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Simultaneous Transformation of Commingled Trichloroethylene, Tetrachloroethylene, and 1,4-Dioxane by a Microbially Driven Fenton Reaction in Batch Liquid Cultures

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ABSTRACT

Improper disposal of 1,4-dioxane and the chlorinated organic solvents trichloroethylene (TCE) and tetrachloroethylene (also known as perchloroethylene [PCE]) has resulted in widespread contamination of soil and groundwater. In the present study, a previously designed microbially driven Fenton reaction system was reconfigured to generate hydroxyl ($HO^{\cdot}$) radicals for simultaneous transformation of source zone levels of single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane. The reconfigured Fenton reaction system was driven by fed batch cultures of the Fe(III)-reducing facultative anaerobe *Shewanella oneidensis* amended with lactate, Fe(III), and contaminants and exposed to alternating anaerobic and aerobic conditions. To avoid contaminant loss due to volatility, the Fe(II)-generating, hydrogen peroxide-generating, and contaminant transformation phases of the microbially driven Fenton reaction system were separated. The reconfigured Fenton reaction system transformed TCE, PCE, and 1,4-dioxane either as single contaminants or as binary and ternary mixtures. In the presence of equimolar concentrations of PCE and TCE, the ratio of the experimentally derived rates of PCE and TCE transformation was nearly identical to the ratio of the corresponding $HO^{\cdot}$ radical reaction rate constants. The reconfigured Fenton reaction system may be applied as an *ex situ* platform for simultaneous degradation of commingled TCE, PCE, and 1,4-dioxane and provides valuable information for future development of *in situ* remediation technologies.

IMPORTANCE

A microbially driven Fenton reaction system [driven by the Fe(III)-reducing facultative anaerobe *S. oneidensis*] was reconfigured to transform source zone levels of TCE, PCE, and 1,4-dioxane as single contaminants or as binary and ternary mixtures. The microbially driven Fenton reaction may thus be applied as an *ex situ* platform for simultaneous degradation of at least three (and potentially more) commingled contaminants. Additional targets for *ex situ* and *in situ* degradation by the microbially driven Fenton reaction developed in the present study include multiple combinations of environmental contaminants susceptible to attack by Fenton reaction-generated $HO^{\cdot}$ radicals, including commingled plumes of 1,4-dioxane, pentachlorophenol (PCP), PCE, TCE, 1,1,2-trichloroethane (TCA), and perfluoroalkylated substances (PFAS).

The chlorinated organic solvents trichloroethylene (TCE) and tetrachloroethylene (also known as perchloroethylene [PCE]) have been historically employed as solvents in a variety of industrial processes, including vapor degreasing of metal surfaces, paint stripping, and dry cleaning (1, 2). TCE and PCE are carcinogenic, and improper disposal practices at industrial sites have resulted in widespread contamination of soil and groundwater (1–6). Due to their high density and low aqueous phase solubility, TCE and PCE are also highly persistent in contaminated environments (3, 4). The potential carcinogen 1,4-dioxane is generally employed as a stabilizer for TCE and PCE in industrial processes (7–11), and thus, TCE- and PCE-contaminated groundwater is often commingled with 1,4-dioxane (9, 12–20).

Current TCE and PCE remediation technologies are based on photolysis, sonolysis, and reductive transformations by iron-bearing minerals or dechlorinating microorganisms (6, 21–26). Photolysis and sonolysis, however, are limited by UV light penetration and low energy transfer efficiency, respectively (27–30), while microbially catalyzed reductive dehalogenation reactions are limited by microbial nutrient requirements, contaminant bioavailability, and incomplete dechlorination, leading to the production of toxic intermediates (31). Current 1,4-dioxane remediation technologies such as carbon absorption, air stripping, and distillation are limited by problems associated with 1,4-dioxane solubility, boiling point, and vapor pressure, respectively (32).

Chemical oxidation is also an attractive remediation technology for degradation of TCE, PCE, and 1,4-dioxane (2, 5, 12, 33). Currently deployed chemical oxidants include ozone, titanium oxide, persulfate, permanganate, peroxide, zero valent iron (ZVI), and hydroxyl ($HO^{\cdot}$) radicals (2, 5). $HO^{\cdot}$ radicals represent a powerful oxidant with an oxidation potential (+2.76 V) higher than those of ozone (+2.07 V), persulfate (+2.01 V), and permanga-

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nate (+1.68 V) (33). $HO^{\cdot}$ radicals may be produced by the conventional Fenton reaction (equation 1) in which ferrous iron [Fe(II)] catalyzes the decomposition of hydrogen peroxide (H_2O_2) to produce Fe(III), hydroxyl ion (OH^-), and $HO^{\cdot}$ radical:

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + HO^{\cdot} \quad (1)$$

Conventional Fenton reaction-generated $HO^{\cdot}$ radicals oxidatively degrade a number of hazardous compounds, including chlorinated aliphatic and aromatic compounds (34), pentachlorophenol (PCP) (35, 36), PCE (24), TCE (1, 5, 23, 26, 33), 1,1,2-trichloroethane (TCA) (37), 1,4-dioxane (32), and petroleum hydrocarbons (38). Conventional Fenton reaction-driven transformation of TCE, PCE, and 1,4-dioxane is limited, however, by the high concentrations of the Fenton reagents Fe(II) and H_2O_2 that must be continuously supplied to produce $HO^{\cdot}$ radicals and drive TCE, PCE, and 1,4-dioxane transformation (1, 24). At neutral pH, addition of Fe(III)-complexing ligands may improve conventional Fenton reaction efficiencies by preventing Fe(III) oxide precipitation (39). UV irradiation is often employed to induce Fe(III) rereduction and photolytic radical production in photo-Fenton systems. The UV irradiation systems, however, are limited by UV light penetration, and H_2O_2 must still be continuously supplied to drive the conventional Fenton reaction (32).

