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Diversity of anaerobic microbial processes in chlorobenzoate degradation: nitrate, iron, sulfate and carbonate as electron acceptors

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Abstract The utilization of monochlorobenzoate isomers (2-, 3- and 4-chlorobenzoate) by anaerobic microbial consortia in River Nile sediments was systematically evaluated under denitrifying, Fe-reducing, sulfidogenic and methanogenic conditions. Loss of all three chlorobenzoates was noted in denitrifying cultures; furthermore, the initial utilization of chlorobenzoates was fastest under denitrifying conditions. Loss of 3-chlorobenzoate was seen under all four reducing conditions and the degradation of chlorobenzoates was coupled stoichiometrically to NO_3^- loss, Fe^{2+} production, SO_4^{2-} loss or CH_4 production, indicating that the chlorobenzoates were oxidized to CO_2. To our knowledge, this is the first observation of halogenated aromatic degradation coupled to Fe reduction.

Introduction

In the absence of oxygen, organic compounds can be metabolized by various microbial communities in the environment using alternative electron acceptors such as nitrate, Mn(IV), Fe(III), sulfate and carbonate in the processes of denitrification, Mn and Fe reduction, sulfidogenesis and methanogenesis respectively. Aquatic sediments, subject to anthropogenic loadings of sewage, wastewater and agricultural run-off or submarine discharge of contaminated groundwater, often become oxygen-depleted, and anaerobic microbial processes may be of significance in the environmental fate of organic contaminants. For example, denitrification can be enhanced in shallow estuarine sediments where the groundwater input of nitrate is high (Slater and Capone 1987). Fe reduction is considered to be important in both electron and carbon flow in some riverine and marine sediments (Aller et al. 1986; Hines et al. 1991), while sulfate reduction can account for more than 50% of carbon metabolized in marine sediments because of high sulfate levels in these sediments (Howarth 1984). In addition, methanogenesis is the primary route (more than 80%) for carbon and electron flow in fresh-water sediments, especially in areas of high organic loading (Lovley and Klug 1986).

Given the diversity and importance of diagenetic microbial processes in carbon and electron flow in the environment, we aimed to determine the potential for anaerobic microbial degradation of different isomers of monochlorobenzoates in fresh water sediments from the River Nile, Egypt. In this study, chlorobenzoates serve as model compounds for investigating the degradability of chlorinated aromatic compounds under different reducing conditions. Chlorobenzoates are a by-product in the bacterial metabolism of polychlorobiphenyls (Furukawa et al. 1983) and herbicides (Häggblom 1992) and their degradation under anoxic conditions has been previously demonstrated with inocula from a number of different sources, including lake sediments (Horowitz et al. 1983; Suflita et al. 1983; Linkfield et al. 1989), aquifer sediments (Gibson and Suflita 1986), estuarine and riverine sediments (Genthner et al. 1989; Häggblom et al. 1993), as well as sewage sludge (Suflita et al. 1983). The diversity in sediment sources suggests that microbes from different geographical locations have the potential for chloroaromatic degradation. Thus far, most studies have focused on haloaromatic degradation under methanogenic conditions (e.g. Shelton and Tiedje 1984) although there are a few reports of chlorobenzoate metabolism by microbial consortia under sulfidogenic (Genthner et al. 1989; Häggblom et al. 1993) and denitrifying (Genthner et al. 1989; Häggblom et al. 1993) conditions.

In this study, microbial degradation of 2-, 3- and 4-chlorobenzoate was systematically evaluated under

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denitrifying, Fe-reducing, sulfidogenic and methanogenic conditions using the same sediment inocula from the River Nile. Our results indicate that chlorobenzoates can be metabolized in enrichment cultures under denitrifying, sulfidogenic and methanogenic conditions. Furthermore, we report the first observation of halogenated aromatic degradation coupled to Fe reduction.

Materials and methods

Sediment source

Grab samples of sediments were taken from two sites along the River Nile, one in Cairo and a second near Komombo, Egypt, and were transferred to air-tight glass jars. Sediments from Cairo were a dark brown mud rich in organic carbon, while sediments from Komombo were a dark grey sandy silt.

