



Effect of sorption and desorption-resistance on biodegradation of chlorobenzene in two wetland soils

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ABSTRACT

Bioavailability of chlorobenzenes (CBs) in soils to microbial populations has implications for remediation of waste sites with long histories of contamination. Bioavailability of CB was assessed using mineralization assays for two types of wetland soils with contrasting properties. The rate and extent of CB mineralization were greater than predicted by mathematical models which assume instantaneous desorption followed by biodegradation. The freshly added CB was degraded with initial mineralization rates (IMRs) of $0.14 \mu\text{g L}^{-1} \text{h}^{-1}$ and $1.92 \mu\text{g L}^{-1} \text{h}^{-1}$ for marsh soil and wetland soil respectively. These values indicate that CB-degrading bacteria had an access to the sorbed CB. Mineralization assays were also performed for wetland soils after the CB was aged for 1, 7 and 31 days. The results revealed that even a desorption-resistant part of the sorbed CB was degraded although the degradation occurred at lower rates and to a lesser extent.

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1. Introduction

Physicochemical processes such as sorption and desorption have been shown to affect biodegradation, bioavailability, and subsurface transport of various organic chemicals [1–4]. Biodegradation rates of sorbed contaminants are thought to be lower than those of soluble contaminants since it has been reported that sorbed chemicals are unavailable to microorganisms unless desorption occurs [5]. Pollutant sequestration reduces the bioavailability to microorganisms although bacteria have been reported that are capable of degrading soil-sorbed naphthalene [1,6,7]. It has also been confirmed that biodegradation of organic compounds can be limited by the slow rate of desorption [8–10]. The rate and extent of desorption are affected by soil organic carbon content, cation-exchange capacity, specific surface area and water solubility [11–15].

Desorption rates of persistent organic pollutants (POPs) have been shown to be markedly lower upon prolonged contact with soil [13–15]. Therefore, reversible sorption models are not able to explain the long-term persistence of organic contaminants at many contaminated sites [16]. A desorption-resistant fraction that

is not readily desorbed from soil organic matter has been observed. A rapid desorption of a labile fraction is followed by the slow release of a non-labile fraction. Desorption-resistance is related to the “contaminant aging” phenomenon, which results from the partial dissipation or degradation of more labile fractions of the contaminant before collection [3,17,18]. Previous findings have shown that the extent of bioavailability of sorbed contaminants decreases with increased aging for contaminants such as biphenyl [19], styrene [20], phenanthrene [21–23], and DDT [24] respectively.

Chlorobenzene (CB) has been used as a solvent and can be produced as a daughter product from higher chlorinated benzenes during biodegradation in the environment. Several aerobic CB-degrading strains have been isolated [25,26], and they have been frequently observed in soil samples with histories of CB exposure. Batch studies have demonstrated the ability of removing CB sorbed in soil by CB-degrading organisms [26–28]. In this study, mineralization of chlorobenzene, a non-ionic contaminants, by CB-degrading bacteria was investigated by conducting experiments under various conditions regarding water content in soil-slurry, aging and desorption effects. The work presented herein was conducted in support of remediation activities at a Louisiana Superfund site known as Petro Processors Inc. (PPI) located north of Baton Rouge, LA. Results of this study may have implications as evidence is being gathered to address issues such as “how clean is clean?”.

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2. Materials and methods

2.1. Chemical preparation and analysis

Non-labeled CB (99.5% purity, spectrophotometric grade) was purchased from Aldrich Chemical Co. (Milwaukee, WI). CB solutions were prepared by dissolving aliquots of CB (neat) in deionized water to obtain the desired contaminant concentrations. Radio-labeled chlorobenzene (Sigma Chemical Co., St. Louis, Missouri, 27 mCi/mmol) was used as a tracer. For extraction procedures, isopropanol (99.8% purity, nanograde, Fisher Scientific) and methanol (99% purity, Fisher Scientific) were used.

2.2. Soils

Two types of soil were used for this study. The first soil was collected from the surface (0–20 cm) of a bottomland hardwood swamp located adjacent to the Brooklawn site, one of two Superfund sites collectively known as the Petro Processors Inc. site located in north Baton Rouge, LA. Large lumps of soil were manually broken apart, and the material was oven dried at 55 °C for 24 h. The soil was then pulverized and homogenized by passage through a sieve with 150 µm openings (US standard sieve No. 100). The soil was then autoclaved at 124 °C and 20 psi for 30 min in a pressure steam sterilizer (Model 25X, Wisconsin Aluminum Foundry Co. Inc., Manitowoc, WI).