Microbially driven Fenton reactions that alternately produce the Fenton reagents H_2O_2 (via microbial O_2 respiration) and Fe(II) [via microbial Fe(III) reduction] alleviate the need for continual addition of H_2O_2 and Fe(II) to drive $HO^{\cdot}$ radical production (12, 17, 32, 40). The Fe(III)-reducing facultative anaerobe *Shewanella oneidensis* was recently employed to drive the Fenton reaction for oxidative degradation of PCP and 1,4-dioxane (32, 36). In the *S. oneidensis*-driven Fenton reaction, batch liquid cultures were amended with Fe(III) and 1,4-dioxane and subsequently exposed to alternating anaerobic and aerobic conditions. During the anaerobic period, *S. oneidensis* produced Fe(II) via microbial Fe(III) reduction, while during the aerobic period, *S. oneidensis* produced H_2O_2 via microbial O_2 respiration. During the transition from anaerobic to aerobic conditions, Fe(II) and H_2O_2 interacted chemically via the Fenton reaction to produce $HO^{\cdot}$ radicals that completely degraded 1,4-dioxane at source zone concentrations (32).

Although TCE and PCE are often commingled with 1,4-dioxane in contaminated groundwater, only a limited number of studies have examined simultaneous transformation of multiple contaminants, with emphasis on binary mixtures of TCE and 1,4-dioxane or TCE and PCE (26, 41–43). The main objectives of the present study were to do the following: (i) design a new fed batch, microbially driven Fenton reaction system that minimizes contaminant loss due to volatility by separating the Fe(II)-generating, H_2O_2-generating, and contaminant transformation phases and (ii) apply the new microbially driven Fenton reaction system to simultaneously transform single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane.

MATERIALS AND METHODS

Bacterial growth medium, growth conditions, and chemical reagents. To prepare *S. oneidensis* stock cultures for use in contaminant transformation experiments, *S. oneidensis* was grown aerobically for 48 h until stationary phase (optical density at 600 nm [OD_{600}] of 3.0) in LB medium (10 g/liter tryptone, 5 g/liter yeast extract, and 10 g/liter NaCl) (44). Stationary-phase cells were harvested and washed three times in minimal salt solution (LS) (pH 7.0) (see Table S1 in the supplemental material). TCE, PCE, and 1,4-dioxane transformation experiments were conducted in LS medium amended with 50 mM lactate as the carbon and energy source and 10 mM Fe(III)-citrate as the anaerobic electron acceptor (36, 45). Fe(III)-citrate was prepared by previously described methods (46). TCE, PCE, 1,4-dioxane, acetonitrile, ferrozine, phosphoric acid, mannitol, sodium acetate, sodium nitrate, sodium lactate, and thiourea were obtained from Sigma-Aldrich. In the contaminant transformation experiments, contaminants were added to the fed batch reactor system at final concentrations that reflected the source zone levels: TCE, 100 μM; PCE, 100 μM; and 1,4-dioxane, 2 mM.

TABLE 1 Limit of detection and limit of quantitation of analytes in this study[a]

Compound	LOD	LOQ
1,4-Dioxane	0.2 mM	0.7 mM
Acetate	40.4 μM	133.3 μM
Lactate	18.0 μM	59.4 μM
Trichloroethylene	7.6 μM	25.1 μM
Tetrachloroethylene	8.9 μM	29.4 μM

[a] Abbreviations: LOD, limit of detection; LOQ, limit of quantitation.

Design of fed batch reactor system for simultaneous transformation of TCE, PCE, and 1,4-dioxane by the microbially driven Fenton reaction. The toxicity of single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane was tested in LS medium by comparing aerobic growth of *S. oneidensis* batch cultures in LS medium in the presence and absence of the contaminant mixtures. To avoid inadvertent loss of contaminants due to volatility during injection of compressed nitrogen or compressed air (Henry's law constant for TCE, 1.1×10^{-3} mol kg^{-1} kPa^{-1}; for PCE, 5×10^{-4} mol kg^{-1} kPa^{-1}; and for 1,4-dioxane, 2.2 mol kg^{-1} kPa^{-1}) (32, 47–50), the batch reactor system previously employed for 1,4-dioxane degradation (32) was modified, and the Fe(II)-generating, H_2O_2-generating, and contaminant transformation phases were separated (see Fig. S4 in the supplemental material). In the Fe(II)-generating phase, contaminant-free *S. oneidensis* liquid cultures [10^9 cells ml^{-1} in LS medium amended with 10 mM Fe(III)] were incubated in 60-ml glass serum bottles under anaerobic conditions by injecting (hydrated) compressed nitrogen until the entire 10 mM pool of Fe(III) was reduced to approximately 10 mM Fe(II) [approximately 24 h of anaerobic incubation; the Fe(II)-generating phase]. The compressed nitrogen line was then replaced by a (hydrated) compressed air line, and the 10 mM Fe(II)-containing *S. oneidensis* liquid culture was incubated under aerobic conditions until the 10 mM pool of Fe(II) was oxidized to approximately 4 mM (approximately 24 h of aerobic incubation; the H_2O_2-generating phase). The 4 mM residual Fe(II) was carried into the contaminant transformation phase to interact with the pool of microbially produced H_2O_2 and generate $HO^{\cdot}$ radicals (similar to $HO^{\cdot}$ radical production by the previously reported 1,4-dioxane-degrading batch reactor system) (32).

Following 24 h of the H_2O_2-generating phase (aerobic incubation), the contaminant transformation phase was initiated by adding single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane with a sterile syringe. The contaminant transformation phase was carried out for approximately 5 days (without gas injection), and the contaminant concentrations were monitored via high-pressure liquid chromatography (HPLC). Following completion of the first 5-day contaminant transformation phase (i.e., after contaminants were transformed to below detection limits) (Table 1), a second cycle of Fe(II)-generating and H_2O_2-generating phases was carried out during the time periods from day 7 to day 8 and from day 8 to day 9, respectively, followed by respiking of contaminant mixtures to initial concentrations and carrying out a second 5-day contaminant transformation phase during the time period from day 9 to day 14. An identical third cycle of Fe(II)-generating, H_2O_2-generating, and contaminant transformation phases was carried during the time period from day 14 to day 19. Reactor temperature (25°C) and pH (7.0) were held constant throughout all reactor phases.