Media

All media were prepared using standard anaerobic techniques. Each liter of denitrifying medium contained 4.2 g Na_2HPO_4, 1.5 g KH_2PO_4, 0.3 g NH_4Cl, 3.3 g KNO_3, 60 mg $MgSO_4 \cdot 7H_2O$, 1.3 mg $FeSO_4 \cdot H_2O$ and 0.2 mg $CuSO_4 \cdot 5H_2O$ (Bossert et al. 1986). Medium for the Fe-reducing enrichments was prepared in an identical manner to that described by Lovley and Phillips (1988); each liter of medium contained freshly precipitated amorphous Fe (as ferric oxyhydroxide), 2.5 g $NaHCO_3$, 0.1 g $CaCl_2 \cdot H_2O$, 0.1 g KCl, 1.5 g NH_4Cl and 0.6 g $NaH_2PO_4 \cdot H_2O$. Each liter of sulfidogenic medium contained 1.17 g NaCl, 0.41 g $MgCl_2$, 0.3 g KCl, 0.11 g $CaCl_2$, 0.27 g NH_4Cl, 0.20 g KH_2PO_4, 2.84 g Na_2SO_4, 0.1 mg resazurin, 0.17 mg $NaMoO_4$, 2.52 g $NaHCO_3$ and 0.35 g $Na_2S \cdot 9H_2O$ (Widdel 1980). Each liter of methanogenic medium contained 40 mg $(NH_4)_2PO_4$, 0.37 g $FeCl_2 \cdot 4H_2O$, 0.5 g $Na_2S \cdot 9H_2O$, 2.64 g $NaHCO_3$ and 1.0 mg resazurin (Owen et al. 1979). Trace salts and vitamins were added to all media (Owen et al. 1979). The initial concentration of the electron acceptors was 30 mM nitrate, 200 mM Fe(III), and 20 mM sulfate for denitrifying, Fe-reducing and sulfidogenic media respectively.

Enrichment preparation

Sediment slurries (1:10 vol:vol sediment and the appropriate media) were divided into aliquots in serum vials (50 ml nominal volume) capped with black butyl rubber stoppers and crimped with aluminum seals. Each vial contained 50 ml slurry and had a 10-ml headspace. One set of cultures was incubated with distilled water without electron acceptor added. The headspace of the vials was N_2:CO_2 (70:30 v/v) for water, Fe-reducing, sulfidogenic and methanogenic cultures, and Ar for denitrifying cultures. Each substrate (benzoate, 2-, 3-, 4-chlorobenzoate; Aldrich Chemical Co., Milwaukee, Wis.) was maintained as a deoxygenated stock solution in 0.1 M NaOH, and was added to separate vials to a final concentration of 100 μM. Background controls were prepared in the same manner as the experimental cultures except that no substrate was added. These controls were to account for NO_3^- loss, Fe^{2+} production, SO_4^{2-} loss or CH_4 production due to metabolism of existing carbon in the sediment inoculum. Sterile controls were autoclaved three times on consecutive days before the experiment was initiated. Strict anaerobic microbial techniques were used throughout in experimental manipulations. Syringes and needles used for substrate addition and sample collection were flushed with Ar or with N_2:CO_2 passed over hot reduced copper filings to remove traces of O_2. All enrichments, with autoclaved controls, were made in duplicate and incubated under static conditions in the dark at 30 °C. A total of 140 vials was established, which included the experimental cultures, background and sterile controls.

In the halobenzoate experiment, a culture enriched for 3-chlorobenzoate degradation under Fe-reducing conditions from Cairo sediments was subdivided into nine vials. 3-Bromobenzoate or 3-iodobenzoate (Aldrich Chemical Co.) was fed to vials (two replicates plus one autoclaved control) each to a final concentration of 200 μM. The remaining three vials were fed 3-chlorobenzoate (200 μM) to ensure that the culture was active in utilizing this substrate. The headspace of the vials was N_2:CO_2 (70:30). Sterile controls were autoclaved three times on consecutive days before the experiment was started.

Analytical methods

Organic substrate

At each assay time, the cultures were mixed well to distribute the sediment and 0.5 ml sediment/water slurry was withdrawn from the vials into a deoxygenated, sterile syringe. The samples were centrifuged and the supernatant filtered (0.45 μM), then frozen (−20 °C) prior to analysis. Loss of substrate was monitored by injecting samples into a high-pressure liquid chromatograph (HPLC; Beckman System Gold models 126/166, San Ramon, Calif.) with a C-18 column (Supelco, 25 cm × 4.6 mm, 5-μm particle size, Bellefonte, Pa.). A solvent system of 60:38:2 water/methanol/acetic acid at a flow rate of 1.0 $\mathrm{ml\,min^{-1}}$ was used. The detector wavelength was set at 280 nm. Substrate concentrations were monitored with a Spectra-Physics SP4400 integrator (San Jose, Calif.) calibrated with standards of benzoate and 2-,3- and 4-chlorobenzoate. In the halobenzoate experiment, standards of 3-bromobenzoate and 3-iodobenzoate were used.

