Reductive Dehalogenation and Mineralization of 3-Chlorobenzoate in the Presence of Sulfate by Microorganisms from a Methanogenic Aquifer

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We investigated the anaerobic biodegradation of 3-chlorobenzoate (3CBz) by microorganisms from an aquifer where chloroaromatic compounds were previously found to resist decay in the presence of sulfate. After a lengthy lag period, 3CBz was degraded in the presence of sulfate and concurrently with sulfate reduction. Chlorine removal from 2,5- or 3,5-dichlorobenzoates and the transient appearance of benzoate from 3CBz confirmed that reductive dehalogenation was the initial fate process for these substrates. Sulfate did not influence 3CBz degradation rates in acclimated enrichment cultures but accelerated the development of 3CBz degradation activity in fresh transfers. Benzoate degradation was more rapid in the presence of sulfate regardless of the enrichment history. Nitrate, sulfite, and a headspace of air inhibited 3CBz dehalogenation, while thiosulfate had no effect. Mass balance determinations revealed that 71 to 107% of the theoretically expected amount of methane was produced from 3CBz and benzoate oxidation in the absence of sulfate. In parallel cultures containing 15 mM sulfate, methanogenesis was reduced to 48 to 71% of that theoretically expected, while sulfate reduction accounted for 12 to 50% of the reducing equivalents. In either the presence or absence of sulfate, steady-state dissolved hydrogen concentrations were similar to those reported for sulfate-reducing or methanogenic environments, respectively. Molybdate inhibited sulfate reduction and 3CBz dehalogenation to a similar extent but did not affect benzoate biodegradation. Sulfate-dependent 3CBz biodegradation was not observed. We conclude that reductive dehalogenation and sulfate reduction occur concurrently in these enrichments and that the sulfate-dependent stimulation in fresh transfers was likely due to the acceleration of benzoate oxidation.

Since the recognition of biologically catalyzed reductive dehalogenation reactions over a decade ago, the anaerobic metabolism of chlorinated aromatic compounds has been the subject of many investigations. During this period, chloroaromatic compounds, including many chemicals that are known environmental contaminants and public health concerns, have been found to be widely susceptible to reductive dehalogenation. Furthermore, aryl reductive dehalogenation reactions have been demonstrated in samples from ecologically and geographically diverse environments. Reductive dehalogenation is now widely held to be (i) the predominant fate process for chloroaromatic compound biodegradation in anaerobic environments and (ii) the rate-limiting step for chloroaromatic compound mineralization under anaerobic conditions (for reviews, see references 20, 27, and 39).

We have studied the biodegradation of a variety of halogenated aromatic compounds in sediments obtained from an anoxic aquifer polluted by leachate from a municipal landfill (4). We found that the reductive dehalogenation of chlorophenols, chlorobenzoates, chlorophenoxyacetates, chloroanilines, and several halogenated heterocyclic compounds is severely inhibited or, more commonly, is not detected in samples held

under sulfate-reducing conditions (1, 14, 21, 33, 38). The inhibitory effect of sulfate and other sulfur oxyanions on aryl reductive dehalogenation reactions has also been observed by other investigators. Chlorophenol dehalogenation was inhibited by sulfate in consortia enriched from municipal digester sludge (24), anaerobic sludge granules (26), and pond sediments (16, 19), as was chlorocatechol dehalogenation in enrichments from sediment samples containing high sulfate levels (3). Microorganisms from two freshwater sediments and one estuarine sediment reductively dehalogenated polychlorinated biphenyls (PCBs) under methanogenic conditions, but no dehalogenation activity was detected under sulfate-reducing conditions (2); in a separate study, sulfate greatly inhibited PCB dehalogenation activity (29). Recently, PCB dehalogenation has been demonstrated in estuarine sediments, but this activity occurred more rapidly and reliably when sulfate was excluded from the incubation mixture (5). Chlorinated benzenes were dehalogenated in soil slurries under methanogenic conditions but not in the presence of sulfate; removal of the sulfur oxyanion by stimulating its biological removal resulted in increased dehalogenation (32). In a comparison of the dehalogenation potential of three freshwater and two estuarine sediments, primary enrichments from all sites in which sulfate was periodically replenished failed to degrade monochlorinated benzoates and phenols (12).