Inhibition of the microbially driven Fenton reaction in control incubations. A series of four control incubations were carried out to confirm that the single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane were transformed by HO˙ radicals generated by the *S. oneidensis*-driven Fenton reaction. The four control incubations were carried out in the fed batch reactor system described above with the following changes. In the first set of control incubations, the H_2O_2-generating and contaminant transformation phases were carried out with 15 mM NO_3^- replacing 10 mM Fe(III) as the electron acceptor. In the second set of control incubations, the HO˙ radical-scavenging compounds mannitol (120 mM) and thiourea (20 mM) were added to the fed batch reactor system prior to initiating the H_2O_2-generating phase. The toxicities of mannitol and thiourea were tested in LS medium by comparing aerobic growth of *S. oneidensis* batch cultures in the presence and absence of mannitol or thiourea and single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane. In the third set of control incubations, Fe(III) or *S. oneidensis* cells (abiotic control) were omitted from the H_2O_2-generating and contaminant transformation phases. In the fourth set of control incubations, contaminant concentrations were monitored in abiotic sealed serum bottles maintained under strict anaerobic conditions without injection of compressed nitrogen for a 20-day anaerobic incubation period.

Analytical methods. Samples (200-μl samples) were withdrawn every 24 h with a sterile syringe and centrifuged at 6,000 × *g* for 10 min. HCl-extracted Fe(II) concentrations were determined by a previously described ferrozine-based colorimetric technique (51). 1,4-Dioxane concentrations were determined via HPLC using a Zorbax SB-C_{18} column with 20% aqueous acetonitrile as the mobile phase and a constant flow rate of 1.0 ml min^{-1} (15). Chromatograms were generated at 190 nm for 1,4-dioxane with a retention time of 2.4 min. TCE and PCE samples were extracted with ethyl acetate (1:1 volume ratio), and the organic phase was directly injected into the HPLC system using a Zorbax SB-C_{18} column with 60% aqueous acetonitrile as the mobile phase and a constant flow rate of 1.0 ml/min. Chromatograms were generated at 214 nm for TCE and PCE with retention times of 4.5 and 7.5 min, respectively. Lactate and acetate were analyzed via HPLC using a Supelcogel C-610H column with 0.1% H_3PO_4 as the mobile phase and a constant flow rate of 0.3 ml min^{-1}. Chromatograms were generated at 210 nm for lactate and acetate with retention times of 29.41 and 35.06 min, respectively. Calibration curves were generated from standards to determine the concentrations of each compound.

RESULTS

TCE transformation in the presence and absence of PCE and 1,4-dioxane. In Results, the single, binary, and ternary mixtures of TCE, PCE, and 1,4-dioxane are shown abbreviated as follows: TCE alone = T; PCE alone = P; 1,4-dioxane alone = D; TCE + PCE = TP; TCE + 1,4-dioxane = TD; PCE + 1,4-dioxane = PD; and TCE + PCE + 1,4-dioxane = TPD. To test for T, P, and D toxicity, *S. oneidensis* was grown aerobically in LS growth medium supplemented with a ternary mixture of T (100 μM), P (100 μM), and D (2 mM). Aerobic growth rates in the presence and absence of TPD were nearly identical, which indicated that the TPD concentrations did not inhibit *S. oneidensis* metabolic activity (see Fig. S1 in the supplemental material).

In the anaerobic phase of the TCE degradation experiments, Fe(III)-containing *S. oneidensis* cultures reduced the pool of 10 mM Fe(III) at a rate of 417 μM h^{-1}, and Fe(II) concentrations increased to 10 mM [the Fe(II)-generating phase; gray-shaded area in Fig. 1a]. In the subsequent aerobic phase, Fe(II) was air oxidized at rates of 192 to 321 μM h^{-1}, and Fe(II) concentrations decreased from 10.0 mM to 2.3 to 5.4 mM (the first H_2O_2-generating phase; yellow-shaded area in Fig. 1a). During the first contaminant transformation phase, Fe(III) was microbially reduced at rates of 39.2 to 41.7 μM h^{-1}, and Fe(II) concentrations increased to 7.0 to 10.4 mM (unshaded area in Fig. 1a). During the first contaminant transformation phase with T as the sole contaminant (T1), T was transformed at an initial rate of 1.1 μM h^{-1} (Fig. 1b and Table 2). By comparison, T was transformed in TP, TD, and TPD mixtures at initial rates of 71%, 82%, and 74%, respectively, of the T transformation rate with T as the sole contaminant (i.e., percentage of the T1 transformation rate; Fig. 1b and Table 2). T was transformed to below detection limits (Table 1) by the end of the first transformation phase (day 7 time point) with T as the sole contaminant and in the TD, TP, and TPD mixtures (Fig. 1b).

In the second Fe(II)- and H_2O_2-generating phase, Fe(II) was air oxidized at rates of 196 to 238 μM h^{-1}, and Fe(II) concentrations decreased from 7.0 to 10.4 mM to 2.3 to 4.7 mM (the second H_2O_2-generating phase; yellow-shaded area in Fig. 1a). During the second contaminant transformation phase, Fe(III) was microbially reduced at rates of 36.7 to 50.0 μM h^{-1}, and Fe(II) concentrations increased to 6.7 to 10.7 mM. During the second contaminant transformation phase with T as the sole contaminant (T2), T was transformed at an initial rate of 1.5 μM h^{-1} (Fig. 1b and Table 2). By comparison, T was transformed in the TP, TD, and TPD mixtures at initial rates of 51%, 67%, and 55%, respectively, of the T transformation rate with T as the sole contaminant (i.e., percentage of the T2 transformation rate; Fig. 1b and Table 2). T was transformed to below detection limits (Table 1) by the end of the second transformation phase (day 14 time point) with T as the sole contaminant and in the TD, TP, and TPD mixtures (Fig. 1b).