Fe^{3+} reduction

Fe^{2+} production was determined by a modification of the method described by Lovley and Phillips (1988) (E. Roden; personal communication). Briefly, the method involves adding a subsample of the sediment slurry to dilute HCl to extract acid-soluble Fe. The HCl solution was then centrifuged and an aliquot of the supernatant was added to a solution of ferrozine (Aldrich Chemical Co.). The HCl-extractable Fe^{2+} reacts with ferrozine to form a colored compound, which was then measured by spectrophotometry (Shimadzu UV-240 UV-visible spectrophotometer; Shimadzu Corp. Kyoto, Japan) at 562 nm. Fe^{2+} standards were made from ferrousethylenediammonium sulfate (Fluka Chemical Co., Ronkonkoma, N.Y.). The sensitivity of this method was 1 μM for Fe^{2+}.

NO_3^- and SO_4^{2-} reduction

Nitrate and sulfate loss was determined by taking an aliquot of the filtered culture supernatant, diluting it appropriately, then injecting into an ion chromatograph (Dionex DX-100, Sunnyvale, Calif.) with conductivity detection and equipped with an anion-exchange column (IonPac AS9). The eluant was Na_2CO_3/$NaHCO_3$ (2.0 mM:0.75 mM) at a flow of 2.0 $\mathrm{ml\,min^{-1}}$. Nitrate and sulfate standards were made from KNO_3 and Na_2SO_4 respectively. The sensitivity of this method was 1 μM for either nitrate or sulfate.

Methane production

The volume of gas produced was measured with a water-lubricated glass syringe. Methane was monitored in the headspace of the vials by injecting a sample into a gas chromatograph with thermal conductivity detection (Fisher model 1200).

All measurements for NO_3^- loss, Fe^{2+} production, SO_4^{2-} loss and CH_4 production that could be coupled to substrate loss in the cultures were corrected for background carbon metabolism by subtracting those measurements taken from cultures to which only the electron acceptor, but no substrate, had been added.

Bromide and iodide

In the halobenzoate experiment, bromide and iodide release was monitored by ion chromatography. Bromide and iodide standards were made from NaBr and NaI (Aldrich Chemical Co.). The sensitivity of the method was 1 μM for either Br^- or I^-.

Results

Loss of chlorobenzoates

Metabolism in Cairo sediments of each chlorobenzoate isomer under denitrifying, Fe-reducing, sulfate-reducing and methanogenic conditions is summarized in Fig. 1. Of the three chlorobenzoate isomers added to Cairo sediments, loss of 3-chlorobenzoate (100 μM) occurred initially within 40–130 days under all four reducing conditions, with the most rapid substrate utilization occurring in the denitrifying enrichments. Loss of 4-chlorobenzoate (100 μM) occurred within 30 days under denitrifying conditions and took over 200 days under sulfidogenic conditions. There was no utilization of 4-chlorobenzoate in the methanogenic or Fe-reducing enrichments within 180 days. Metabolism of 2-chlorobenzoate (100 μM) occurred within 130 days under denitrifying conditions; but no utilization of this substrate occurred within 180 days in the other enrichments. Benzoate was readily utilized under all reducing conditions within 14 days (data not shown). There was no loss of the chlorobenzoate isomer or benzoate in the autoclaved controls over 180 days. As noted in Fig. 1, degradation of the chlorobenzoate isomers in all of the active cultures could be sustained upon re-feeding of each of the respective substrate.

Some similarities were observed with cultures inoculated with Komombo sediment (Fig. 2). In these enrichments, 3-chlorobenzoate (100 μM) was utilized within 30–130 days under all reducing conditions. Loss of 4-chlorobenzoate (100 μM) took place within 30 days in the denitrifying enrichments and within 180 days in the Fe-reducing enrichments. There was no loss of 4-chlorobenzoate within 180 days in methanogenic or sulfidogenic cultures. This is in contrast to Cairo sediments where 4-chlorobenzoate was degraded in the sulfidogenic, but not in the Fe-reducing enrichments. No loss of 2-chlorobenzoate was observed under any of the reducing conditions in Komombo sediments. This is in contrast to Cairo sediments, where 2-chlorobenzoate was metabolized under denitrifying conditions. Benzoate was degraded within 14 days under all reducing conditions (data not shown).

