquinones (representative of fungi-produced halogenated aromatics) by various *Desulfitobacterium* species provided evidence for reductive dehalogenation of conjugated organohalides under anaerobic conditions (*11*). *Desulfitobacterium* spp. have also been reported to be capable of *ortho*-, *meta*-, and *para*-dechlorination of chlorophenolic compounds (*12*−*15*), although the spectrum of chlorophenols dechlorinated by each *Desulfitobacterium* isolate has not been comprehensively evaluated. *Desulfitobacterium* sp. strain Viet1, which utilizes tetrachloroethene (*16*, *17*) as a terminal electron acceptor, has been reported to reduce chlorophenols (*18*) and contains a putative *o*-chlorophenol reductive dehalogenase (GenBank accession number AF259791). *Desulfitobacterium* spp. are widely distributed in anoxic environments and may play

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important roles in contaminant cycling and the long-term fate of plant-sequestered recalcitrant contaminants (e.g., 2,4-DCP), and hence, understanding the interactions of contaminants with both aquatic plants and microorganisms is relevant.

This work investigated 2,4-DCP sequestration by *L. minor* and evaluated contaminant release and bioavailability after plant death and disruption. ^{14}C-labeled 2,4-DCP was used to demonstrate that active *L. minor* systems removed 2,4-DCP from the aqueous phase and sequestered the contaminant internally. Strain Viet1, which dechlorinated 2,4-DCP to 4-chlorophenol (4-CP), was used to assess the fate and transformation of 2,4-DCP sequestered internal to *L. minor* and to determine the readily bioavailable contaminant fraction. This study provides novel information on the long-term fate and bioavailability of plant-sequestered 2,4-DCP. The results have implications for management of phytoremediation systems as well as contaminant cycling in sediments and the water column.

Materials and Methods

Microbial Dechlorination Studies with *Desulfitobacterium* sp. strain Viet1. *Microbial Medium Preparation and Growth Conditions.* A pure culture of *Desulfitobacterium* sp. strain Viet1 (*16*, *17*) was used in this study. Reduced anaerobic mineral salts medium was prepared as described previously (*19*). Triplicate cultures were incubated at 22 °C in either 160 mL serum bottles containing 100 mL of medium or 60 mL serum bottles containing 30 mL of medium. Each vial received a 2% (v/v) inoculum of a stock culture of strain Viet1 grown with tetrachloroethene (0.33 mM) as the electron acceptor and 5 mM lactate or 10 mM pyruvate as the electron donor.

Chlorophenol Dechlorination by Desulfitobacterium *sp. strain Viet1*. The dechlorinating activity of strain Viet1 in plant-free systems was examined with the following chlorophenols (initial chlorophenol concentrations tested are presented in parentheses): 3,4-dichlorophenol (3,4-DCP; 28.2 μM), 2,5-dichlorophenol (2,5-DCP; 17.2 μM), 2,6-dichlorophenol (2,6-DCP; 35.0 μM), 2,4,6-trichlorophenol (2,4,6-TCP; 25.8 μM), 2,3,5-trichlorophenol (2,3,5-TCP; 32.5 μM), and 2,4,5-trichlorophenol (2,4,5-TCP; 10.6 μM). Dechlorination of 2,4-DCP was examined at 26.4, 30.0, 38.3, and 60.0 μM, and uninoculated vials served as abiotic controls. Chlorophenols were added from an anoxic, filter-sterilized stock solution of chlorophenol dissolved in anoxic methanol. Methanol comprised <0.1% (v/v) of the aqueous volume during incubations to avoid potential toxic effects of the alcohol on strain Viet1. Pyruvate or lactate (5 mM each) was provided as the electron donor. Aqueous-phase concentrations of chlorinated phenols were monitored periodically as described below.

Plant Exposure to 2,4-DCP. All plant mass units provided are fresh (wet) weight. Fresh weight is measured by removing plants from the nutrient medium, gently blotting plant fronds dry to remove water, and immediately weighing. Contaminants were dissolved in modified duckweed nutrient medium made in accordance with method 8211B (*20*) with modifications of 4 mM total P (K_2HPO_4 or KH_2PO_4), 2 mM total C ($NaHCO_3$), and substitution of sulfate salts for chloride salts. NaOH (2 N) or H_2SO_4 (2 N) was used to adjust the medium pH. All water used was deionized water.