More recently, researchers have had success in demonstrating chloroaromatic biodegradation under sulfate-reducing conditions. Chlorophenol mineralization in estuarine sediments was found to occur in the absence of methanogenesis and was dependent on the presence of sulfate (17); dichlorophenols were transformed to monochlorophenols, confirming

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reductive dehalogenation as the initial fate process. In river sediments, monochlorophenols and 3-chlorobenzoate (3CBz) disappeared from nonsterile incubations containing sulfate, but no metabolites were observed (15). Similarly, both 3- and 4CBz were degraded by microorganisms from river sediments with the consumption of sulfate to near stoichiometrically expected amounts, but it is unclear whether reductive dehalogenation was specifically involved in this transformation (18). PCB dechlorination occurred in both methanogenic and sulfate-amended enrichments of marine sediments at similar rates (31). Such disparate observations prompted us to reexamine the influence of sulfate on the enrichment of microorganisms capable of chloroaromatic metabolism.

MATERIALS AND METHODS

Sample collection and aquifer slurry construction. Aquifer sediments and groundwater were obtained from a methanogenic sampling site located adjacent to the Norman Municipal Landfill as previously described (4). Although the site is normally methanogenic, sulfate periodically impacts this site, and at the time of sampling, the groundwater sulfate concentration was 6.4 mM. Aquifer slurries were constructed in sealed serum bottles, also, as previously described (4). Resazurin (0.0002%) was added as a redox indicator, and Na_2S (1 mM) was used as a reductant to ensure anoxic conditions.

Enrichment and biodegradation experiments. All amendments of halogenated substrates, electron donors, electron acceptors, and inhibitors were made from anoxic, sterile stock solutions made with distilled water. All incubations were carried out in the dark, without shaking at room temperature. Triplicate aquifer slurries were amended with 3CBz to an initial concentration of 300 μM . Triplicate autoclaved (90 min, 121°C) incubation mixtures served as controls. Samples were periodically withdrawn from aquifer slurries by syringe and analyzed by high-pressure liquid chromatography for substrate disappearance. Following complete disappearance of 3CBz, aquifer slurries were routinely reamended with the halogenated substrate (300 to 500 μM). Sediment-free enrichments capable of 3CBz biodegradation were obtained by repeated periodic transfer (10 to 20%) of the culture into fresh medium (36) amended with 3CBz (500 μM). Further biodegradation experiments were conducted by the inoculation of fresh media with sediment-free enrichment cultures as described for each experiment in Results. When necessary, sulfate was removed from the cultures by centrifugation of the cells ($20,000 \times g$ for 20 min) and subsequent resuspension in sulfate-free anaerobic media (36). All manipulations were performed in an anaerobic glove box. In order to determine the fate of aromatic substrates, 3CBz and benzoate losses as well as the production of methane and the reduction of sulfate were measured and compared with the following expected stoichiometries:

Data were corrected by the subtraction of endogenous methane production and sulfate reduction by parallel enrichments that were not amended with 3CBz or benzoate.

Analytical methods. Liquid subsamples were taken by syringe from aquifer slurries or enrichments and either analyzed immediately or stored at -10°C until analyzed. Subsamples were assayed for the aromatic substrate concentration by reverse-phase high pressure liquid chromatography as previously described (14). Methane and dissolved hydrogen concentrations were determined by analysis of the culture headspace by gas chromatography (28). Sulfate and acetate were quantitated with an ion chromatography system (model DX 500 equipped with a CD20 conductivity detector and an Ionpac AS4A column; Dionex Corp., Sunnyvale, Calif.). The optical density of each culture was determined by measuring the absorbance at 600 nm.

RESULTS

3CBz biodegradation in enrichments. No evidence for 3CBz biodegradation was observed for 12 weeks in aquifer slurries that initially contained 6 mM sulfate and had been amended with 300 μM 3CBz. However, after 17 weeks of incubation, 3CBz was no longer detectable in nonsterile slurries, while autoclaved controls showed no evidence of abiotic loss (Fig. 1). Sulfate was not closely monitored in these initial experiments, but when nonsterile incubation mixtures were reamended with

FIG. 1. Disappearance of 3CBz in initial active aquifer slurries (■) compared to autoclaved control aquifer slurries (□). Data points and error bars are the means and standard deviations, respectively, of measurements from triplicate aquifer slurries.

both 3CBz (500 μM) and sulfate (6 mM), both substrates were consumed without a lag period (data not shown).