In the third Fe(II)- and H_2O_2-generating phase, Fe(II) was air oxidized at rates of 133 to 208 μM h^{-1}, and Fe(II) concentrations decreased to 3.5 to 5.7 mM (the third H_2O_2-generating phase; yellow-shaded area in Fig. 1a). During the third contaminant transformation phase, Fe(III) was microbially reduced at rates of 33.3 to 48.6 μM h^{-1}, and Fe(II) concentrations increased to 5.9 to 9.2 mM. During the third contaminant transformation phase with T as the sole contaminant (T3), T was transformed at an initial rate of 1.4 μM h^{-1} (Fig. 1b and Table 2). By comparison, T was transformed in the TP, TD, and TPD mixtures at initial rates of 46%, 65%, and 74%, respectively, of the T transformation rate with T as the sole contaminant (i.e., percentage of the T3 transformation rate; Fig. 1b and Table 2). T was transformed to below detection limits (Table 1) by the end of the third transformation phase (day 19 time point) with T as the sole contaminant. Due to early termination of the third contaminant transformation phase, T was transformed to 37, 56, and 28 μM levels in the TD, TP, and TPD mixtures, respectively (Fig. 1b).

T was transformed as the sole contaminant in the three successive contaminant transformation phases (days 2 to 7, 9 to 14, and 16 to 19; Fig. 1b and Table 2). T concentrations remained constant at 100 μM in parallel control incubations lacking Fe(III) or *S. oneidensis* cells (abiotic control), but including three identical cycles of successive anaerobic [Fe(II)-generating], aerobic (H_2O_2-generating), and contaminant (T, TP, TD, and TPD) transformation phases. Identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction and chemical (O_2-catalyzed) Fe(II) oxidation were observed in parallel control incubations with contaminants omitted (Fig. 1a), which indicated that rates of microbially catalyzed Fe(III) reduction and O_2-catalyzed Fe(II) oxida-