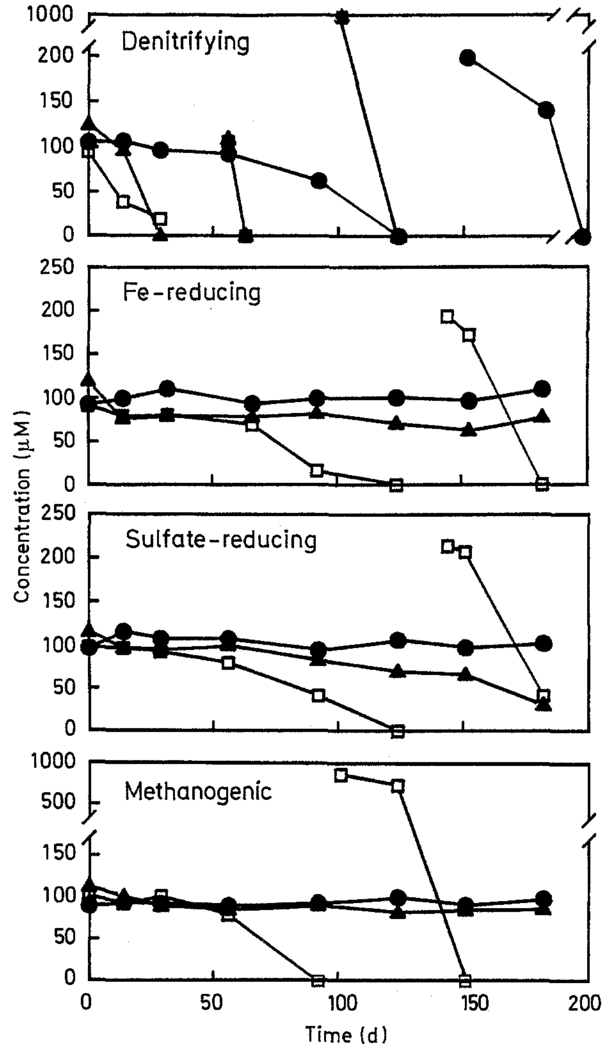

Fig. 1 Loss of chlorobenzoate isomers under denitrifying, Fe-reducing, sulfate-reducing and methanogenic conditions in cultures with Cairo sediments: ● 2-chlorobenzoate, □, 3-chlorobenzoate, ▲ 4-chlorobenzoate

Chlorobenzoate degradation was dependent on the addition of an electron acceptor in cultures from both Komombo (Fig. 3), and Cairo sediments (data not shown). In both cases, the addition of NO_3^-, Fe^{3+}, SO_4^{2-} or CO_3^{2-} promoted the degradation of 3-chlorobenzoate within 30–130 days; however, in the absence of an electron acceptor (only water added to the sediment slurry) there was no loss of the compound for up to 280 days. This was also noted for the other isomers in cultures from both sites (data not shown). The 3-chlorobenzoate enrichments with only water added became methanogenic (with methane detectable in the headspace) after approximately 300 days.

Summarized in Table 1 is the t_{50} value of substrate loss for each isomer and each reducing condition. The t_{50} is the time at which 50% of the isomer has disappeared, and values were estimated from the graphs of substrate loss with time. As shown in this table, there was no difference in t_{50} for benzoate under all four reducing conditions. For all susceptible chlorobenzoate isomers for which data were obtained, t_{50} values were shorter under denitrifying compared to other conditions. For example, for 3-chlorobenzoate, t_{50} values were similar among Fe-reducing, sulfidogenic and methanogenic cultures; for 4-chlorobenzoate, the t_{50} was shorter in Fe-reducing, than in sulfate-reducing enrichments.