^{14}C Tracer Studies. Experimental reactors were 500 mL Erlenmeyer flasks sealed with rubber stoppers. Rubber stoppers were fitted with two 16 gauge stainless steel needles capped with stopcocks, one which reached the bottom of the reactor for aqueous sample extraction and one extending 5 cm into the reactor for headspace removal. ^{14}C-amended reactors received 50 μL of ^{14}C-labeled 2,4-DCP stock solution (American Radiolabeled Chemicals, Inc., St. Louis MO; 20.9 mCi/mmol, 1.28 μCi/mL) so that the ^{14}C-labeled 2,4-DCP comprised 0.1% of the total 2,4-DCP concentration. ^{14}C-amended reactors were run in parallel with reactors containing only unlabeled 2,4-DCP, and all reactors contained 12.3 μM 2,4-DCP in 200 mL of medium at pH 8. Chlorinated phenol concentrations in reactors containing only unlabeled 2,4-DCP were monitored via HPLC as described below. After addition of radiolabeled contaminant and mixing with the medium, an initial sample (6 mL) was collected. Next, 4.5 g plants (fresh weight) were added to each reactor, and the reactors were sealed with rubber stoppers.

Triplicate reactors were sacrificed after 24 and 48 h of incubation. The entire aqueous volume was removed from the reactor via the stainless steel needle with an equivalent volume of N_2 gas added to maintain atmospheric pressure. The mass of medium remaining associated with the plants was the difference between the plant mass after the medium was removed from the reactor and the plant fresh weight after the plants were removed from the reactor and blotted dry to remove excess water. After aqueous sampling, a positive pressure of N_2 gas was used to purge the headspace for 20 min through two gastight traps in series. The headspace traps contained 10 mL of a xylene-based scintillation cocktail (R. J. Harvey Instrumentation Co., Hilldale, NJ), designed for trapping gaseous organic or inorganic carbon (subsequently referred to as "trapping solution"). The second trap never contained any radioactive material, indicating that all volatile ^{14}C-labeled compounds were caught in the first trap. After the headspace was purged, the plants were removed from the reactor, rinsed with 20 mL of water, blotted dry, and weighed. Reactors were rinsed with water, the reactor rinse water was combined with plant rinseate, and the total rinse volume was measured. The ^{14}C contained in the plant/reactor rinse was the total activity in the rinse water minus the activity of medium that remained associated with the plant after the medium removal.

Radioactive materials contained in the plant material were quantified using an R. J. Harvey biological oxidizer, where organic ^{14}C was oxidized to $[^{14}C]CO_2$. Before analysis, the plants were frozen and then ground with a mortar and pestle. Ground plant material was combusted in 1 g aliquots at 900 °C with an input of pure O_2 for 4 min as a carrier gas. The combustion product CO_2 was trapped in 15 mL of trapping solution. Triplicate samples of plant material were oxidized for each reactor, and the average relative standard deviation (RSD) of radioactive materials retained after plant oxidation was 6.8%. ^{14}C standards (2,4-dichlorophenol or bicarbonate) obtained from American Radiolabeled Chemicals, Inc. were oxidized in triplicate to calculate the oxidation recovery. Oxidation recovery ranged from 81% to 99%, and a correction factor calculated from oxidation of standard material was applied to plant oxidation measurements. Radioactive materials were quantified via liquid scintillation on a Beckman LS II (Fullerton, CA).

Plant Exposure for Microcosm Studies. In preparation for developing microcosms, *L. minor* (6.5 g fresh weight) was exposed to 120.2 μM 2,4-DCP in an unsealed 500 mL Erlenmeyer flask containing 400 mL of medium (pH 5). After 67 h of exposure, plants were separated from the medium using a screened scoop, rinsed with 100 mL of water, and frozen at −80 °C for later use. As a result of plant sequestration of 2,4-DCP, the aqueous-phase concentration of 2,4-DCP dropped to 58.9 μM at 67 h, yielding 51% uptake of the contaminant by *L. minor*. The percent uptake is defined as the percentage of total contaminant in the flask accumulated by the plants (i.e., percent uptake = contaminant mass internal to the plants/initial contaminant mass in the aqueous phase). The percent uptake depends on the time of plant exposure to contaminants, initial contaminant concentration, and plant mass per aqueous volume. On the basis of the

2,4-DCP uptake and the plant biomass, an internal plant concentration of sequestered 2,4-DCP equivalents, representing both parent 2,4-DCP and plant metabolites of 2,4-DCP, was calculated to be 3.8 μmol of 2,4-DCP equilavents/g of fresh weight plant.