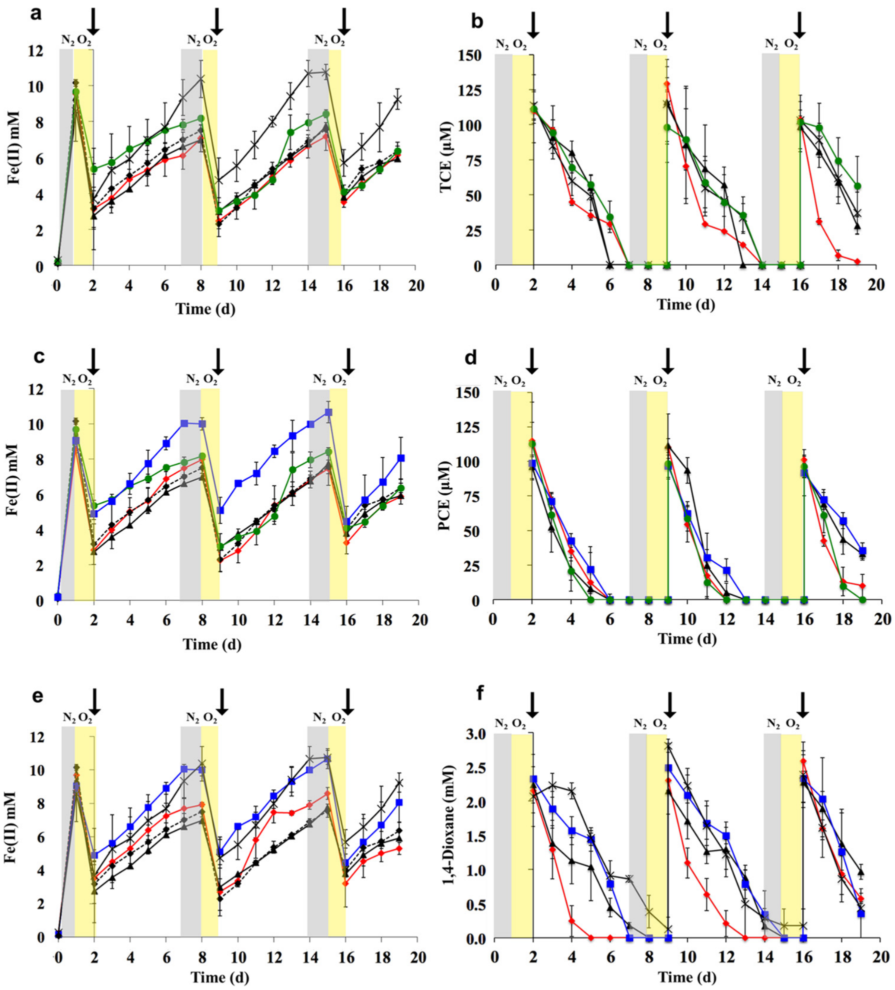

FIG 1 Concentration profiles during microbial Fenton transformation of TCE, PCE, and 1,4-dioxane in fed batch liquid cultures of *S. oneidensis* amended with 10 mM Fe(III)-citrate, single, binary, and ternary mixtures of TPD (100 µM TCE, 100 µM PCE, and 2 mM 1,4-dioxane) and subjected to anaerobic and aerobic conditions and TPD transformation phases. The cultures were subjected to anaerobic (day 0 to 1, 7 to 8, and 14 to 15) and aerobic (day 1 to 2, 8 to 9, and 15 to 16) conditions. The cultures were also subjected to TPD transformation (days 2 to 7, 9 to 14, and 16 to 19) phases: (a) Fe(II) (for TCE reactions); (b) TCE; (c) Fe(II) (for PCE reactions); (d) PCE; (e) Fe(II) (for 1,4-dioxane reactions); (f) 1,4-dioxane. In panels a to f, red diamonds indicate single contaminant only, black mults indicate TCE plus 1,4-dioxane, green circles indicate TCE plus PCE; blue squares indicate PCE plus 1,4-dioxane, black triangles indicate TCE plus PCE plus 1,4-dioxane, and black diamonds indicate no-contaminant control. Time is shown in days (d) on the *x* axes. Gray shaded areas correspond to the Fe(II)-generating phase, yellow shaded areas correspond to the H_2O_2-generating phase, and unshaded areas correspond to the contaminant transformation phase. The arrows indicate the time of addition (day 2) and respiking (days 9 and 16) of contaminants. The points plotted represent the means of results from duplicate measurements carried out in two parallel yet independent incubations. The error bars represent the ranges of the values. In some cases, the error bar is smaller than the symbol.

tion were not affected by the presence of the T, TP, TD, or TPD mixtures.

PCE transformation in the presence and absence of TCE and 1,4-dioxane. Nearly identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction and chemical (O_2-catalyzed) Fe(II) oxidation were observed in analogous P, TP, PD, and TPD contaminant transformation experiments (Fig. 1c). P was transformed at an initial rate of 1.4 µM h^{-1} during the contaminant transformation phase with P as the sole contaminant (Fig. 1d and Table 2). By comparison, P was transformed in the PT, PD, and TPD mixtures at initial rates of 110%, 75%, and 62%, respectively, of the P transformation rate with P as the sole contaminant (i.e., percentage of the P1 [the first contaminant transformation phase with P as the sole contaminant] transformation rate; Fig. 1d and Table 2). P was transformed to below detection limits (Table 1) by the end of the first transformation phase (day 7 time point) with P as the sole contaminant and in the PT, PD, and TPD mixtures (Fig. 1d).

During the second contaminant transformation phase with P as the sole contaminant (P2), P was transformed at an initial rate of 1.6 µM h^{-1} (Fig. 1d and Table 2). By comparison, P was transformed at initial rates of 88%, 69%, and 96%, respectively, of the P transformation rate with P as the sole contaminant (i.e., percentage of the P2 transformation rate; Fig. 1d and Table 2). P was transformed to below detection limits (Table 1) by the end of the

TABLE 2 Initial rates of TCE, PCE, and 1,4-dioxane transformation in single, binary, and ternary mixtures during the microbially driven Fenton reaction in a fed-batch reactor

Contaminant	Cycle	Initial rate of contaminant transformation (μM h^{-1})[a]			
TCE		T	TP	TD	TPD
	1	1.1 ± 0.07	0.8 ± 0.19 (71)	0.9 ± 0.12 (82)	0.8 ± 0.05 (74)
	2	1.5 ± 0.01	0.8 ± 0.14 (51)	1.0 ± 0.23 (67)	0.8 ± 0.30 (55)
	3	1.4 ± 0.11	0.6 ± 0.07 (46)	0.9 ± 0.02 (65)	1.0 ± 0.20 (74)
PCE		P	PT	PD	TPD
	1	1.4 ± 0.32	1.6 ± 0.28 (110)	1.1 ± 0.28 (75)	0.9 ± 0.04 (62)
	2	1.6 ± 0.11	1.4 ± 0.22 (88)	1.1 ± 0.04 (69)	1.5 ± 0.10 (96)
	3	1.2 ± 0.12	1.2 ± 0.07 (98)	0.8 ± 0.20 (69)	0.9 ± 0.08 (72)
1,4-Dioxane		D	DT	DP	TPD
	1	30.8 ± 8.25	10.9 ± 4.14 (35)	12.1 ± 1.12 (39)	16.8 ± 5.55 (54)
	2	36.2 ± 4.13	22.2 ± 3.46 (61)	13.9 ± 4.14 (38)	13.0 ± 1.47 (35)
	3	27.9 ± 3.37	27.2 ± 6.74 (97)	28.3 ± 4.44 (101)	19.7 ± 5.45 (70)

[a] The values in parentheses are the percentages of T1, T2, and T3 for cycles 1, 2, and 3, respectively, for TCE, the percentages of P1, P2, and P3 for cycles 1, 2, and 3, respectively, for PCE, and the percentages of D1, D2, and D3 for cycles 1, 2, and 3, respectively, for 1,4-dioxane.

second transformation phase (day 14 time point) with P as the sole contaminant and in the PT, PD, and TPD mixtures (Fig. 1d).

During the third contaminant transformation phase with P as the sole contaminant (P3), P was transformed at an initial rate of 1.2 μM h^{-1} (Fig. 1d and Table 2). By comparison, P was transformed at initial rates of 98%, 69%, and 72%, respectively, of the P transformation rate with P as the sole contaminant (i.e., percentage of the P3 transformation rate; Fig. 1d and Table 2). P was transformed to below detection limits (Table 1) by the end of the third transformation phase (day 19 time point) with PT as contaminant mixture. Due to early termination of the third contaminant transformation phase (day 19 time point), P was transformed to 10, 36, and 33 μM levels in the P, PD, and TPD mixtures, respectively (Fig. 1d).

P was transformed as the sole contaminant in the three successive contaminant transformation phases (days 2 to 7, 9 to 14, and 16 to 19; Fig. 1d and Table 2). P concentrations remained constant at 100 μM in parallel control incubations lacking Fe(III) or *S. oneidensis* cells (abiotic control), but including three identical cycles of successive anaerobic [Fe(II)-generating], aerobic (H_2O_2-generating), and contaminant (P, PT, PD, and TPD) transformation phases. Identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction and chemical (O_2-catalyzed) Fe(II) oxidation were observed in parallel control incubations with contaminants omitted (Fig. 1c), which indicated that rates of microbially catalyzed Fe(III) reduction and O_2-catalyzed Fe(II) oxidation were not affected by the presence of the P, PT, PD, or TPD mixture.

