Influence of Sulfur Oxyanions on Reductive Dehalogenation Activities in Desulfomonile tiedjei

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Received 26 December 1996/Accepted 10 June 1997

The inhibition of aryl reductive dehalogenation reactions by sulfur oxyanions has been demonstrated in environmental samples, dehalogenating enrichments, and the sulfate-reducing bacterium Desulfomonile tiedjei; however, this phenomenon is not well understood. We examined the effects of sulfate, sulfite, and thiosulfate on reductive dehalogenation in the model microorganism D. tiedjei and found separate mechanisms of inhibition due to these oxyanions under growth versus nongrowth conditions. Dehalogenation activity was greatly reduced in extracts of cells grown in the presence of both 3-chlorobenzoate, the substrate or inducer for the aryl dehalogenation activity, and either sulfate, sulfite, or thiosulfate, indicating that sulfur oxyanions repress the requisite enzymes. In extracts of fully induced cells, thiosulfate and sulfite, but not sulfate, were potent inhibitors of aryl dehalogenation activity even in membrane fractions lacking the cytoplasmically located sulfur oxyanion reductase. These results suggest that under growth conditions, sulfur oxyanions serve as preferred electron acceptors and negatively influence dehalogenation activity in D. tiedjei by regulating the amount of active aryl dehalogenase in cells. Additionally, in vitro inhibition by sulfur oxyanions is due to the interaction of the reactive species with enzymes involved in dehalogenation and need not involve competition between two respiratory processes for reducing equivalents. Sulfur oxyanions also inhibited tetrachloroethylene dehalogenation by the same mechanisms, further indicating that chloroethylenes are fortuitously dehalogenated by the aryl dehalogenase. The commonly observed inhibition of reductive dehalogenation reactions under sulfate-reducing conditions may be due to similar regulation mechanisms in other dehalogenating microorganisms that contain multiple respiratory activities.

Reductive dehalogenation is the predominant bioconversion responsible for the metabolism of haloorganic compounds under anaerobic conditions and the only known fate process for many highly halogenated pollutants. Although reductive dehalogenation of a wide variety of aromatic and aliphatic compounds has been demonstrated, isolation of the anaerobic microorganisms which catalyze this reaction has been difficult. Research has been restricted largely to enrichments and undefined microbial consortia, and consequently our understanding of the biochemistry and physiology of reductive dehalogenation is constrained. This situation is likely to change in the near future due to the recent isolation of a number of microorganisms capable of catalyzing reductive dehalogenation reactions (2, 3, 10, 15, 18, 36, 37, 45).

The biochemistry of reductive dehalogenation in Desulfomonile tiedjei, a sulfate-reducing bacterium which dehalogenates 3-chlorobenzoate (3CBz) to benzoate (7, 39) and metabolically dehalogenates tetra- and trichloroethylene (44), has been extensively studied. The dehalogenation reactions are catalyzed by a membrane-bound reductive dehalogenase (8, 34) which is believed to function as the terminal reductase for anaerobic halobenzoate respiration. Dehalogenation of 3CBz by resting cells results in the acidification of external media and an increase in intracellular levels of ATP, indicating that dehalogenation in this organism is coupled to energy conservation via a chemiosmotic mechanism (30). Components of the electron transport chain involved in reductive dehalogenation have not been identified; in vitro activity is dependent on reduced methyl viologen, which can donate electrons directly to the dehalogenase (8, 34). The dehalogenase is a regulated enzyme, requiring 3CBz or a suitable analog for induction (5, 8).

The biochemical basis or energetic benefit of reductive dehalogenation in other isolates capable of catalyzing these reactions has been investigated to various extents. Growth on nonfermentable substrates, such as acetate, hydrogen, or formate, in the presence of chloroaromatic electron acceptors suggests that aryl reductive dehalogenation serves as a form of anaerobic respiration in many of these microorganisms as well (3, 10, 36). The reductive dehalogenation of chloroaromatic compounds has been studied in stock cultures which have had no known previous exposure to halogenated substrates and catalyze the reactions fortuitously with little or no energetic gain. More recently, a number of chloroethylene-dehalogenating microorganisms that have a requirement for chloroaromatic substrates to support their growth on nonfermentable electron donors have been isolated from sites impacted by chlorinated solvents (10, 15, 18, 37). Such findings suggest that alkyl dehalogenation can also serve as a form of anaerobic respiration. Biochemical studies with Dehalospirillum multivorum and “Dehalobacter restrictus” confirm the respiratory nature of alkyl dehalogenation in these microorganisms (32, 38). A dehalogenase from the former organism has been purified and characterized, and the kinetic constants and substrate specificity indicate that this enzyme is designed specifically for the dehalogenation of tetrachloroethylene (33). Unlike microorganisms that dehalogenate cometabolically, chloroorganic-respiring microorganisms dehalogenate substrates at high rates and with an energetic benefit. It is therefore likely that these microorganisms are more ecologically significant in contaminated environments and potentially more
useful as biotechnological agents for the destruction of chlorinated compounds.