Following the depletion of three such amendments of 3CBz, a primary enrichment culture was washed free of sulfate and transferred to media containing 3CBz (400 μM) with and without sulfate (5 mM). 3CBz was degraded in both the presence and absence of sulfate in these secondary enrichments (data not shown). The latter cultures were maintained under sulfate-reducing and methanogenic conditions for 12 weeks, and the effect of sulfate on 3CBz biodegradation was evaluated by still another transfer into sulfate-containing and sulfate-free media. As shown by the results in Fig. 2, 3CBz was degraded in both the presence and absence of sulfate by both secondary enrichments, but the effect of sulfate depended on the enrichment history. Sulfate resulted in reduced rates of 3CBz decay in enrichments maintained under sulfate-free conditions (Fig. 2A), while the anion stimulated 3CBz metabolism in cultures enriched in the presence of sulfate (Fig. 2B).

Dehalogenation of dichlorobenzoate isomers. The depletion of 3CBz in aquifer slurries and initial enrichments occurred without the accumulation of benzoate, the expected product of reductive dehalogenation. In order to implicate reductive dehalogenation as the predominant fate process for 3CBz decay by these enrichments, 3,5-dichlorobenzoate was provided as a parent substrate for a sulfate-amended secondary enrichment. The substrate was reductively dehalogenated to near stoichiometric amounts of 3CBz (Fig. 3), thus demonstrating *meta*-chlorine release in the presence of sulfate. Comparable enrichments amended with 2,5-dichlorobenzoate accumulated 2CBz (data not shown). In addition, small amounts of benzoate (up to 100 μM) were observed sporadically in 3CBz-amended enrichments in either the presence or absence of sulfate (data not shown).

Effect of alternative electron acceptors. We examined the effect of sulfate, sulfite, thiosulfate, nitrate, and oxygen on 3CBz disappearance in enrichments. Media containing various electron acceptors or a headspace of air received a 25% inoculum from a 3CBz-degrading incubation mixture which was enriched in the absence of sulfate. Unamended, sulfate-amended, and thiosulfate-amended enrichments quickly degraded 3CBz at identical rates (Fig. 4). Biodegradation of 3CBz was immediately and completely inhibited by sulfite, while inhibition due to nitrate or an air headspace was ob-

FIG. 2. Effect of sulfate on 3CBz disappearance by enrichments previously maintained without sulfate (A) or under sulfate-reducing conditions (B). Data points are single determinations from sterile (□), sulfate-amended (■), and sulfate-free (●) cultures.

served after a lag. The similar rates of 3CBz biodegradation observed in the presence and absence of sulfate were typical for acclimated enrichments.

Effect of electron donors and sulfate on the establishment of 3CBz biodegradation activity. In fresh transfers, the addition of sulfate consistently stimulated 3CBz biodegradation, while the same effect was not apparent in acclimated cultures. Such findings suggest that the stimulatory effect of sulfate may be a growth-related phenomenon. We investigated the effect of supplemental electron donors and sulfate on the establishment of

FIG. 3. Reductive dehalogenation of 3,5-dichlorobenzoate (■) to 3CBz (▲) in the presence of 20 mM sulfate. Data points are single determinations. In a sterile control, 3,5-dichlorobenzoate (□) was not transformed.

FIG. 4. Disappearance of 3CBz in the presence of sulfite (●), sulfate (○), thiosulfate (Δ), nitrate (▲), and a headspace of air (+) compared to the unamended (■) and autoclaved (□) controls. Electron acceptors were present at 10 mM concentrations; initial 3CBz concentrations were 400 to 600 μ M. Data points are means of duplicate incubations.

3CBz biodegradation activity in a fresh basal medium inoculated with a minimal transfer (3% [vol/vol]) from an enrichment culture acclimated to the degradation of 3CBz in the absence of sulfate. In enrichments amended with lactate, pyruvate, or hydrogen as electron donors, we observed complete consumption of the supplemental electron donor, a substantial increase in biomass, and complete reduction of sulfate when present (Table 1). In enrichment cultures with either acetate, which was not readily metabolized, or no supplemental electron donor, only a modest increase in biomass was observed and relatively little sulfate, when present, was consumed. 3CBz biodegradation was markedly stimulated in cultures which contained sulfate only in the absence of a readily metabolized electron donor (Table 1). These data demonstrate that even though sulfate does stimulate the development of 3CBz biodegradation activity, the nonspecific stimulation of growth by the inclusion of an electron donor is counterselective. In parallel transfers, benzoate was degraded more than three times faster in the presence of sulfate than in its absence (data not shown).