1,4-Dioxane transformation in the presence and absence of TCE and PCE. Nearly identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction and chemical [O_2-catalyzed Fe(II) oxidation] were observed in analogous D, DT, DP, and TPD contaminant transformation experiments (Fig. 1e). D was transformed at an initial rate of 30.8 μM h^{-1} (Fig. 1f and Table 2). By comparison, D was transformed in the DT, DP, and TPD mixtures at initial rates of 35%, 39%, and 54%, respectively, of the D transformation rate with D as the sole contaminant (i.e., percentage of the D1 [the first contaminant transformation phase for D used alone] transformation rate; Fig. 1f and Table 2). D was transformed to below detection limits (Table 1) by the end of the first transformation phase (day 7 time point) in the D and DP mixtures. However, D was transformed to only 0.17 mM and 0.85 mM levels in the DT and TPD mixtures, respectively (Fig. 1f).

During the second contaminant transformation phase with D as the sole contaminant (D2), D was transformed at an initial rate of 36.2 μM h^{-1} (Fig. 1f and Table 2). By comparison, D was transformed at initial rates of 61%, 38%, and 36%, respectively, of the D transformation rate with D as the sole contaminant (i.e., percentage of the D2 transformation rate; Fig. 1f and Table 2). D was transformed to below detection limits (Table 1) by the end of the second transformation phase (day 14 time point) with D as the sole contaminant. However, D was transformed to only 0.34, 0.30, and 0.16 mM levels in the DT, DP, and TDP mixtures, respectively (Fig. 1f).

During the third contaminant transformation phase with D as the sole contaminant (D3), D was transformed at an initial rate of 27.9 μM h^{-1} (Fig. 1f and Table 2). By comparison, D was transformed at initial rates of 97%, 101%, and 70%, respectively, of the D transformation rate with D as the sole contaminant (i.e., percentage of the D3 transformation rate; Fig. 1f and Table 2). Due to early termination of the third transformation phase (day 19 time point), D was transformed to 0.6, 0.43, 0.48, and 0.96 mM levels in the D, DT, DP, and TPD mixtures, respectively (Fig. 1f).

D was transformed as the sole contaminant in the three successive contaminant transformation phases (days 2 to 7, 9 to 14, and 16 to 19; Fig. 1f and Table 2). D concentrations remained constant at 100 μM in parallel control incubations lacking Fe(III) or *S. oneidensis* cells (abiotic control), but including three identical cycles of successive anaerobic [Fe(II)-generating], aerobic (H_2O_2-generating), and contaminant (D, DT, DP, and TPD) transformation phases. Identical patterns of microbial (*S. oneidensis*-catalyzed) Fe(III) reduction and chemical (O_2-catalyzed) Fe(II) oxidation were observed in parallel control incubations with contaminants omitted (Fig. 1e), which indicated that the rates of microbially catalyzed Fe(III) reduction and O_2-catalyzed Fe(II) oxidation were not affected by the presence of the D, DT, DP, or TPD mixture.

The rates of TCE transformation (in single, binary, and ternary mixtures of contaminants) ranged from 84 to 137% between the first, second, and third TCE transformation phases (i.e., by comparing rates of TCE transformation in phases T1, T2, and T3, or TP1, TP2, and TP3 or TD1, TD2, and TD3 or TPD1, TPD2, and TPD3; Table 2). In a similar fashion, the rates of PCE and 1,4-dioxane transformation (in single, binary, and ternary mixtures of contaminants) also ranged from 77 to 168%. However, during the second (DT mixture) and third (DT and DP mixtures) 1,4-dioxane transformation cycles, 1,4-dioxane transformation rates were 104%, 150%, and 133% higher, respectively. The higher 1,4-dioxane transformation rates were most likely due to a measurement error or amendment of slightly higher than required 1,4-dioxane concentrations in the subsequent cycles (Fig. 1b, d, and f and Table 2). Furthermore, lactate was depleted to only approximately 15 to 20 mM levels after three successive cycles (i.e., 30 to 40% of the initial 50 mM lactate feed remained after the third cycle) (see Fig. S3 in the supplemental material).

Inhibition of the microbially driven Fenton reaction in control incubations. To confirm that the single, binary, and ternary mixtures of TPD were transformed by $HO^{\cdot}$ radicals generated by the *S. oneidensis*-driven Fenton reaction, a series of control incubations were carried out in the presence of the $HO^{\cdot}$ radical-scavenging compound mannitol or thiourea. The single, binary, or ternary mixtures of TPD were not transformed in the contaminant transformation phase in the presence of mannitol or thiourea (see Fig. S2 in the supplemental material). To test for toxicity of mannitol and thiourea in the presence of TPD, *S. oneidensis* cultures were grown aerobically in LS growth medium supplemented with mannitol or thiourea and the ternary TPD mixture. Aerobic growth rates in the presence or absence of mannitol or thiourea were nearly identical, which indicates that a combination of mannitol or thiourea and TPD did not inhibit *S. oneidensis* metabolic activity (Fig. S1). The ability of the $HO^{\cdot}$ radical-scavenging compounds mannitol and thiourea to inhibit TPD transformation indicates that $HO^{\cdot}$ radicals derived from the microbially driven Fenton reaction are involved in the transformation of single, binary, and ternary TPD mixtures.

The requirement for microbial Fe(III) reduction was tested by replacing Fe(III) with NO_3^- and carrying out an otherwise identical set of TPD transformation experiments with NO_3^--containing fed batch reactors. TPD was not transformed with NO_3^- as the electron acceptor (see Fig. S2 in the supplemental material) or in the absence of *S. oneidensis* cells (Fig. S2). These results indicated that microbial Fe(III) reduction was required to drive the TPD transformation process. Furthermore, in all TPD transformation and control incubations, lactate was consumed at similar rates (87.5 to 100 $\mu M\ h^{-1}$), while lactate concentrations remained constant during the 17-day abiotic control incubations (Fig. S3).