Multiple respiratory activities often coexist within individual microorganisms and within microbial communities. In the presence of multiple electron acceptors, one respiratory activity, often the most energetically favorable one, may be the dominant terminal electron-accepting process, resulting in the preferential use of the respective electron acceptor. Reductive dehalogenation activities that function as respiratory processes may likewise be influenced by the presence of alternate electron acceptors. It is well documented that the reductive dehalogenation of aromatic substrates in environmental samples is inhibited by alternate electron acceptors, especially sulfur oxyanions; inhibition of aryl dehalogenation due to sulfate-reducing conditions has been observed for a wide variety of chloroaromatic compounds and at a variety of geographic and ecological locales (1, 11, 13, 17, 19, 26, 29). Sulfur oxyanions also inhibit reductive dehalogenation in *D. tiedjei*, justifying its use as a model microorganism to investigate this process. Dehalogenation is inhibited in cells grown in the presence of sulfate, sulfite, or thiosulfate (21). However, in either resting cell suspensions or cell extracts, sulfate does not influence dehalogenation activity, while sulfite and thiosulfate are potent inhibitors (6, 8). In this study, the effects of sulfur oxyanions on dehalogenation activity in *D. tiedjei* are examined in order to clarify the mechanisms of inhibition.

**MATERIALS AND METHODS**

**Cultures.** *D. tiedjei* was cultivated in an anaerobic mineral medium containing pyruvate (40 mM) and 0.01% yeast extract as previously described (7). Starter cultures for all experiments were grown fermentatively in the absence of an electron acceptor. The medium was amended with 3CBz (2 mM) and/or sulfur oxyanions (0.5 to 20 mM) as electron acceptors and inoculated with *D. tiedjei* (5% transfer). Amendments to the medium were made from anoxic, sterile stock solutions. Samples of culture fluid were removed by syringe and analyzed by high-pressure liquid chromatography for chlorobenzoate disappearance and benzoate appearance.

**Preparation of extracts.** When cultures reached maximal optical density (*A*~600~ = 0.450), cells were harvested by centrifugation at 15,000 × g for 20 min at 4°C. The cell pellets were washed once and resuspended in potassium phosphate buffer (50 mM, pH 8.0) at a concentration of 1 g (wet weight)/5 ml of buffer. The cell suspension was broken twice by French pressure cell disruption (124 MPa) and centrifuged at 15,000 × g for 20 min at 4°C to remove unbroken cells. To separate membranes from the cytoplasmic and periplasmic contents, the cell extract was ultracentrifuged at 150,000 × g for 2 h at 4°C, and the pellet was resuspended in buffer. This membrane suspension was twice ultracentrifuged and resuspended in buffer. This membrane suspension was twice ultracentrifuged and resuspended in buffer (50 mM phosphate [pH 8.0], 100 mM NaCl) to remove contaminating soluble proteins from the membrane preparation.

**Dehalogenation assays and analytical methods.** Dehalogenation activities present in cell extracts were measured as described elsewhere (8, 44). Aryl and alkyl dehalogenation activities were quantified by measurement of the rate of benzoate or trichloroethylene appearance, respectively (8, 44). Growth of cultures was measured by optical density at 600 nm. Protein concentrations were determined by the colorimetric biochinonic acid method (40).