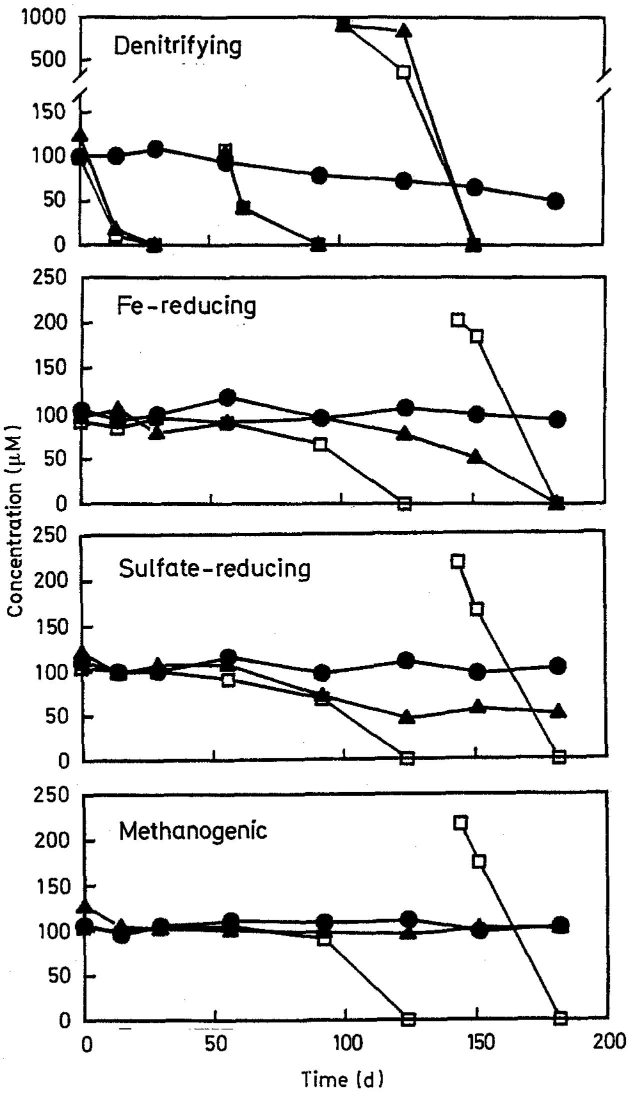

Fig. 2 Loss of chlorobenzoate isomers under denitrifying, Fe-reducing, sulfate-reducing and methanogenic conditions in cultures with Komombo sediments: ● 2-chlorobenzoate, □, 3-chlorobenzoate, ▲ 4-chlorobenzoate

Stoichiometry

In order to examine whether chlorobenzoate degradation could be coupled to denitrification, Fe-reduction, sulfidogenesis or methanogenesis, predicted values determined from stoichiometric equations of nitrate loss, Fe^{2+} production, sulfate loss, and methane production were compared to those measured in chlorobenzoate-degrading enrichments. These equations assume that chlorobenzoate is completely mineralized to CO_2

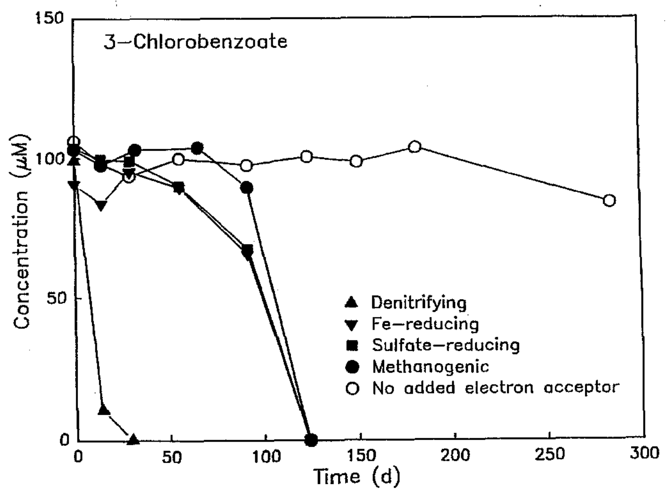

Fig. 3 Initial loss of 3-chlorobenzoate in cultures with Komombo sediments under all four reducing conditions and no added electron acceptor

Table 1 Summary of t_{50} values (the time at which 50% of substrate is utilized) for initial utilization of 100 μM substrate under each reducing condition. –No loss was observed within 57 weeks

Substrate	t_{50} (weeks) Denitrifying	Fe-reducing	Sulfate-reducing	Methanogenic
Cairo				
Benzoate	1	1	1	1
2-Chlorobenzoate	14	—	—	—
3-Chlorobenzoate	2	11	12	10
4-Chlorobenzoate	3	13	23	—
Komombo				
Benzoate	1	1	1	1
2-Chlorobenzoate	26	—	—	—
3-Chlorobenzoate	1	15	14	15
4-Chlorobenzoate	1	22	—	—

and/or CH_4 as follows:

$$C_7H_5O_2Cl + 5.6\,NO_3^- + 4.6\,H^+ \rightarrow 7\,CO_2 + 2.8\,N_2 + 4.8\,H_2O + Cl^-$$

$$C_7H_5O_2Cl + 29\,Fe^{3+} + 19\,H_2O \rightarrow 7\,HCO_3^- + 29\,Fe^{2+} + 36\,H^+ + Cl^-$$

$$C_7H_5O_2Cl + 3.5\,SO_4^{2-} + 5\,H_2O \rightarrow 7\,HCO_3^- + 3.5\,H_2S + H^+ + Cl^-$$

$$C_7H_5O_2Cl + 5\,H_2O \rightarrow 3.5\,CH_4 + 3.5\,CO_2 + H^+ + Cl^-$$

Cultures were repeatedly fed the substrates until approximately 1 mmol/l had been utilized. In this manner, the amount of electron acceptor used for substrate degradation can be readily determined and is sufficiently above that used for background metabolism of carbon in the sediment.