DISCUSSION

The high oxidation potential of $HO^{\cdot}$ radicals derived from conventional Fenton reactions drives oxidative degradation of a variety of hazardous contaminants, including TCE, PCE, and 1,4-dioxane (23, 26, 32, 52). Conventional Fenton reactions are hampered, however, by the need for continuous supply of the Fenton reagents Fe(II) and H_2O_2 to sustain contaminant degradation (1, 17, 23, 25, 33, 40). Microbially driven Fenton reactions, on the other hand, alternately regenerate Fe(II) [via microbial Fe(III) reduction under anaerobic conditions] and H_2O_2 (via microbial O_2 respiration under aerobic conditions), thus alleviating the need to continually supply the Fenton reagents to drive $HO^{\cdot}$ radical production and contaminant degradation (32, 36). The Fe(III)-reducing facultative anaerobe *S. oneidensis* was recently employed to drive the Fenton reaction for $HO^{\cdot}$ radical production and 1,4-dioxane degradation in a batch reactor system that included *S. oneidensis* cells, Fe(III), and alternate injections of compressed nitrogen [to facilitate microbial Fe(III) reduction] and compressed air (to facilitate microbial H_2O_2 production) (32). The Henry's law constants for TCE (0.11 mol kg^{-1} 100 kPa^{-1}) and PCE (0.05 mol kg^{-1} 100 kPa^{-1}), however, are more than 3 orders of magnitude lower than the Henry's law constant for 1,4-dioxane (220 mol kg^{-1} 100 kPa^{-1}) (32, 47–50). Thus, in initial batch reactor designs, the compressed gas injections quickly volatilized TCE and PCE to below detection levels (data not shown) (Table 1), while the concentrations of TCE (and similarly PCE) remained constant in the absence of compressed gas injection (see Fig. S2 in the supplemental material). The batch reactor system was therefore modified to avoid TCE and PCE loss due to volatility. In the modified fed batch reactor system, the Fe(II)-generating [microbial Fe(III) reduction stimulated by injection of compressed nitrogen], H_2O_2-generating (microbial H_2O_2 production stimulated by injection of compressed air), and contaminant transformation phases (without injection of compressed gases) were separated, and the fed batch reactor system was cycled three times through each of the three separate phases (Fig. S4).

Although TCE and PCE are often commingled with 1,4-dioxane in contaminated soil and groundwater, only a limited number of studies have examined simultaneous degradation of multiple contaminants, with emphasis on binary mixtures of TCE and 1,4-dioxane or TCE and PCE (26, 41–43). The present study is the first report of simultaneous transformation of source zone levels of ternary mixtures of TCE, PCE, and 1,4-dioxane. In control incubations held under strictly anaerobic Fe(III)-reducing conditions, the TPD mixtures were not transformed, which demonstrated that *S. oneidensis* was unable to enzymatically transform TCE, PCE, or 1,4-dioxane in the contaminant transformation phase. In addition, under a normal cycle of alternating anaerobic and aerobic conditions, the three contaminant mixtures were not transformed in the absence of Fe(III), in the absence of *S. oneidensis* cells, or in the presence of the $HO^{\cdot}$ radical-scavenging compound mannitol or thiourea. These findings indicate that the TPD mixtures were simultaneously transformed by $HO^{\cdot}$ radicals produced by the microbially driven Fenton reaction (14).

The rates of TCE, PCE, and 1,4-dioxane transformation ranged from 77 to 168%, indicating that the microbially driven Fenton system can handle at least three repeated feeds of ternary mixtures of TCE, PCE, and 1,4-dioxane without compromising the efficiency of contaminant transformation (Table 2). Lactate was depleted to only approximately 15 to 20 mM levels after three successive cycles, indicating that the microbially driven Fenton reaction was not limited by lactate depletion upon completion of the third cycle (see Fig. S3 in the supplemental material).

The rates of contaminant transformation by $HO^{\cdot}$ radicals are a function of the contaminant-specific $HO^{\cdot}$ radical reaction rate constants ($k_{HO^{\cdot}}$) (TCE range, 0.85×10^9 to 1.59×10^9 $M^{-1}\ s^{-1}$; TCE average, 1.22×10^9 $M^{-1}\ s^{-1}$; PCE range, 1.62×10^9 to 2.33×10^9 $M^{-1}\ s^{-1}$; PCE average, 1.98×10^9 $M^{-1}\ s^{-1}$; 1,4-dioxane range, 1.1×10^9 to 2.4×10^9 $M^{-1}\ s^{-1}$; 1,4-dioxane average, 1.75×10^9 $M^{-1}\ s^{-1}$) (25, 53), the initial concentrations of contaminant, and the number of double bonds in the contaminant molecular structure (26). Since the predicted rates of $HO^{\cdot}$ radical production are high compared to contaminant degradation rates, other unidentified sinks are likely removing excess $HO^{\cdot}$ radicals, thus suggesting that the $HO^{\cdot}$ radical concentration is low under all reactor conditions. These unidentified sinks impacting the Fenton degradation of TCE, PCE, and 1,4-dioxane include the myriad of $HO^{\cdot}$ radical-interacting intermediates produced during contaminant degradation (12, 37).