**RESULTS**

**Dehalogenase activity in extracts of *D. tiedjei* grown in the presence of sulfur oxyanions.** It has been previously reported that the aryl dehalogenase activity in *D. tiedjei* is induced by 3CBz (8); however, little is known regarding the influence of other electron acceptors on the expression of the dehalogenation activity. We examined the amount of dehalogenation activity in extracts of cells grown alone and in combination with 3CBz (2 mM) and a variety of oxyanions (5 mM). Following 17 days of incubation, the cultures reached their maximal optical density and were harvested, disrupted, and assayed for 3CBz dehalogenase activity (Fig. 1). Cells grown in the absence of 3CBz exhibited only a basal level of activity, while the presence of this electron acceptor in cultures resulted in a 40-fold increase in dehalogenation, confirming the inducibility of this activity. Cells grown in the presence of both 3CBz and sulfate, thiosulfate, or sulfite contained levels of dehalogenation activity which were markedly lower than those in cells grown with 3CBz as the sole electron acceptor, suggesting that sulfur oxyanions play a role in the regulation of dehalogenase production or expression. Dehalogenation activity in cells grown in the presence of both 3CBz and a sulfur oxyanion, however, was increased compared to that of cells grown with the respective sulfur oxyanion alone. Thus, sulfur oxyanions under growth conditions repressed dehalogenation activity. Growth in the presence of nitrate, which is reduced for assimilatory purposes by *D. tiedjei* but not for respiration, did not influence the amount of dehalogenation activity in cell extracts (Fig. 1).

To further examine the effect of sulfur oxyanions on the expression of the dehalogenase, fermentatively grown cells of *D. tiedjei* were used to inoculate a medium containing 2 mM 3CBz and sulfate at concentrations ranging from 0 to 10 mM. We monitored 3CBz disappearance throughout the growth of the cultures and found that increasing initial concentrations of sulfate resulted in decreasing amounts of 3CBz dehalogenation (Fig. 2). When cultures reached their maximal optical density, cells were harvested and assayed for 3CBz dehalogenation activity. The presence of sulfate did not influence the growth yields of *D. tiedjei*, and all cultures achieved similar final optical densities (*A*~600~ = 0.439 ± 0.027 [mean ± standard deviation]). However, sulfate influenced the amount of dehalogenation activity in extracts generated from these cultures in a concentration-dependent manner; cells grown with increasing initial amounts of sulfate were found to contain decreasing amounts of aryl dehalogenase activity (Fig. 3). We observed a similar phenomenon for *D. tiedjei* cells grown in increasing concentrations of thiosulfate (data not shown).

To examine the time course of reductive dehalogenation induction in the presence and absence of sulfur oxyanions, parallel cultures of *D. tiedjei* grown with 3CBz (2 mM) alone and in combination with sulfate (2 mM) were harvested at various optical densities and assayed for dehalogenase activity. In the absence of sulfate, the specific activity of the cells in-
creased throughout the growth curve, while in the presence of sulfate, dehalogenase activity was maintained at a low level (Fig. 4).

Effect of sulfur oxyanions on 3CBz dehalogenase activity in previously induced cell extracts. Although sulfate, sulfite, and thiosulfate inhibit dehalogenation under growth conditions, in fully induced resting cells or cell extracts, sulfate does not interfere with dehalogenation, while sulfite and thiosulfate are both potent inhibitors (6, 8). The in vitro inhibition of dehalogenation activity exhibited by the former electron acceptors may be due to their ability to effectively compete with dehalogenation for reducing equivalents or, alternatively, their chemical reactivity. The cytoplasm of D. tiedjei contains copious amounts of the sulfite reductase desulfoviridin, whereas the 3CBz dehalogenase is an integral membrane protein (8); therefore, these respiratory processes were physically separated by ultracentrifugation in order to examine the effect of sulfur oxyanions on dehalogenation activity in the absence of their reductases. Sulfate and nitrate did not effect dehalogenation activity in either cell extracts, as previously reported, or membrane fractions. Sulfite and thiosulfate, however, inhibited the reductive dehalogenase in whole-cell extracts and membrane fractions to similar extents (Fig. 5). Dialysis of extracts treated with sulfite completely restored dehalogenation activity, indicating that the inhibition of the dehalogenase by sulfite is reversible (Fig. 5).

Effect of sulfur oxyanions on alkyl dehalogenation of tetrachloroethylene by D. tiedjei. D. tiedjei also reductively dehalogenates tetra- and trichloroethylene, reactions that are cometabolic transformations catalyzed by the 3CBz dehalogenase (4, 44). Therefore, we investigated the effect of sulfur oxyanions serving

FIG. 2. Effect of initial sulfate concentration on dehalogenation of 3CBz during growth of D. tiedjei. Sulfate concentrations were 0 (■), 0.5 (●), 1 (▲), 2 (▼), 5 (♦), and 10 (♦) mM. Data points and standard deviations from triplicate cultures are shown.