The second type of soil was collected from the top layer (0–20 cm) of a freshwater marsh located in Madisonville, LA. Marsh soil samples were first chopped with scissors to homogenize the material and then sterilized by autoclaving as described above. Samples both types of soil were methanol-extracted, analyzed, and found to be free of CB. All soil samples were autoclaved again before use in subsequent experiments. Soil properties were determined according to standard methods in the Huffman Laboratories Inc., Golden, CO, and soil characteristics are summarized in Table 1.

2.3. Microbial culture

An aerobic enrichment of cultures was prepared using a mixed culture obtained from the Central Wastewater Treatment Plant in Baton Rouge, LA using liquid nutrient medium previously described by Dorn et al. [29]. Prior to use in bioavailability assays, the undefined mixed culture was grown in nutrient medium containing 3 mg/L of CB. Organisms were repeatedly transferred to fresh liquid mineral medium and cultured for approximately 2 months prior to the initiation of CB mineralization studies. A preliminary experiment (data not shown) indicated that the cultured organisms were capable of utilizing CB as a sole carbon and energy source. To minimize the potential for variability based on the size or composition of the microbial population, equal volumes of the enrichment culture was added simultaneously to all of the artificially aged soils tested. In addition, measurements of porewater CB concentration were conducted in control microcosms containing 0.1 M NaN₃ as a bacterial inhibitor.

2.4. Mineralization studies

Mineralization experiments were conducted to evaluate the availability of soil-sorbed CB to CB-degrading bacteria utilizing

a method described by Guerin and Boyd [1] and Feng et al. [19]. All treatments for these experiments were set up in 200-mL capacity flasks, capped with Teflon-lined silicone septa and then placed on a rotary shaker at 100 rpm and maintained at approximately 25 °C. Table 2 summarizes the preparation of mineralization studies in the aqueous and soil-slurry phases. A CO₂ trap containing 2 mL of 1 N NaOH was placed inside each reactor. At various time intervals, the contents of the base trap were removed from the reactor, and 1.0 mL of the base solution was counted by liquid scintillation to quantify ¹⁴C-¹⁴CO₂ production. Hionic-Fluor (Packard Bioscience Company, Meriden, CT) was the scintillant in a 1:10 sample-to-scintillant ratio for the mineralization study. Each time base-trap sampling was conducted, 2.0 mL of 1 N NaOH was replaced in each reactor. The sampling procedure was conducted in a temperature-controlled room at 5 °C to minimize CB volatilization losses.

Using the procedure outlined above, three mineralization experiments were conducted: (i) comparison of CB mineralization in soil-slurries with different water contents; (ii) examination of the mineralization of CB in a freshly contaminated soil; (iii) measurement of CB mineralization in the desorption-resistant fraction of soil.

For the first experiment, soil-slurries containing three different soil:water ratios were prepared. For the PPI soil, the ratios of 0.04, 0.44, and 0.89 (w/v on a dry mass basis) were used and the ratios of 0.01, 0.04 and 0.08 (w/v) were also used for the marsh soil. These ratios were selected to yield approximately the same CB concentrations for two soils.

For the second experiments, 60 g of each PPI and marsh soil was placed in replicate 125-mL capacity glass bottles (*I-Chem*). An aqueous solution spiked with CB (~5 mg/L) and ¹⁴C-CB as a tracer was added to each bottle, the bottles were mixed vigorously, filled to capacity with minimal headspace, and then capped with Teflon fluorocarbon resin/silicone septa. The bottles were equilibrated in a rotary tumbler for 2 days at 80 rpm, and then the bottles were then centrifuged to obtain a clear soil–water interface. The supernatant was then decanted, and a portion of the soil pellet was used to measure the soil activity of ¹⁴C-CB using a biological oxidizer (Model OX600, R.J. Harvey Instrument Corporation, Hillsdale, NJ). The remainder of the soil was immediately added to flasks as described above for mineralization assay. In this case, 8.01 g of PPI soil or 1.64 g of marsh soil (on a dry mass basis) was added to each reactor, and then a mineralization study was conducted as described previously.