Anaerobic Microcosms with strain Viet1 and *L. minor*. Microcosms contained *Desulfitobacterium* sp. strain Viet1 and *L. minor* previously exposed to 2,4-DCP (3.8 μmol of 2,4-DCP equivalents/g of fresh weight plant). Construction of microcosms involved grinding 2,4-DCP-exposed plants (exposure described above) with a mortar and pestle which was cooled to −80 °C. Aliquots (0.5 g) of 2,4-DCP-exposed, ground plant material were added to 30 mL of anaerobic microbial growth medium (*19*) in an anaerobic glovebox (Coy Laboratory Products, Grass Lake, MI). After addition of ground plant material to each bottle, the bottles were sealed with butyl rubber septa and removed from the glovebox, followed by inoculation (2%, v/v) with a stock culture of strain Viet1. On the basis of the mass of contaminant per gram of plant and the total mass of plant added to the microcosms, ~1.9 μmol of plant-sequestered DCP equivalents was added to each microcosm, resulting in a maximum aqueous-phase concentration of ~63 μM 2,4-DCP equivalents (i.e., the mass of plant-sequestered 2,4-DCP equivalents added and normalized to the aqueous volume for each microcosm). One triplicate set of microcosms was autoclaved to serve as an abiotic control (autoclaved microcosms), and one triplicate set served as the experimental vessels (active microcosms). Initial aqueous samples (1.5 mL) were taken approximately 30 min (0.02 d) after addition of the plants to the microcosms (10 min after inoculation with strain Viet1) to assess initial contaminant concentrations. After these initial samples were collected, the control microcosms were autoclaved. Additional samples (1.5 mL) were taken at 6, 36, and 118 d, and concentrations of 2,4-DCP and 4-CP were assessed. The percent contaminant recovered was calculated by comparing the total number of moles of 2,4-DCP and 4-CP measured in the aqueous phase with the total quantity of contaminant expected in the aqueous phase if all parent 2,4-DCP and conjugated metabolites in the plant were released and recovered in the aqueous phase as 2,4-DCP or 4-CP.

Analytical Methods. Aqueous samples were filtered through a 0.2 μm PTFE syringe filter and acidified with an addition of glacial acetic acid equal to 0.2% of the original sample volume prior to analysis. Chlorinated phenols were separated using reversed-phase liquid chromatography (HP 1100 and Zorbax SB-C_{18}, 5 μm, 2.1 × 150 mm column), quantified with a diode array UV/vis detector (λ = 220 nm), and confirmed using mass spectrometry. The elution protocol (0.3 mL/min) consisted of a 3 min hold at 70:30 A/B after injection, 10 min linear gradient to 65% B, and 5 min hold at 100% B, where A and B were 0.1% acetic acid in water and acetonitrile, respectively. All data shown herein represent aqueous concentrations of chlorinated phenols quantified using liquid chromatography except those which are explicitly described as associated with the ^{14}C tracer experiment.

Analysis of Literature Data. An analysis of published data on 2,4-DCP uptake and 2,4-DCP plant metabolites (*21*) was performed to shed light on the bioavailability of plant-sequestered 2,4-DCP after plant death. The data analyzed were generated using plant exposure systems similar to the systems described in this work. The data extracted from reference *21* were used to calculate the percent uptake for each data point (i.e., percent uptake = contaminant mass internal to the plants/initial mass in the aqueous phase). In addition, the fraction of sequestered 2,4-DCP that remained as parent 2,4-DCP (defined as $F_{DCP\text{-}I}$) was calculated for each data point by comparing the measured concentrations of parent 2,4-DCP and all three measured 2,4-DCP metabolites (i.e., DCP-G, DCP-MG, and DCP-AG) (*21*).

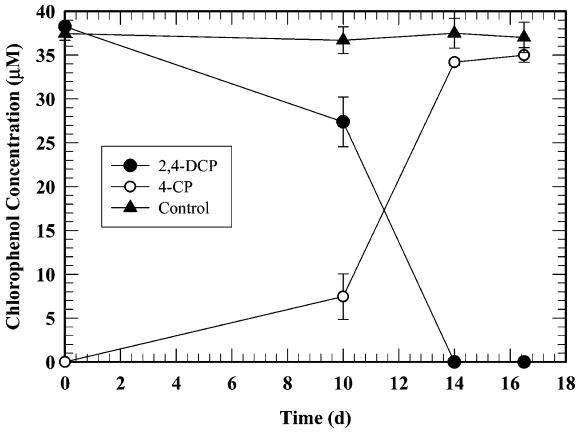

FIGURE 1. Dechlorination of 2,4-DCP to 4-CP by strain Viet1 over a 16.5 d time period. Uninoculated control data are shown for reference. Error bars represent one standard deviation of triplicates.