FIG. 3. Effect of initial sulfate concentration on 3CBz dehalogenation activities of D. tiedjei cells. Cells were harvested at their maximal optical density. Columns and error bars represent the averages and standard deviations of triplicate cultures.

FIG. 4. Levels of dehalogenation activity found in D. tiedjei during growth on pyruvate-3CBz media in the presence (♦) and absence (■) of sulfate. Both electron acceptors were initially present at a 2 mM concentration. Data are single determinations from parallel cultures.
Nitrate 0.399

Thiosulfate 0.034

Sulfate 0.022

ion.

were present at a 10 mM concentration. Columns and error bars show the (closed bars) and washed membrane fractions (open bars) and the rescue of None 0.410

D. tied- inhibitors on alkyl reductive dehalogenation activity in (21), this inhibition has not been observed under nongrowth to inhibit dehalogenation in equivalents (1, 12, 13, 19, 26). Although sulfate has been found inhibit aryl reductive dehalogenation due to a competition between sulfate reduction and dehalogenation for reducing activities in (25). This suggests that reduced amounts of in vitro dehalogenation activity are due to regulation of the aryl dehalogenase specifically. Respiratory activities are most commonly controlled by regulation of the requisite terminal reductases at the level of transcription (20, 24), but translational and posttranslational regulation may also be involved (16). Although components of the dehalogenation respiratory chain have not been identified, a unique c-type cytochrome located in membrane fractions of D. tiedjei has been reported to be coinduced with 3CBz dehalogenation (34). This suggests that regulation of electron transfer proteins specifically associated with the dehalogenation process may also be involved.

We also found that sulfate and thiosulfate caused severe inhibition of 3CBz dehalogenation in washed membrane preparations from D. tiedjei cells induced for dehalogenation, while sulfate had no effect. It has been speculated that either reducing equivalents used for dehalogenation are preferentially used for sulfite and thiosulfate reduction but not sulfate reduction or sulfite and thiosulfate directly inhibit dehalogenation activities. Inhibition of aryl dehalogenation by sulfite and thiosulfate in the absence of cytoplasmic bisulfite reductase and thiosulfate reductase suggests that in vitro inhibition is due to a direct interaction of these reactive oxyanions with membrane-associated proteins involved in dehalogenation. Since it is believed that intermediate respiratory components are not required for in vitro activity (34), sulfite and thiosulfate may interact directly with the aryl dehalogenase itself, although their interaction with associated respiratory components in the membrane preparations cannot be ruled out. The inhibition of aryl reductive dehalogenation under nongrowth conditions by

der sulfate, sulfite, or thiosulfate. The latter sulfur oxyanions also serve as terminal electron acceptors for D. tiedjei (41), and the enzymes responsible for their reduction are constitutively expressed (25). Our results suggest that during growth in the presence of electron acceptors for both reductive dehalogenation and sulfur oxyanion reduction, D. tiedjei performs the latter process preferentially and that dehalogenation is inhibited largely due to the repression of components involved in aryl dehalogenation. Although dehalogenation activity was greatly reduced in cells grown in the presence of both 3CBz and sulfur oxyanions, it was not absent and was higher than the basal level detected in fermentatively grown cells. A low level of dehalogenation activity in cells grown in the presence of 3CBz and sulfur oxyanions may be advantageous, since D. tiedjei can use the dehalogenated metabolite benzoate as a substrate in the presence of sulfite or thiosulfate (7).

Increasing initial amounts of sulfate or thiosulfate in the growth medium resulted in a delay in the onset of dehalogenation, a decrease in the amount of 3CBz transformed, and a decrease in the amount of dehalogenase activity found in cell extracts. These data may reflect the time required to consume the sulfur oxyanions below some concentration at which repression due to these alternate electron acceptors is relieved. We measured the dehalogenation activity of growing cells over time under similar growth conditions in the absence of sulfate and found that the specific activity did not rapidly reach a steady-state level but continually increased throughout the growth curve. From this data, we would predict that increases in the delay in induction due to the presence of increasing amounts of sulfur oxyanions would be reflected in incremental decreases in the amount of dehalogenation activity recovered in early-stationary-phase cells, and this was, in fact, observed.