Despite these complicating factors, the predicted and experimentally derived contaminant transformation rates in the present study may be compared for the case of binary mixtures of TCE and PCE, because both contaminants were added at identical concentrations and both contain an identical number of double bonds. Subsequently, the ratio of the experimentally derived rates of PCE and TCE transformation in binary mixtures of PCE and TCE (designated P^{exp}/T^{exp}) is predicted to be equal to the ratio of PCE- and TCE-specific $k_{HO^{\cdot}}$ [designated $k_{HO^{\cdot}}(P)/k_{HO^{\cdot}}(T)$]. P^{exp}/T^{exp} ratios ranged from 1.8 to 2.1 in the three transformation phases of the PCE and TCE binary mixture experiments (Table 2), which were comparable to the $k_{HO^{\cdot}}(P)/k_{HO^{\cdot}}(T)$ ratios (1.5 to 1.9) previously reported for TCE and PCE transformation by purely chemical Fenton reactions (Table 2) (25). Similar rate comparisons with binary mixtures of 1,4-dioxane and TCE or 1,4-dioxane and PCE were not possible because the source zone levels of 1,4-dioxane (2 mM) used in the present study were 20-fold greater than the source zone levels of TCE and PCE (100 μM) (54, 55). Correspondingly, the 20-fold-higher 1,4-dioxane concentrations led to 1,4-dioxane transformation rates that were 9-to-34-fold higher than the TCE and PCE transformation rates in all contaminant mixtures (Table 2). Regardless of the concentration-dependent differences in contaminant transformation rates, the microbially driven Fenton reaction transformed source zone levels of ternary mixtures of TCE, PCE, and 1,4-dioxane to below detection limits (Table 1) when contaminant transformation phases were extended to <5-day time periods (first and second cycles; Fig. 1b, d, and f).

The rates of transformation of TCE as the sole contaminant (in the first, second, and third TCE transformation phases) were 18 to 54% lower in binary and ternary mixtures of PCE and 1,4-dioxane (i.e., by comparing rates of TCE transformation in phases T1, TP1, TD1, and TPD1, phases T2, TP2, TD2, and TPD2, and phases T3, TP3, TD3, and TPD3; Table 2). Decreases in TCE transformation rates in the binary and ternary contaminant mixtures are most likely due to competition for $HO^{\cdot}$ radicals by PCE (with a $k_{HO^{\cdot}}$ 62% higher than that of TCE) or 1,4-dioxane (with a $k_{HO^{\cdot}}$ approximately 43% higher than that of TCE and amended at a 20-fold-higher concentration than that of TCE). The rates of transformation of PCE as the sole contaminant (in the first, second, and third PCE transformation phases) were not affected by TCE in binary mixtures of TCE and PCE, but were 4 to 38% lower in binary and ternary mixtures containing 1,4-dioxane (i.e., by comparing rates of PCE transformation in phases P1, TP1, PD1, and TPD1, phases P2, TP2, PD2, and TPD2, and phases P3, TP3, PD3, and TPD3; Table 2). The inability of TCE to affect PCE transformation rates is most likely due to the 62% higher $k_{HO^{\cdot}}$ for PCE than TCE. Decreases in PCE transformation rates in the binary and ternary contaminant mixtures containing 1,4-dioxane are most likely due to competition for $HO^{\cdot}$ radicals by 1,4-dioxane, which was amended at 20-fold-higher concentrations than those of PCE. The rates of transformation of 1,4-dioxane as the sole contaminant (in the first, second, and third TCE transformation phases) were 39 to 65% lower in binary and ternary mixtures of TCE and PCE (i.e., by comparing rates of 1,4-dioxane transformation in phases D1, TD1, PD1, and TPD1, phases D2, TD2, PD2, and TPD2, and phases T3, TD3, PD3, and TPD3; Table 2). Decreases in 1,4-dioxane transformation rates in the binary and ternary contaminant mixtures are most likely due to competition for $HO^{\cdot}$ radicals by PCE (with a $k_{HO^{\cdot}}$ 13% higher than that of 1,4-dioxane; Table 2). During the third 1,4-dioxane transformation cycle, however, 1,4-dioxane transformation rates rebounded to 70 to 101% of the transformation rates with 1,4-dioxane as the sole contaminant, most likely due to decreases in PCE concentrations to levels below the threshold required for PCE to compete with 1,4-dioxane for $HO^{\cdot}$ radicals during the third contaminant degradation cycle (Table 2).

Similar to previous results reported with purely chemical Fenton reactions, the results of the present study indicate that contaminant transformation rates in the microbially driven Fenton reaction depend on the $k_{HO^{\cdot}}$ of competing contaminants, the initial contaminant concentration, and the number of double bonds in the contaminant molecular structure. In binary and ternary mixtures, contaminants with greater $k_{HO^{\cdot}}$ values suppress the transformation of other contaminants with lower $k_{HO^{\cdot}}$ values, and the contaminant transformation phase must be extended for longer time periods to transform contaminants below detection limits (26). The microbially driven Fenton reaction may thus be applied as an effective *ex situ* platform for simultaneous degradation of at least three (and potentially more) commingled contaminants at source zone levels.

Microbial respiratory processes catalyze both H_2O_2 production and Fe(III) rereduction in the microbially driven Fenton reaction designed in the present study. Microbial Fe(III) reduction is a dominant anaerobic respiratory process in many contaminated subsurface environments (56), which suggests that the microbially driven Fenton reaction may be stimulated *in situ* by exposing Fe(III)-reducing facultative anaerobes in Fe(III)-containing contaminated environments to alternating anaerobic and aerobic conditions. Other *in situ* remediation strategies may include stimulation of the microbially driven Fenton reaction by placing reactive iron barriers in the flow path of contaminated subsurface aquifers and exposing Fe(III)-reducing bacteria attached to the iron barriers to alternating anaerobic and aerobic conditions (57). Additional targets for *ex situ* and *in situ* degradation by the microbially driven Fenton reaction developed in the present study include multiple combinations of environmental contaminants susceptible to attack by Fenton reaction-generated $HO^{\cdot}$ radicals, including commingled plumes of 1,4-dioxane, PCP (35, 36), PCE (24), TCE (23), TCA (37), and PFAS (58).

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R.S. performed all experiments, developed part of the protocol, and cowrote the manuscript. M.T. coanalyzed all data. T.J.D. developed the concept and part of the protocol, coanalyzed all data, and cowrote the manuscript.

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