Fate of benzoate and 3CBz in the presence and absence of sulfate. An enrichment culture acclimated to the biodegradation of 3CBz in the presence of sulfate was studied further to assess the effect of sulfate on the rate of both 3CBz and benzoate biodegradation and the metabolic fate of these substrates. Triplicate aliquots of the enrichment culture were provided with 3CBz (500 μ M) and benzoate (3,500 μ M) as substrates separately and in combination in both the presence and the absence of sulfate (15 mM). 3CBz was rapidly depleted at identical rates in both the presence and absence of sulfate, a common observation for well-acclimated cultures suggesting that sulfate does not influence dehalogenation per se. However, in the absence of sulfate, benzoate biodegradation was greatly retarded (Fig. 5). Following the complete biodegradation of benzoate and 3CBz, methane formation and sulfate loss were measured and compared to the expected stoichiometries for the complete oxidation of substrates to CO_2 . As summarized in Table 2, 71 to 107% of the predicted methane was recovered in enrichment cultures without sulfate. In enrichment cultures amended with sulfate, methane production was

TABLE 1. Effect of sulfate (2 mM) and supplemental substrates (2 mM) on the propagation of a 3CBz-degrading community^a

Supplemental substrate	Result for incubation						
	Without sulfate			With sulfate			
	3CBz consumed (μ M)	Supplemental substrate consumed	Growth ^b	3CBz consumed (μ M)	Sulfate consumed (mM)	Supplemental substrate consumed	Growth ^b
None	74		+	524	0.2		+
Acetate	62	No	+	512	0.4	No	+
Lactate	0	Yes	+++	24	2.0	Yes	+++
Pyruvate	130	Yes	+++	43	2.0	Yes	+++
H ₂ /CO ₂ ^c	83	Yes	+++	166	2.0	Yes	+++

^a A 3% (vol/vol) inoculum was transferred into fresh basal mineral medium containing 3CBz (500 μ M) and incubated for 110 days. The initial sulfate concentration was 2 mM when present.

^b +, A_{600} of <0.10; +++, A_{600} of >0.20.

^c H₂/CO₂ ratio of 4:1 (70 kPa).

reduced, representing approximately 48 to 61% of the theoretically expected amount of methane. Sulfate reduction was also stimulated above that for the no-substrate control and could account for 12 to 50% of the predicted reducing equivalents generated from benzoate oxidation. We also monitored hydrogen concentrations throughout the course of these enrichment cultures. In substrate-unamended controls, dissolved hydrogen concentrations in either the presence or absence of sulfate were reduced to levels typically reported for sulfate-reducing (<3 nM) and methanogenic (5 to 10 nM) environments, respectively. In substrate-amended enrichment cultures, hydrogen concentrations were elevated during the consumption of aromatic compounds with one exception; in incubations containing 3CBz and sulfate, hydrogen concentrations were maintained at low levels throughout the experiment. Hydrogen concentrations in substrate-amended incubations with or without sulfate eventually reached steady-state levels typical for either sulfate-reducing or methanogenic environments, respectively.

Effect of molybdate on dehalogenation and benzoate biodegradation in the presence of sulfate. Molybdate is a well-known inhibitor of sulfate reduction, so we examined its effect on benzoate and 3CBz biodegradation as well as sulfate reduction and methanogenesis. Triplicate aliquots of a 3CBz-degrading enrichment were amended with 500 μ M benzoate, 500 μ M 3CBz, and molybdate at concentrations varying from 0 to 2 mM (Table 3). Benzoate was rapidly degraded in the presence of all concentrations of molybdate at identical rates. Although molybdate did not influence benzoate disappearance, it did influence the fate of reducing equivalents derived from its oxidation; sulfate reduction was depressed and methanogenesis was stimulated in the presence of molybdate. We also examined acetate production and found that acetate accumulated to near the predicted 3 mol per benzoate metabolized. In contrast to benzoate decay, 3CBz biodegradation was greatly inhibited by 250 μ M molybdate, with complete inhibition at a concentration of 1 mM or greater. Interestingly, sulfate reduction exhibited sensitivity to molybdate similar to that of 3CBz degradation.