The specific mechanism of repression of dehalogenation activity by sulfur oxyanions under growth conditions is not known. Methyl viologen has been reported to directly reduce purified aryl dehalogenase from D. tiedjei (34). This suggests that reduced amounts of in vitro dehalogenation activity are due to regulation of the aryl dehalogenase specifically. Respiratory activities are most commonly controlled by regulation of the requisite terminal reductases at the level of transcription (20, 24), but translational and posttranslational regulation may also be involved (16). Although components of the dehalogenation respiratory chain have not been identified, a unique c-type cytochrome located in membrane fractions of D. tiedjei has been reported to be coinduced with 3CBz dehalogenation (25), suggesting that regulation of electron transfer proteins specifically associated with the dehalogenation process may also be involved.

Dehalogenation activity (nmol · min⁻¹ · mg⁻¹)

amendments

Amendment Electron acceptor* Inhibitor*

None 0.410 ± 0.041 0.301 ± 0.009

Sulfate 0.022 ± 0.004 0.300 ± 0.004

Sulfite 0.028 ± 0.006 0.013 ± 0.003

Thiosulfate 0.034 ± 0.009 0.036 ± 0.009

Nitrate 0.399 ± 0.031 0.278 ± 0.007

* Data are means and standard deviations of triplicate assays.

* For the electron acceptor survey, extracts of cells grown in the presence of 2 mM 3CBz and 5 mM oxyanion were assayed for dehalogenation activity.

* For the inhibitor survey, extracts of cells grown in the presence of 2 mM 3CBz were assayed for dehalogenation activity in the presence of 10 mM oxyanion.

DISCUSSION

It has been widely speculated that sulfur oxyanions can inhibit aryl reductive dehalogenation due to a competition between sulfate reduction and dehalogenation for reducing equivalents (1, 12, 13, 19, 26). Although sulfate has been found to inhibit dehalogenation in D. tiedjei under growth conditions (21), this inhibition has not been observed under nongrowth conditions (6, 8), suggesting other mechanisms of inhibition. Markedly reduced levels of dehalogenation activity were found in cell extracts of D. tiedjei cultured in the presence of both 3CBz, the aryl dehalogenase’s substrate and inducer, and either sulfate, sulfite, or thiosulfate. The latter sulfur oxyanions also serve as terminal electron acceptors for D. tiedjei (41), and the enzymes responsible for their reduction are constitutively expressed (25). Our results suggest that during growth in the presence of electron acceptors for both reductive dehalogenation and sulfur oxyanion reduction, D. tiedjei performs the latter process preferentially and that dehalogenation is inhibited largely due to the repression of components involved in aryl dehalogenation. Although dehalogenation activity was greatly reduced in cells grown in the presence of both 3CBz and sulfur oxyanions, it was not absent and was higher than the basal level detected in fermentatively grown cells. A low level of dehalogenation activity in cells grown in the presence of 3CBz and sulfur oxyanions may be advantageous, since D. tiedjei can use the dehalogenated metabolite benzoate as a substrate in the presence of sulfite or thiosulfate (7).

Increasing initial amounts of sulfate or thiosulfate in the growth medium resulted in a delay in the onset of dehalogenation, a decrease in the amount of 3CBz transformed, and a decrease in the amount of dehalogenase activity found in cell extracts. These data may reflect the time required to consume the sulfur oxyanions below some concentration at which repression due to these alternate electron acceptors is relieved. 

We measured the dehalogenation activity of growing cells over time under similar growth conditions in the absence of sulfate and found that the specific activity did not rapidly reach a steady-state level but continually increased throughout the growth curve. From this data, we would predict that increases in the delay in induction due to the presence of increasing amounts of sulfur oxyanions would be reflected in incremental decreases in the amount of dehalogenation activity recovered in early-stationary-phase cells, and this was, in fact, observed.

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FIG. 5. Effect of oxyanions on 3CBz dehalogenation by whole-cell extracts (closed bars) and washed membrane fractions (open bars) and the rescue of sulfite-dependent inhibition in cell extracts by dialysis (striped bars). Oxyanions were present at a 10 mM concentration. Columns and error bars show the averages and standard deviations of triplicate determinations.
sulfite or thiosulfate but not sulfate has also been previously described for dehalogenating enrichments (13, 26, 43), cell extracts of a dehalogenating enrichment (47), and a novel isolate (23). Sulfite is a powerful nucleophile known to inactivate proteins by a variety of mechanisms, including the disruption of disulfide bridges between cysteine residues (46) and the formation of adducts with flavins (31, 42). Sulfite-flavin adducts are formed with glucose oxidase at the atom which transfers reducing equivalents during catalysis, but the native enzyme is regenerated following dialysis (42). Interestingly, we too found that inhibition of reductive dehalogenation by sulfite was also reversible, as also reported by Zhang et al. for dehalogenation activity in extracts of a chlorophenol-dehalogenating enrichment (47).