Results

Reductive Dechlorination of Chlorophenols by *Desulfitobacterium* sp. strain Viet1. Prior to using strain Viet1 to examine the bioavailability of plant-sequestered contaminants, the spectrum of chlorophenols reductively dechlorinated by strain Viet1 was assessed. The dechlorination activity of strain Viet1 was examined with seven chlorophenols with a variety of substituent numbers and positions. Dechlorination in the *ortho* position was observed for chlorophenols containing only *o*- and *p*-chlorines (i.e., 2,4-DCP and 2,4,6-TCP), yielding 4-CP. Dechlorination was not observed for *o*-chlorophenols that did not contain a substituent in the *para* position (i.e., 2,6-DCP). Several *m*-chlorophenols examined were not dechlorinated (2,5-DCP, 3,4-DCP, and 2,3,5-TCP), even if both *o*- and *p*-chlorines were present (2,4,5-TCP). Reductive dechlorination of 2,4-DCP to 4-CP by strain Viet1 is shown in Figure 1 with data for uninoculated controls for reference. The mass recovered as 4-CP after 16.5 d was 91.5% (±1.7%) of the mass of 2,4-DCP added to the experimental system. 2,4-DCP was dechlorinated to 4-CP for all four initial 2,4-DCP concentrations tested, dechlorination beyond 4-CP was not observed, and phenol was not detected even after 32 d of incubation (data not shown). These results demonstrated that strain Viet1 dechlorinated 2,4-DCP, forming 4-CP as a stable, quantifiable dechlorination end product. Therefore, *Desulfitobacterium* sp. strain Viet1 was used to assay the bioavailability of 2,4-DCP after sequestration by *L. minor*.

2,4-DCP Sequestration by *L. minor*. *Radiolabeled Tracer Studies.* The ^{14}C tracer recoveries in the uptake experiments were 97.1% (±2.1%) and 104.1% (±6.2%) of the ^{14}C material at times of 24 and 48 h, respectively, indicating adequate material-balance closures and radiolabel recovery. 2,4-DCP uptake by *L. minor* was established with HPLC measurements of aqueous-phase samples in reactors containing only unlabeled 2,4-DCP (Figure 2). Parallel reactors amended with ^{14}C-labeled 2,4-DCP demonstrated that ^{14}C material was taken up by plants at a rate similar to that of 2,4-DCP in reactors containing only unlabeled 2,4-DCP (Figure 2). Data for the fraction of ^{14}C material detected in the aqueous phase, plant, plant/reactor rinse, and headspace are provided in Table 1. No radiolabel tracer partitioned into the headspace. A small amount of the tracer was found in the plant/reactor rinse water (<2.5%), and this small fraction represented the quantity of ^{14}C material associated with plant surfaces. Aqueous-phase data indicated that only 22.0% (±3.0%) and 9.4% (±2.1%) of the ^{14}C material remained in the aqueous phase after 24 and 48 h of exposure, respectively. These data

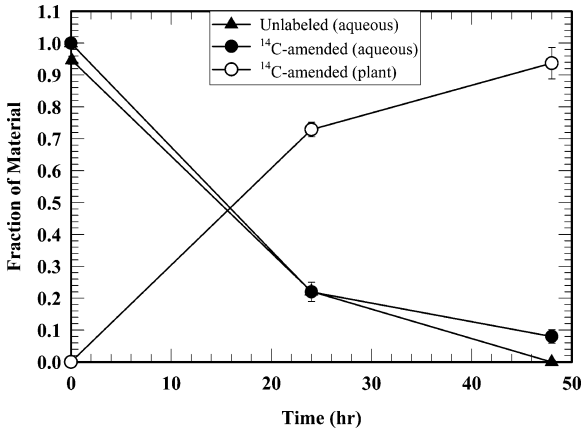

FIGURE 2. 2,4-DCP removed from the aqueous phase in plant reactors amended with unlabeled 2,4-DCP and parallel systems containing a ^{14}C tracer. 2,4-DCP removed from the aqueous phase was sequestered in *L. minor* as evidenced by ^{14}C tracer accumulation in plants. Error bars represent one standard deviation of triplicates.