For the third experiments, the soil was further prepared using an isopropanol co-solvent extraction method developed by Liu et al. [30]. In this method, equal volumes of isopropanol and electrolyte solution (0.01 M NaCl, 0.01 M CaCl₂) were combined to prepare an extraction solution. Soils were contaminated as described above (20 mg/L of initial concentration of CB). In order to make the soil “aged” artificially, the CB sorbed soil, in *I-Chem* bottles, was stored in the dark for a designated period of time (2, 7, and 31 days—see Table 2) before further preparation. After aging, the contaminated soil was mixed vigorously with co-solvent solution (120 mL/g of soil) in a tumbler for 24 h, and then the co-solvent solution was separated from the soil via centrifugation maintained at approximately 25 °C at 100 rpm. The separated soil was rinsed with electrolyte solution two times to remove the residual isopropanol. The soil was then mixed with co-solvent solution again, and placed in a tumbler for another 24 h. Additional rinse was conducted with electrolyte solution after which CB concentration in the aqueous phase was measured. The CB concentration remaining in the soil phase was measured using a biological oxidizer (Model OX600, R.J. Harvey Instrument Corporation, Hillsdale, NJ).

Table 1
Properties of two wetland soils used in this study

Soils	%H	%O	%N	%S	%Ash	%OC
PPI	0.3	2.0	0.1	0.04	96.4	1.4
Wetland	3.9	23.3	2.1	1.0	42.4	27.6

Table 2
Summary for the preparation of mineralization study

Treatments	Soil type	Aging (days)	Soil wt. (g)	Nutrients (mL)	¹⁴ CB solution (mL)	Bacterial suspension (mL)	<i>R</i> _{s/w} (kg/L)
Soil-free		0	0	20	10 ^a	5	0
	PPI	0	40	30	10 ^b	5	0.89
		0	20	30	10 ^b	5	0.44
		0	2	30	10 ^b	5	0.04
Soil-slurry	Wetland	0	3.68	30	10 ^b	5	0.08
		0	1.84	30	10 ^b	5	0.04
		0	0.18	30	10 ^b	5	0.01
Freshly-contaminated	PPI	2	8.01	15	99.47 ^c	5	0.43
	Wetland	2	1.64	15	71.48 ^c	5	0.08
	PPI	2	7.19	15	111.3 ^c	5	0.32
		7	2.81	15	110.1 ^c	5	0.04
		31	6.6	15	110.7 ^c	5	0.28
Desorption-resistant	Wetland	2	0.92	15	86.5 ^c	5	0.03
		7	6.59	15	83.4 ^c	5	0.28
		31	0.95	15	82.3 ^c	5	0.03

*R*_{s/w}, ratio of soil to water.

^a 2 mg/L of initial concentration was added.

^b 5 mg/L of initial concentration was added.

^c The volume of CB solution (20 mg/L) added into each bottle containing soil was the one when the sorption experiment was initiated.

2.5. Models of mineralization kinetics

Experimental mineralization data, expressed as the percentage (*P*) of the initial activity converted to ¹⁴CO₂ as a function of time (*t*), were fitted to four separate models to describe the rate and extent of mineralization. First, data were fitted to a first-order CO₂ production equation of the form:

$$P = P_{\max}(1 - e^{-kt}) \quad (1)$$

where *P* is the percentage of initial radioactivity mineralized, *P*_{max} is the maximal percentage mineralized, *k* is the first-order rate constant (h⁻¹), and *t* is time (h). Non-linear regression analysis (using Tablecurve 3D, SPSS, Inc.) was used to calculate the parameters *P*_{max} and *k*. Because ¹⁴C-labeled CB conversion to biomass was not accounted for, the resulting model in this approach (as well as those described below) produce conservative estimates of CB degradation for the system studied.

Second, mineralization data were fitted to the coupled degradation-desorption (CDD) model described by Guerin and Boyd [1,7]. The equation is:

$$P = \frac{v_2 t + [(v_1 + v_2)(1 - e^{-kt})]}{k} \quad (2)$$

where *v*₁ represents the initial rate (percent h⁻¹) which provides initial mineralization rates (IMRs, μg L⁻¹ h⁻¹) when normalized to the initial CB concentration (μg L⁻¹). It is comparable to those derived by using the first-order model (Eq. (1)). Parameter of *v*₁/*k*, equivalent of *P*_{max} and *v*₂ represents the mineralization rate resulting from desorption of bound CB, which is related to the kinetics of CB desorption. *P* is the percentage of CB mineralized as a function of time, *t*.

In the third and fourth approaches, a dimensionless bioavailability factor (*B*_f) suggested by Zhang and Bouwer [4] was used to analyze the mineralization data and assess the impact of sorption and desorption behavior on biodegradation in a batch system. The bioavailability factor is defined by the following equation:

$$B_f = \frac{1}{1 + K_d m / v_1} \quad (3)$$

where *K*_d can be obtained from a sorption isotherm study, and *m*/*v*₁ is the soil:water ratio (kg L⁻¹).