Summarized in Table 2 is the utilization of nitrate from the cultures in which 2-, 3- and 4-chlorobenzoate degradation occurred. From the known amount of substrate utilized and the stoichiometric equation above, the amount of nitrate required as an electron acceptor can be calculated. This is then compared to the measured amounts of nitrate consumed. The results indicate that nitrate consumption was 83%–177% of that expected in these cultures and suggest that complete degradation of the compound had taken place. The reason for the high nitrate consumption observed in the Cairo sediments rich in organic material is unclear. One possibility is that chlorobenzoate stimulated co-metabolism of the organic material in the sediment. If we compare the measured amounts of Fe^{2+} produced in the 3-chlorobenzoate- and benzoate-degrading cultures, and the calculated amount required, Fe^{2+} production in these enrichments was 115%–129% of that expected (Table 3). As for sulfate, the measured loss of the electron acceptor in the 3- and 4-chlorobenzoate- utilizing cultures was 94%–106% of that expected (Table 4). The results for the 3-chlorobenzoate-degrading methanogenic enrichments is shown in Table 5. The amount of methane produced in these cultures was 82% of that expected from calculated amounts. All of these results are consistent with the presumption that anaerobic chloroaromatic degradation is coupled stoichiometrically to use of the various electron acceptors.

Loss of halobenzoates and halide release

In order to determine whether halide release occurs during halobenzoate degradation, subcultures of a 3-chlorobenzoate-degrading culture enriched under

Table 2 Consumption of nitrate during degradation of monochlorobenzoates in Nile sediments. Predicted consumption is based on a stoichiometry of 1 mol chlorobenzoate = 5.6 mol NO_3^-. The measured NO_3^- consumption has the background values from control cultures (14 mM in Cairo sediments and 3.5 mM in Komombo sediments within 101 days) subtracted. For 2-chlorobenzoate, the background control cultures lost 14 mM NO_3^- within 421 days

Chlorobenzoate fed	Chlorobenzoate metabolized (mM)	NO_3^- consumption (mM)		*b*/*a* (%)
		Predicted (*a*)	Measured (*b*)	
Cairo				
2-Chlorobenzoate	0.93	5.2	6.84	132
3-Chlorobenzoate	1.00	5.6	9.92	177
4-Chlorobenzoate	1.16	6.5	8.21	126
Komombo				
3-Chlorobenzoate	1.12	6.3	5.78	92
4-Chlorobenzoate	1.13	6.3	5.23	83

Table 3 Production of Fe^{2+} during degradation of monochlorobenzoates in Nile sediments. The predicted Fe^{2+} production is based on a stoichiometry of 1 mol chlorobenzoate = 29 mol Fe^{2+}. The measured production has the background values from control cultures (75 mM in Cairo sediments and 23 mM in Komombo sediments within 298 days) subtracted

Chlorobenzoate fed	Chlorobenzoate metabolized (mM)	Fe^{2+} production (mM)		*b*/*a* (%)
		Predicted (*a*)	Measured (*b*)	
Cairo				
3-Chlorobenzoate	0.82	24	30	125
Komombo				
Benzoate	0.83	24	31	129
3-Chlorobenzoate	0.89	26	30	115

Table 4 Consumption of sulfate during degradation of monochlorobenzoates in Nile sediments. Predicted SO_4^{2-} consumption is based on a stoichiometry of 1 mol chlorobenzoate = 3.5 mol SO_4^{2-}. The measured consumption has the background values from control cultures (8.5 mM in Cairo sediments and 0.5 mM in Komombo sediments within 383 days) subtracted

Chlorobenzoate fed	Chlorobenzoate metabolized (mM)	SO_4^{2-} consumption (mM)		*b*/*a* (%)
		Predicted (*a*)	Measured (*b*)	
Cairo				
3-Chlorobenzoate	0.91	3.2	3.08	94
4-Chlorobenzoate	0.78	2.7	2.87	106
Komombo				
3-Chlorobenzoate	0.57	2.0	1.89	95