TABLE 1. Material Balance for the ^{14}C Tracer in the *L. minor* System

^{14}C portion	material detected, 24 h (%)	material detected, 48 h (%)
aqueous phase	22.0 ± 3.0	9.4 ± 2.1
	22.2 ± 0.6[a]	0.0 ± 0.0[a]
plant	72.9 ± 2.3	93.7 ± 4.9
plant/reactor rinse	2.1 ± 1.3	0.8 ± 0.0
headspace	0.0 ± 0.0	0.0 ± 0.0
material recovery	97.1 ± 2.1	104.1 ± 6.2

[a] 2,4-DCP remaining in the aqueous phase as determined by HPLC measurements in parallel reactors amended with only unlabeled 2,4-DCP.

corresponded with aqueous-phase concentrations determined using HPLC measurements, which indicated 22.2% (±0.6%) of the aqueous-phase contaminant remained after 24 h and none after 48 h. Organic traps capturing $[^{14}C]CO_2$ resulting from plant oxidation indicated that 72.9% (±2.3%) and 93.7% (±4.9%) of the ^{14}C tracer was contained in the plant at 24 and 48 h, respectively. A comparison of ^{14}C accumulated by plants with 2,4-DCP removed from the aqueous phase (Figure 2) verified that the contaminant removed from the aqueous phase was sequestered internal to *L. minor*.

Reduction of Plant-Sequestered 2,4-DCP by strain Viet1. Data for 2,4-DCP and 4-CP concentrations in the microcosms are presented with time in Figure 3A, and the percent contaminant recovered with time is presented in Figure 3B. Initial samples (0.02 d) indicated that 2,4-DCP was released into the aqueous phase at concentrations of 5.27 (±0.48) μM 2,4-DCP and 5.23 (±1.26) μM 2,4-DCP for active and autoclaved microcosms, respectively (Figure 3A). 4-CP was not initially detected in any microcosm. Contaminant recovered as aqueous 2,4-DCP in the initial samples for active and autoclaved microcosms was 8.2% (±1.3%) of the total contaminant present in the microcosms (Figure 3B). Therefore, no significant difference was observed between active and autoclaved microcosms at the initial time point, indicating that biological activity did not influence immediate (0.02 d) contaminant release from plants. Further, these data indicated that a portion of 2,4-DCP accumulated internal to the plants immediately partitioned into the aqueous phase upon addition of plant biomass to the microcosms. Gradual 2,4-DCP release from the plants was observed in autoclaved microcosms after the initial release within 0.02 d of plant addition to the microcosms (Figure 3A). Aqueous concentrations increased from 5.23 (±1.26) μM 2,4-DCP at 0.02 d to 16.82 (±0.59) μM 2,4-DCP at 118 d, with no significant increase in aqueous concentrations observed after 36 d. 4-CP production did not occur in autoclaved microcosms. In autoclaved microcosms, 26.1% (±1.0%) of the contaminant sequestered by the plants was released, indicating that a large fraction of plant-sequestered contaminant was released in abiotic systems.

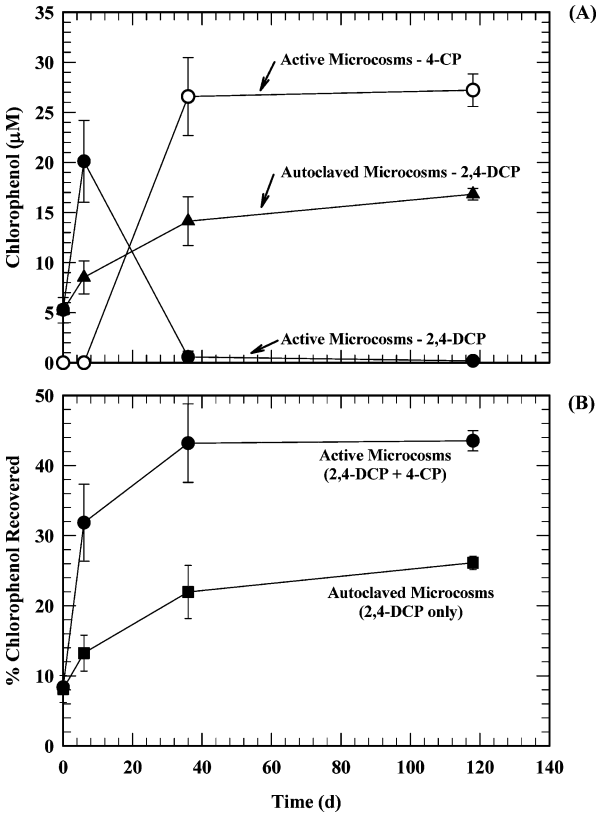

FIGURE 3. Transformation of plant-sequestered 2,4-DCP by active and autoclaved microcosms. Aqueous 2,4-DCP and 4-CP concentrations are shown in (A), and percentages of chlorophenol in plants recovered as 2,4-DCP or 4-CP in the aqueous phase are shown in (B). Error bars represent one standard deviation of triplicates.