Additional equations relating biodegradation to desorption, developed by Feng et al. [19], were used to assess mineralization kinetics. These are expressed as:

$$P_d = P_{\max}(1 - e^{-B_f k t}) \quad (4)$$

$$P_{nd} = B_f P_{\max}(1 - e^{-kt}) \quad (5)$$

The assumption for Eq. (4) is a soil-slurry with instantaneous desorption, while a slurry without desorption is assumed for Eq. (5). *P*_d and *P*_{nd} (%) are the percentage of CO₂ production in the mineralization assay.

Mineralization data for the experiments used to determine biodegradability of freshly added and desorption-resistant CB were plotted together with the theoretical lines, *P*_d (Eq. (4)) and *P*_{nd} (Eq. (5)). If sorbed CB was unavailable to bacteria, the CO₂ production values should be equal to or less than *P*_d values. Cumulative CO₂ production levels lying above *P*_{nd} values indicate that bacteria have an access to sorbed CB. Cumulative CO₂ production rates falling below *P*_{nd} values indicate that bacteria have no access to sorbed CB.

There were no statistical differences in each treatment using pair *t*-test in *P*_{max}, a measure of the extent of mineralization, or the first-order CO₂ production rate constant (*k*), between treatments when compared using Bonferroni's inequality at α = 0.05.

3. Results and discussion

3.1. Effect of soil:water ratios on mineralization

Mineralization of CB in the treatments with various soil:water ratios is presented in Fig. 1. In the absence of soil, approximately 30% of the carbon was converted to CO₂. Mineralization was higher in the presence of soil with approximately 45% and 50% of the carbon converted into CO₂ in the PPI and marsh soil, respectively. Mineralization curves were fitted to the first-order CO₂ production model (Eq. (1)). Parameters are presented in Table 3.

In the PPI soil, the soil:water ratio did not substantially affect mineralization of CB (see Fig. 1, top). No further increase in CO₂ production was detected after approximately 150 h.

In contrast, different extents of mineralization were observed for reactors containing different soil:water ratios in the marsh soil. The *k* values decreased (0.01–0.005) as the soil:water ratio increased and rates were statistically different between all three treatments (α = 0.05). This may be due to lower CB concentration in the aqueous

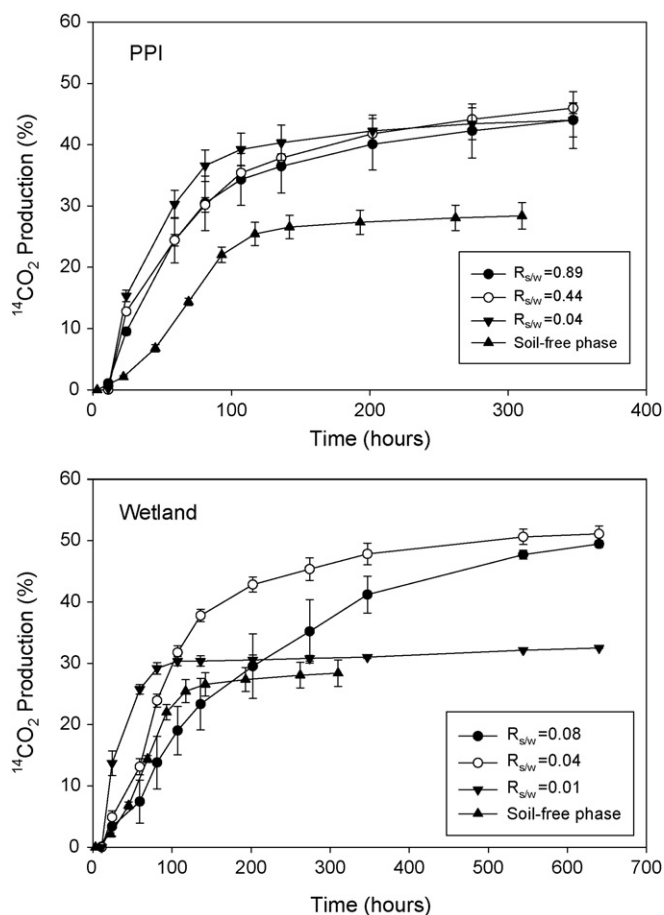


Fig. 1. Patterns of mineralization of chlorobenzene in soil-slurry microcosms containing various moisture contents. $R_{s/w}$, ratio of soil to water.

phase for reactors with higher mass of soil in the early stage of exposure. The increase of soil:water ratio leads to higher soil mass and this will produce higher initial sorption and thereby lower mineralization rate for the aqueous phase CB in the early stage of exposure. The extent of mineralization (P_{max}) in the lowest soil:water ratio (0.01) was not statistically different from the soil-free control. However, the addition of only 0.18 g of marsh soil more than doubled the rate constant (from 0.01 h^{-1} to 0.023 h^{-1}).