DISCUSSION

In previous studies, we have consistently observed the persistence of a wide variety of chloroaromatic compounds in aquifer sediments held under sulfate-reducing conditions (1, 14, 21, 33, 38). Other researchers have reported similar findings using different haloorganic chemicals and sampling locations, confirming the somewhat generalizing nature of this observation (2, 3, 12, 16, 24, 26, 32). In this study, we were able

to demonstrate 3CBz biodegradation by the microbiota of these sediments in the presence of high concentrations of sulfate and concurrently with sulfate reduction. In situ sulfate (>6 mM) at the time of sampling and the periodic reamendment of sulfate to aquifer slurries likely exerted a selection pressure that resulted in the enrichment of a population of 3CBz-degrading microorganisms that is insensitive to the effects of sulfate. The *meta* dehalogenation of dichlorobenzoates, the transient and sporadic accumulation of trace amounts of benzoate in 3CBz-amended enrichment cultures, and the rapid biodegradation of benzoate confirm that 3CBz is degraded by

FIG. 5. 3CBz (A) and benzoate (B) degradation in the presence (■) and absence (●) of 15 mM sulfate and in sterile controls (□). Data points are means of triplicate incubations, with standard deviations (error bars) smaller than the symbols unless shown.

TABLE 2. The mineralization of benzoate (500 μ M) and 3CBz (500 μ M) in the presence and absence of sulfate (1 mM)^a

Substrate	Presence of sulfate	Substrate consumed (μ mol) ^a	Predicted methane formation or sulfate reduction (μ mol)	Methane production		Sulfate reduction	
				Measured (μ mol) ^a	% of expected	Measured (μ mol) ^a	% of expected
3CBz	+	5.0 $\pm$ 0.3	17.5	10.7 $\pm$ 0.4	61	8.7 $\pm$ 4.8	50
	–	5.7 $\pm$ 0.1	20.0	21.3 $\pm$ 2.4	107		
Benzoate	+	37.1 $\pm$ 0.7	139.1	67.2 $\pm$ 2.4	48	16.6 $\pm$ 2.1	12
	–	35.2 $\pm$ 0.3	132.0	105.1 $\pm$ 2.4	80		
Benzoate + 3CBz	+	33.8 $\pm$ 0.2	125.7	73.8 $\pm$ 3.4	59	27.4 $\pm$ 3.9	22
	–	40.7 $\pm$ 0.3	151.2	107.8 $\pm$ 9.8	71		

^a Measurements are means and standard deviations for triplicate cultures.

the cultures via an initial reductive dehalogenation reaction and the subsequent rapid oxidation of benzoate. When actively dehalogenating incubation mixtures were reamended with 3CBz and sulfate, both compounds were consumed simultaneously, demonstrating the coexistence of sulfate reduction and reductive dehalogenation in these aquifer slurries.

Throughout our study, the effect of sulfate on 3CBz biodegradation varied according to the particular incubation mixture's enrichment history. In sulfate-free enrichment cultures that rapidly degraded 3CBz, sulfate amendments had neither a stimulatory nor an inhibitory effect on 3CBz biodegradation rates. In sulfate-containing enrichment cultures which were acclimated to the biodegradation of 3CBz in the presence of sulfate, a modest inhibition of 3CBz biodegradation was observed when sulfate was removed. Marked stimulation of 3CBz biodegradation attributable to sulfate amendments was consistently observed in initial transfers into fresh media, and the stimulation of the development of biodegradation activities was most notably observed in enrichment cultures which received a relatively small inoculum. Sulfate had similar effects on benzoate biodegradation rates. We also observed that the presence of sulfate enabled the microbiota to maintain very low dissolved hydrogen concentrations regardless of the substrate. From these findings, we propose that stimulation of 3CBz biodegradation by sulfate in our enrichments is due to the acceleration of benzoate catabolism and/or growth by this consortium and that sulfate does not influence dehalogenation per se.

Benzoate may be metabolized in these enrichments by at least two conceivable processes: (i) direct oxidization to carbon dioxide by sulfate-reducing bacteria or (ii) incomplete oxidization to acetate by syntrophic microorganisms that couple their metabolism to hydrogen-consuming methanogenic or sulfate-reducing bacteria. Due to the accumulation of 3 mol of acetate per mole of benzoate, the small amount of sulfate reduced, and

the insensitivity of benzoate biodegradation to molybdate, we suggest that benzoate oxidation in our cultures is catalyzed by syntrophic benzoate-degrading microorganisms. The syntrophic biodegradation of benzoate is thermodynamically more favorable when coupled with sulfate reduction rather than with methanogenesis, and this energetic benefit may result in faster growth (30). Thus, the syntrophic biodegradation of benzoate is consistent with the quantitatively minor role that sulfate reduction plays in the overall oxidation of 3CBz and the stimulatory effect that sulfate provides in the development of enrichments.