The 2,4-DCP detected in autoclaved microcosms was hypothesized to be unmetabolized, plant-sequestered 2,4-DCP (i.e., parent 2,4-DCP) released from the plants after inactivation. To validate this hypothesis, data for autoclaved microcosms were compared with literature data (*21*). $F_{DCP\text{-}I}$ and the percent uptake were calculated, and an exponential decrease in $F_{DCP\text{-}I}$ was observed with increasing values of the percent uptake, as shown in Figure 4. High values of $F_{DCP\text{-}I}$ (~100%) were observed at low values of the percent uptake. Low values of $F_{DCP\text{-}I}$ (<20%) were observed for high levels of uptake, and $F_{DCP\text{-}I}$ dropped near zero as systems approached 100% uptake. Data for contaminant released in autoclaved microcosms (current study) are also shown in Figure 4. Plants used in this study were at 51% uptake, and 26.1% (±1.0%) of the contaminant internal to the plants was recovered from the plants in autoclaved reactors after 118 d. The percent recovery after 118 d for the autoclaved microcosms falls within the range of $F_{DCP\text{-}I}$ of data measured by Day (*21*) at similar percent uptakes, indicating that it was reasonable to describe 2,4-DCP released by plants in autoclaved microcosms as parent 2,4-DCP. Therefore, parent 2,4-DCP readily partitions into the aqueous phase and becomes available to bacteria in the bulk aqueous phase.

2,4-DCP release from plants into the aqueous phase was also observed in active microcosms. Samples taken after 6

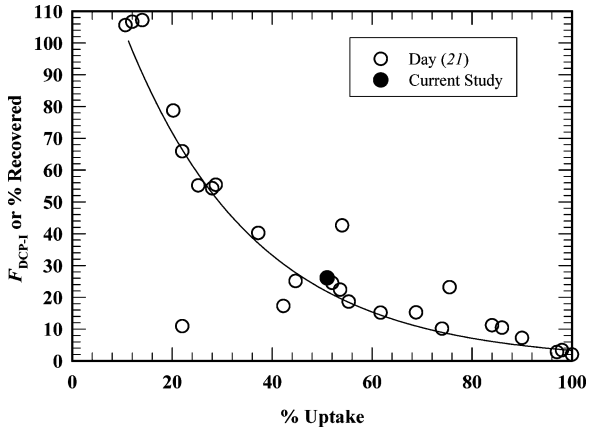

FIGURE 4. F_{DCP-I} is compared with the percent uptake for data from four initial 2,4-DCP concentrations (this represents an analysis of data presented by Day (*21*)). Data for the percent recovered in autoclaved microcosms (current study) are presented for comparison.

d of exposure showed a significant increase in 2,4-DCP concentrations (i.e., 20.11 ± 4.09 μM 2,4-DCP as shown in Figure 3A). After 36 d of exposure, 2,4-DCP concentrations in active microcosms were reduced to very low levels (0.58 ± 0.57 μM 2,4-DCP) and a corresponding production of 4-CP was observed (26.58 ± 3.89 μM 4-CP). No significant increase in 4-CP concentrations was observed between 36 and 118 d (i.e., the 4-CP concentration was 27.21 ± 1.26 μM at 118 d), and no significant decrease in 2,4-DCP concentrations occurred (i.e., the 2,4-DCP concentration was 0.19 ± 0.32 μM at 118 d). Dechlorination beyond 4-CP was not observed, and phenol was not detected. Depletion of 2,4-DCP and accumulation of 4-CP in active microcosms were attributed to reductive dechlorination by strain Viet1. A comparison of the total material recovered in active microcosms for 0.02, 6, 36, and 118 d is presented in Figure 3B. Total material recoveries increased with time in both active and autoclaved microcosms, and significantly more contaminant was recovered in active microcosms than in autoclaved microcosms. After 118 d, 43.5% (±1.4%) of the contaminant in active microcosms was recovered as 4-CP and trace amounts of 2,4-DCP (<0.6 μM) were present. In autoclaved microcosms, 26.1% (±1.0%) of the contaminant was recovered as 2,4-DCP after 118 d, resulting in a 17.4% (±2.4%) difference between recoveries in active and autoclaved microcosms. Therefore, the data indicate that a fraction of contaminant metabolites sequestered internal to the plants were released and transformed, presumably by strain Viet1, although direct evidence for microbial transformation of conjugated plant metabolites of 2,4-DCP was not obtained.