It is noted that differences in the patterns of mineralization between the PPI and marsh soils were observed even though soil:water ratios were selected to result in the same range of CB porewater concentrations for both soils. Despite similar porewater concentrations and the same initial microbial population, the mineralization was greatly affected by soil type. This suggests that other soil properties may affect the rate and extent of mineralization and that the relationship between sorption-desorption and mineralization in soils is not simply a function of the porewater concentration and the initial microbial population. Previous studies reported that biodegradation rates were influenced by soil mois-

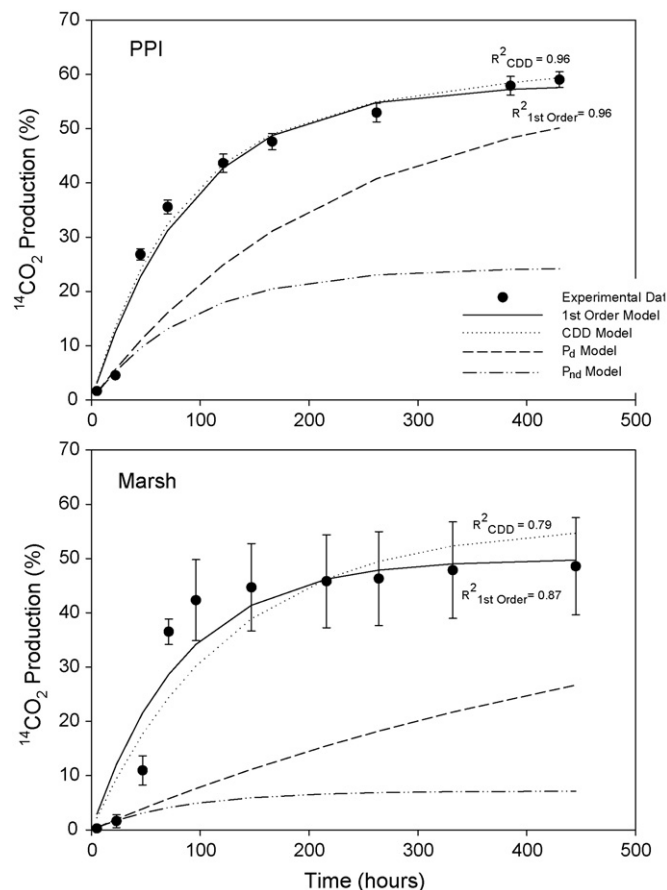


Fig. 2. Plot of mineralization versus incubation time in freshly contaminated PPI and marsh soils.

ture conditions through several mechanisms, including reduced oxygen availability at higher moisture contents and reduced diffusion of the substrate at lower moisture contents [21,31–34]. It is not clear which mechanism was operating in these experiments. Follow-up studies were conducted to assess the effect of sorption on mineralization rates.

3.2. Effect of sorption

To assess the possible role of mass transfer in governing the rate of biodegradation, the rate and extent of mineralization was measured in soils containing freshly added CB. Soil:water ratios (w/v) were selected that are more typical of these soils under field conditions (0.43 for PPI and 0.08 for marsh). As shown in Fig. 2, experimental data were applied to the first-order CO_2 production model, and it proved to fit reasonably well (R^2 values of 0.96 for PPI soil and 0.87 for marsh soil). The observed values of P_{max} were 58.1% and 49.9% in the PPI and the marsh soil, respectively. First-order rate constants were $\sim 0.01 \text{ h}^{-1}$ for both soils when freshly contaminated.

Table 3

Parameter estimates of chlorobenzene mineralization based on the simple first-order and the coupled degradation-desorption models for the freshly contaminated PPI and wetland soil

Soils	First-order model			Coupled degradation-desorption model			
	P_{max}	k	R^2	V_1	V_2	k	R^2
PPI	58.065 (2.647) ^a	0.011 (0.002)	0.962 (3.920)	0.662 (0.092)	0.017 (0.036)	0.013 (0.005)	0.956 (4.161)
Wetland	49.944 (4.285)	0.012 (0.003)	0.867 (6.816)	0.645 (0.125)	−0.037 (0.085)	0.010 (0.006)	0.852 (7.105)

^a Values in parentheses are standard errors of parameter estimates.