Table 5 Production of methane during degradation of monochlorobenzoates in Nile sediments. The predicted CH_4 production is based on a stoichiometry of 1 mol chlorobenzoate = 3.5 mol CH_4. The measured production has the values from background control cultures (239 μmol within 151 days) subtracted

Chlorobenzoate fed	Chlorobenzoate metabolized (μmol)	CH_4 production (μmol)		*b*/*a* (%)
		Predicted (*a*)	Measured (*b*)	
Cairo				
3-Chlorobenzoate	48.3	168	138	82

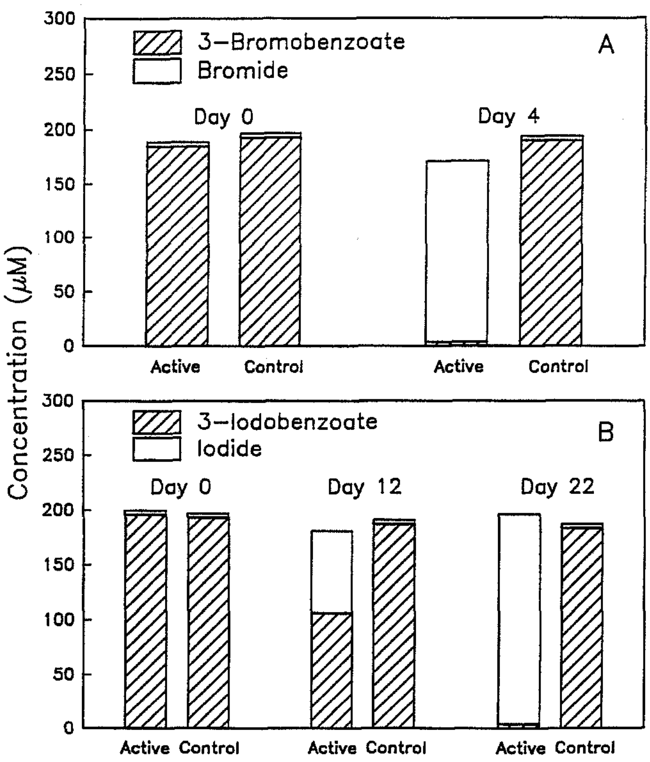

Fig. 4A,B Loss of 3-bromobenzoate (**A**) and 3-iodobenzoate (**B**) with concomitant halide release in cultures with Cairo sediments

Fe-reducing conditions were fed either 3-bromobenzoate or 3-iodobenzoate. Loss of the halobenzoates, as well as release of bromide and of iodide were monitored. 3-chlorobenzoate was not used in this experiment because the release of Cl^- could not be determined given the high concentration of Cl^- in the media. Loss of 200 μM 3-bromobenzoate occurred within 4 days, with the concomitant release of Br^-, and is illustrated in Fig. 4A. Similarly, Fig. 4B shows the loss of 200 μM 3-iodobenzoate, which occurred within 22 days, with the concomitant release of I^-. The amount of Br^- and I^- released was equivalent to all of that which would be expected from the complete degradation of 3-bromobenzoate and 3-iodobenzoate respectively.

Discussion

While dechlorination and degradation of halogenated aromatic compounds have been extensively studied under methanogenic conditions (see review, Häggblom 1992), other reducing conditions have rarely been systematically examined. Environmentally significant electron acceptors such as carbonate, sulfate, iron and nitrate can support microbial communities which differ significantly both physiologically and phylogenetically. Hence, the biodegradative capabilities of these diverse communities have remained largely overlooked. From the results of our study, we show that anaerobic microorganisms in River Nile sediments have the capacity to degrade all three monochlorobenzoate isomers in the absence of oxygen and in the presence of alternative electron acceptors. In general, loss of chlorobenzoate isomers was fastest under denitrifying conditions when compared to the other reducing conditions. Under the experimental conditions used, there was little difference in the rates of initial substrate loss among Fe-reducing, sulfidogenic and methanogenic conditions (Table 1). All three chlorobenzoate isomers were utilized under denitrifying conditions in Cairo and Komombo sediments; however, degradation of 2-chlorobenzoate could not be sustained upon re-feeding in Komombo sediments. It should be noted that a possible explanation for the loss of chlorobenzoate-degradative ability may be due to an inhibitory effect of any accumulated metabolic by-products as a result of incomplete degradation. Another possibility is that metabolism of 2-chlorobenzoate required a co-substrate that was subsequently depleted. These results are somewhat similar to those observed in Upper Hudson River sediments, where 100 µM 3- and 4-chlorobenzoate, although not 2-chlorobenzoate, were degraded within 20 days under denitrifying conditions (Häggblom et al. 1993). In contrast, however, Genthner et al. (1989) found that loss of the different isomers of chlorophenols and chlorobenzoates in enrichments that required nitrate or sulfate occurred less frequently than in cultures that were methanogenic. The different observations may be due to differences in methodology and/or inoculum source.