Discussion

Findings presented herein using a plant−microbial system illustrate that the fate of contaminants in plant systems involves sequestration and release of bioavailable contaminants after plant death and disruption. The ^{14}C tracer data demonstrated that active *L. minor* systems removed 2,4-DCP from the aqueous phase and sequestered the contaminant internal to the plants. A fraction of the plant-sequestered 2,4-DCP was immediately released after addition of inactivated plants to microcosms, and continued release of 2,4-DCP was observed in both active and autoclaved systems. The results demonstrated that partitioning of contaminants out of the plants after plant death and disruption play important roles in contaminant cycling. Significantly more contaminant was released in active microcosms than in autoclaved microcosms. The difference in the quantity of contaminant released was attributed to either microbial cleavage of conjugated metabolites and subsequent dechlorination of 2,4-DCP in active microcosms or equilibrium partitioning. Evidence for microbial deconjugation was provided through examination of the data presented in Figure 3A. In the sample taken at 0.02 d, the concentration of 2,4-DCP was the same in autoclaved and active microcosms. However, in the 6 d sample, there was significantly more aqueous-phase 2,4-DCP present in the active microcosm than in the autoclaved microcosm, and no 4-CP was present in either microcosm. If the behavior of contaminants in the microcosms was dominated by equilibrium partitioning, the concentrations of 2,4-DCP should have been the same in active and autoclaved microcosms prior to dechlorination. Therefore, the results from the initial time points indicated that microbial deconjugation rather than equilibrium partitioning played a dominant role in releasing 2,4-DCP in the active systems compared with the autoclaved systems. These data are supported by the work of Day (*21*), who demonstrated the ability of ambient and plant-associated microorganisms to readily cleave malonyl and glycoside linkages of chemically synthesized DCP-G and DCP-MG, which are *L. minor* metabolites of 2,4-DCP. Although previous experiments were performed under aerobic conditions, the cleavage of conjugated moieties occurs in the presence or absence of oxygen, since glycosyl hydrolases are present and active in aerobic and anaerobic environments (*22*, *23*).

While a fraction of the contaminant sequestered by the plants was released into the aqueous phase and became bioavailable, our results indicate that a substantial portion of plant-sequestered 2,4-DCP was retained in the plant material for an extended time period (>118 d) in the anaerobic systems. Apparently, plants, or parts thereof, provide a reservoir for sequestration of contaminants and subsequent release. It is important to consider the fact that experiments conducted herein used plants at 51% uptake. Previous data (*21*) indicated that at low values of the percent uptake (i.e., 0% to ~35%), contaminant internal to the plants was primarily in the form of parent material (i.e., 2,4-DCP). At midrange values of the percent uptake (i.e., ~35−70%), contaminant internal to the plants was a mixture of parent 2,4-DCP and conjugated metabolites (i.e., primarily a 2,4-DCP−glucose conjugate). At high values of the percent uptake, sequestered contaminant was primarily in the form of conjugated contaminant metabolites. In this study, a midrange percent uptake (plants were at 51% uptake) was targeted so that the plants contained a mixture of parent 2,4-DCP and conjugated metabolites. The readily bioavailable fraction is likely a function of the percent uptake since the metabolites present potentially play different roles in contaminant bioavailability in the contaminant cycling pathway. The quantity of readily bioavailable contaminant as a function of the percent uptake remains an important avenue for future research.

An overview of the proposed contaminant cycling pathway for 2,4-DCP in plant and microbial systems is presented in Figure 5. The diagram depicts the transformation and degradation pathways of 2,4-DCP in both aerobic and anaerobic wetland environments. The pathway presents conjugation of 2,4-DCP into DCP-G, DCP-MG, and DCP-AG by plants and compartmentation of conjugated plant metabolites into vacuoles and cell walls. Figure 5 also shows the proposed transformation pathway expected after plant inactivation (i.e., death). Plants used in microcosms with strain Viet1 were frozen and ground to accelerate the release of 2,4-DCP and plant metabolites into the aqueous phase. The physical stress applied to the plants was assumed to break apart plant tissues and to break open cells and vacuoles, facilitating the release of 2,4-DCP and metabolites contained in the cytosol or vacuoles. Once released into the aqueous phase, parent 2,4-DCP and 2,4-DCP metabolites may have been transformed by microorganisms as proposed in Figure