It has been speculated that sulfate-dependent inhibition of reductive dehalogenation is due to competition for reducing equivalents, either among organisms or among enzyme systems within a single organism (8, 24). Hydrogen has been shown to serve as an electron donor for dehalogenation reactions catalyzed by a variety of enrichments and isolates (10, 24, 29, 37), and there is little doubt that these dehalogenation reactions must compete with other hydrogen-consuming processes that occur in anaerobic habitats such as methanogenesis, acetogenesis, and sulfate reduction. It has been suggested that in the presence of sulfate, hydrogen is consumed by sulfate-reducing bacteria to a concentration at which dehalogenating microorganisms cannot effectively compete. However, the ability of microorganisms to compete for hydrogen depends upon the redox potential of the terminal electron-accepting process (7). Thermodynamic calculations suggest that hydrogen-dependent dehalogenation reactions are energetically more favorable than either sulfate reduction or methanogenesis (11). In consistent fashion, the affinity constant for hydrogen metabolism by a methanogenic tetrachloroethylene-dehalogenating enrichment culture was about 10 times lower for dehalogenation than for methanogenesis (37). We found that dissolved hydrogen concentrations in sulfate-containing enrichments were lower than those in parallel enrichments without sulfate, and these

TABLE 3. Effect of molybdate on the degradation of benzoate and 3CBz^a

Molybdate (μ M)	Mean (μ mol) $\pm$ SD				
	Benzoate degraded	3CBz degraded	Acetate produced	Methane produced	Sulfate reduced
0	4.73 $\pm$ 0.07	2.32 $\pm$ 0.29	21.28 $\pm$ 0.99	1.81 $\pm$ 0.18	2.70 $\pm$ 0.03
50	4.82 $\pm$ 0.11	1.82 $\pm$ 0.05	19.93 $\pm$ 0.78	2.68 $\pm$ 0.13	0.83 $\pm$ 0.12
250	4.93 $\pm$ 0.19	0.21 $\pm$ 0.09	18.03 $\pm$ 0.23	2.80 $\pm$ 0.21	0
1,000	4.92 $\pm$ 0.15	0	16.25 $\pm$ 0.81	2.72 $\pm$ 0.32	0
2,000	4.91 $\pm$ 0.09	0	16.02 $\pm$ 1.06	2.40 $\pm$ 0.14	0
Control ^b			0.17 $\pm$ 0.03	0.14 $\pm$ 0.01	0

^a Measurements were made for triplicate cultures of a 3CBz-degrading enrichment amended with 5 μ mol of benzoate, 5 μ mol of 3CBz, and molybdate at the concentrations indicated following 28 days of incubation.^b Background control incubation without molybdate or substrate.

concentrations were similar to those reported for sulfate-reducing and methanogenic environments, respectively (23). The presence of 3CBz did not influence the steady-state dissolved hydrogen concentration, so it appears that sulfate and bicarbonate, which are present at far greater concentrations, determined the dissolved hydrogen concentrations in these enrichments. Clearly, the dehalogenating population for which we have enriched effectively competes for hydrogen with the indigenous sulfate-reducing populations or utilizes an alternate electron donor.

Desulfomonile tiedjei, a well-studied aryl-dehalogenating bacterium, is capable of reductively dehalogenating 3CBz and performing dissimilatory sulfur oxyanion reduction. However, the latter process interferes with dehalogenation in this organism (9, 36). A number of recently isolated aryl-dehalogenating microorganisms also catalyze the dissimilatory reduction of sulfite and thiosulfate (6, 13, 34, 40), but the effect of sulfur oxyanions on dehalogenation reactions in these microorganisms has not been investigated. Sulfate-reducing bacteria which use electron acceptors other than sulfur oxyanions, such as nitrate, may reduce multiple electron acceptors either preferentially (35, 40) or concomitantly (25). Microorganisms often regulate the expression of their respiratory activities, including dehalogenation, according to the presence of electron acceptors (10, 22, 40), and the often observed inhibition of dehalogenation by sulfate may be due to regulation of respiratory activities by sulfate-reducing bacteria with diverse modes of respiration. Sulfate reduction and dehalogenation by these enrichments demonstrated similar sensitivities to molybdate, an inhibitor of sulfate-reducing bacteria. Although caution must be exercised in interpreting metabolic inhibitor data, these results are consistent with the dehalogenation reaction in these enrichments being performed by a sulfate-reducing bacterium. If so, the organism either performs both reductive processes simultaneously or favors reductive dehalogenation over sulfate reduction as a terminal electron-accepting process.

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