Under Fe-reducing conditions, both 3-chlorobenzoate and benzoate were degraded in Cairo and Komombo sediments. Furthermore, this activity could be sustained upon re-feeding of the compounds. Loss of 4-chlorobenzoate was noted within 200 days in the Komombo cultures; however, this activity could not be maintained upon re-feeding. The degradation of benzoate and 3-chlorobenzoate are consistent with the stoichiometric production of Fe^{2+} predicted for complete oxidation of the carbon substrate to CO_2. Thus, mineralization of the compounds takes place at the expense of microbial Fe reduction. Furthermore, the appearance of Br^- or I^- in stoichiometric amounts concomitant with 3-bromobenzoate or 3-iodobenzoate loss indicates that halide release occurs at some point in the degradation pathway. Complete loss of 3-bromo- and 3-chlorobenzoate (data not shown) occurred within 4 days while loss of 3-iodobenzoate took 22 days. A possible explanation for this difference is the larger size of the 3-iodo derivative, which may be taken up by the cell less readily than the smaller 3-bromo- and 3-chlorobenzoate molecules. It should be noted that these results do not provide information on the dehalogenation mechanism. Possible mechanisms include hydrolytic aryl dehalogenation, reductive aryl dehalogenation, or dehalogenation after ring fission has occurred.

At the present time, it is not clear which bacterial groups are responsible for haloaromatic degradation in our Fe-reducing cultures. A number of non-halogenated compounds, including benzoate, toluene, phenol and *p*-cresol have been reported to be degraded under Fe-reducing conditions by the pure culture, *Geobacter metallireducens* (Lovley et al. 1993). To our knowledge, however, this is the first study to report that haloaromatic compounds are microbially degraded in a process coupled to Fe reduction.

Under sulfate-reducing conditions, both 3- and 4-chlorobenzoate were degraded and with re-feeding of the substrates. Moreover, the measured consumption of sulfate in these cultures agreed with the consumption as predicted by the stoichiometry of chlorobenzoate mineralization coupled to sulfate reduction (Table 4). These results support our earlier work, which shows the degradation of 3-chlorobenzoate as well as chlorophenols to be coupled to sulfate reduction (Häggblom et al. 1993; Häggblom and Young 1990). Other studies, on the other hand, report that neither 3- nor 4-chlorobenzoate was degraded under sulfidogenic conditions (Gibson and Suflita 1986). It was suggested that the potential for dehalogenation and haloaromatic metabolism was present in sulfidogenic aquifer sediments, but that the potential was at least partly inhibited by endogenous levels of sulfate (2 mM) in the aquifer sediment (Beeman and Suflita 1987). Differences in habitat (subsurface aquifer versus river sediments) and/or methodology may account for the different observations. From our studies, 20 mM sulfate did not appear to inhibit, but rather promoted 3- and 4-chlorobenzoate degradation, since cultures to which no electron acceptor was added showed no loss of substrate (Fig. 3; data for 4-chlorobenzoate not shown).

Under methanogenic conditions, only 3-chlorobenzoate was degraded in Cairo and Komombo sediments. This result is consistent with numerous other studies that show dechlorination and degradation of 3-, but not 4-chlorobenzoate, in methanogenic cultures of lake (Horowitz et al. 1983), aquifer (Gibson and Suflita 1986), estuarine and river (Genthner et al. 1989) sediments.

Noteworthy is the fact that the addition of alternative electron acceptors promoted the degradation of chlorobenzoates when compared to cultures to which no electron acceptors were added (Fig. 3). This further indicates that microbial consortia in sediments have the potential for contaminant degradation and may be electron-acceptor-limited. Moreover, it suggests a potential strategy for remediating contaminated soils and sediments.

Degradation of monochlorobenzoate isomers was dependent not only on the electron acceptor present, but also on the position of the Cl^- substituent. The results from the present study are consistent with previous results using cultures from the Hudson and East Rivers (N.Y.), in which the relative degradability of the chlorobenzoate isomers followed the pattern: *meta-* > *para-* > *ortho-* (Häggblom et al. 1993). It is interesting to note that, despite the broad geographical difference in sediment source, similar patterns of chloroaromatic degradation can still be demonstrated.

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