In anaerobic bacterial respiratory processes, a remarkably diverse array of terminal electron acceptors is used, including a wide variety of oxyanions, organic compounds, and transition metals, and within a single microorganism, multiple respiratory processes are often present. The regulation of respiratory activities has been well studied in some model microorganisms. *Escherichia coli* uses oxygen, nitrate, nitrite, fumarate, dimethyl sulfoxide, and trimethylamine-N-oxide as terminal electron acceptors; specific terminal reductases are involved in each process, and reductases are regulated at the transcriptional level, allowing for the preferential utilization of the electron acceptor with the most positive redox potential (20). The hierarchical control of respiratory activities in order to utilize the electron acceptor which yields the greatest energetic gain is not universal in microorganisms. *Wolinella succinogenes* displays diverse respiratory activities in its ability to use nitrate, nitrite, nitrous oxide, fumarate, and elemental sulfur as electron acceptors. However, in this microorganism, the presence of sulfur, which has the most negative redox potential among potential electron acceptors, results in repression of the synthesis of terminal reductases for electron acceptors which would provide a greater energy yield (24). Likewise, although sulfur oxyanion reduction is preferred by *D. tiedjei*, this process actually provides less energy than 3CBz dehalogenation (9), so the regulation of respiratory activities in *D. tiedjei* is also not dictated solely by thermodynamic benefit.

Dehalogenating microorganisms generally contain multiple respiratory activities, but an understanding of how these processes are regulated in the presence of alternate electron acceptors is far from clear. Strain 2-CP-1 uses ortho-chlorinated phenols and fumarate as terminal electron acceptors. Dehalogenation activity is inducible, but dehalogenation did not occur when the organism was grown in the presence of high concentrations of fumarate (3). *Dehalospirillum multivorans* uses tetrachloroethylene, nitrate, fumarate, and possibly polysulfide as electron acceptors (32, 37). Fumarate inhibited dehalogenation under growth conditions, but the inhibitory effect was greatly reduced in resting cell suspensions (32). The regulation of multiple respiratory activities in other dehalogenating isolates has not been probed. “*Dehalobacter restrictus*” is currently unique among isolated microorganisms in having an absolute requirement for a chlorinated compound as its sole electron acceptor for growth (15).

Chloroaromatic contaminants often persist in laboratory incubations held under sulfate-reducing conditions. Our results presented here suggest that this inhibition may be due to sulfate oxyanions serving as preferred electron acceptors and repressing the expression of reductive dehalogenases in bacteria which contain both sulfur oxyanion and chloroaromatic respiratory activities. The prevalence of dissimilatory sulfur oxyanion reduction among isolates that perform reductive dehalogenation reactions lends credence to this hypothesis (2, 7, 10, 36, 45). With the coexistence of aryl and alkyl dehalogenation activities in a *Desulfobacterium* sp. (10) and *D. tiedjei* (44) and the presence of multiple modes of respiration in alkyl-dehalogenating microorganisms (10, 32, 36, 37, 45), alternate electron acceptors have the potential to repress alkyl dehalogenation activities as well (32).

The ability of sulfate to regulate dehalogenation activities may explain several laboratory observations. Aryl reductive dehalogenation activities often display an extended acclimation period prior to the onset of measurable activity. This acclimation period has been attributed primarily to a requirement for the induction of dehalogenation activity (22), but repression of activity due to the presence of alternate electron acceptors may play a role as well. The observation that sulfate may inhibit the development of dehalogenation activity but is not inhibitory when added to incubation mixtures after dehalogenation activity has commenced (1, 17, 27) may be due to sulfate’s ability to prevent the expression of dehalogenase activity but not inhibit previously expressed dehalogenase. Sulfate amendments can also influence the dehalogenation pathway of polychlorinated aromatic compounds (19, 28), and this phenomenon may be due to different regulatory controls for different dehalogenation activities. Recently, aryl reductive dehalogenation has been shown to occur under sulfate-reducing conditions in samples from environments in which a continued selective pressure for the enrichment of sulfate-insensitive dehalogenating microorganisms was present (14, 35, 43). Further studies investigating the role of alternate electron acceptors in the regulation of both aryl and alkyl dehalogenation activities by other available isolates are needed to determine whether repression of reductive dehalogenation by the presence of alternate electron acceptors is a general phenomenon or is restricted to *D. tiedjei*.

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