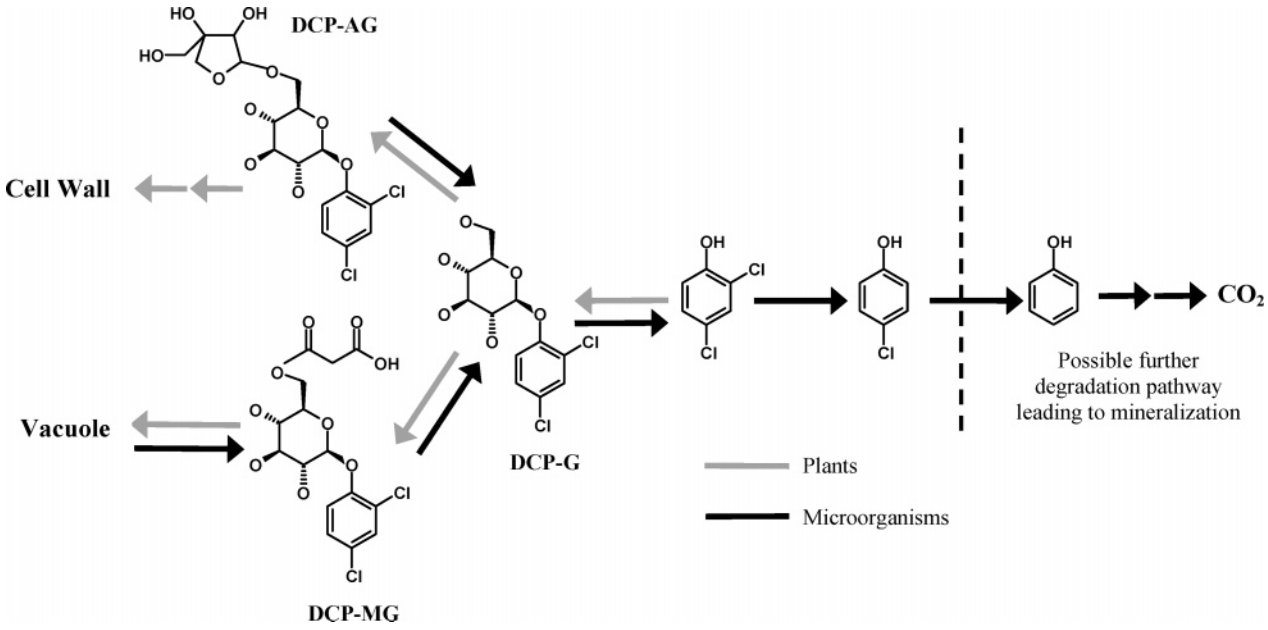

FIGURE 5. Proposed pathway for 2,4-DCP cycling in plant and microbial systems. Microbial degradation shown to the right of the dashed line did not occur in the systems used herein, but can occur in more microbially diverse systems. Plant metabolites DCP-G, DCP-MG, and DCP-AG are shown.

5. The possibility exists that inactivated and disrupted plants released enzymes with the ability to deconjugate 2,4-DCP metabolites; however, the contribution of enzymes from inactivated and disrupted plants in releasing 2,4-DCP in the experimental microcosms was assumed to be negligible. Day (*21*) established that plant metabolites are stable during the process of grinding, and it is assumed freezing and grinding do not affect chemical bonds that bind contaminants to plant cell walls. Therefore, the fraction of contaminant that was not released into the aqueous phase was assumed not to be readily bioavailable. The contaminant cycling pathway shown in Figure 5, therefore, does not indicate release of contaminant from cell walls. Dechlorination of the parent contaminant proceeds through reductive microbial processes, forming 4-CP, and complete mineralization may occur in natural systems (*24*−*26*). Therefore, long-term bioavailability of contaminants in sediments cannot be assigned strictly on the basis of studies described herein. It is likely that organisms present in sediments contribute to the release of additional plant-sequestered contaminant, emphasizing the relevance of long-term evaluation of phytoremediation.

The bioavailability of plant-sequestered contaminants has implications for contaminant cycling and ultimate contaminant fate in engineered and natural aquatic systems. The microcosm experiments demonstrated bacterial transformation of plant metabolites of 2,4-DCP released from dead and disrupted plants, indicating that previous sequestration and metabolism by plants does not necessarily indicate lasting contaminant removal. In fact, contaminant accumulation by plants may promote degradation of certain contaminants by placing plant-sequestered contaminants in an organic-rich, anaerobic environment ideal for reductive dechlorination (*27*). Additionally, transformation of plant-sequestered contaminants may be promoted by natural plant metabolites (e.g., salicylate), which have been shown to stimulate microbial biotransformation of organic pollutants (*28*−*30*). In contrast, a significant portion of plant-sequestered contaminant was not readily bioavailable in the systems used herein, indicating that plants may provide a long-term sink for a fraction of the contaminant that has been sequestered and metabolized by plants. The release of contaminant into the aqueous phase after plant death and disruption shows that contaminant uptake by plants is reversible, and predictions on long-term removal of contaminants from the water column must be interpreted cautiously.

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Supporting Information Available

Pathway for uptake, conjugation, and complexation of 2,4-DCP by *L. minor* after Day and Saunders (*7*). This material is available free of charge via the Internet at http://pubs.acs.org.

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