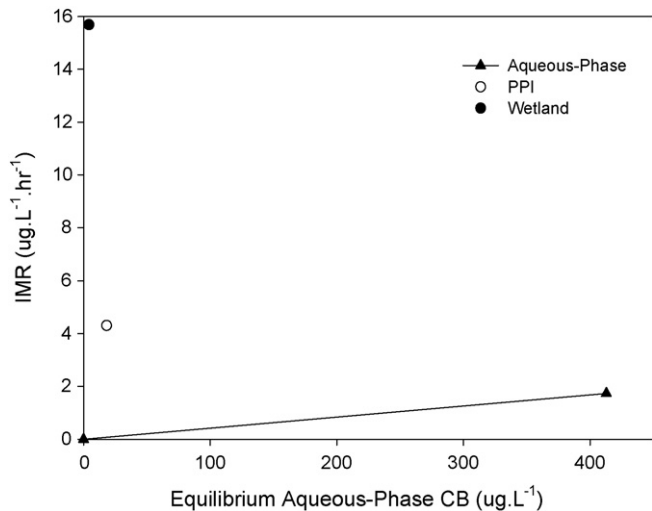


Fig. 3. Plot of an initial mineralization rate under the equilibrium aqueous fraction of chlorobenzene in soil-slurry.

Experimental data were plotted together with theoretical lines, P_d (Eq. (4)) and P_{nd} (Eq. (5)), to evaluate sorbed CB bioavailability. If sorbed CB is not available to bacteria and if only aqueous-phase CB can be degraded in soil-slurries, the amount of initial CO_2 production should be equal or less than P_d , which accounts for instantaneous desorption. The amount of initial CO_2 production should be above P_{nd} , which assumes no desorption. As shown in Fig. 2, the initial CO_2 production levels in soil-slurries are well above P_d values, indicating that mineralization rates are faster than those expected with instantaneous desorption. This implies that bacteria have an access to sorbed CB.

To further investigate the bioavailability of the sorbed CB, data were fit to the CDD model. Estimated parameters of P_{max} ($\approx v_1/k$) are reasonably related with those from the simple first-order model (Eq. (1)). No significant differences were found in the values of initial mineralization rates, v_1 , in either soil. Values of v_2 were low relative to v_1 , confirming that desorption did not contribute significantly to the CB mineralization observed in both soils during the initial stage of transformation. Surprisingly, there were no statistical differences between fitted parameters for the two soils despite large differences in soil properties. The estimate of v_2 in soil-free controls was greater than zero as previously observed [1], indicating the limitations of the model in distinguishing slow desorption rates from experimental noise (Table 3). Since parameter estimates from the first-order and CDD models agreed well, first-order model parameters were used to obtain additional evidence of the sorbed-CB mineralization.

To obtain further evidence of the bioavailability of sorbed CB in soils, initial mineralization rates were calculated from parameters obtained from first-order model fits. The initial mineralization rate ($\mu\text{g L}^{-1} \text{ h}^{-1}$) is the product of P_{max} , k (h^{-1}), and the initial CB concentration ($\mu\text{g L}^{-1}$) and is an estimate of the initial slope of the CO_2 production curve [1,7,19]. IMR values were calculated from mineralization data and plotted as a function of equilibrium aqueous phase CB concentration (Fig. 3). A reference line was constructed using the IMR from the soil-free control and the corresponding initial CB concentration [1]. IMRs plotting above the reference line indicating rates faster than would be predicted from porewater degradation alone proving further evidence for direct degradation of sorbed CB. As shown in Fig. 3, experimental data for both the PPI soil and the marsh soil were above the reference line based on the aqueous phase concentration. This provides further evidence that sorbed CB in both soils was available to microorganisms. In conclu-

sion, mineralization of CB occurred, and bacteria appeared to have access to the pool of freshly added, sorbed CB.

3.3. Effect of desorption-resistance and aging

An experiment was conducted with soils where the sorbed CB was “aged” in the laboratory and then extracted with an isopropanol co-solvent to remove the remaining reversibly bound portion of CB. Two hypotheses were tested from this experiment. An initial hypothesis was such that extraction of the “aged” CB with isopropanol would result in the same concentration regardless of aging time. Secondly, we hypothesized that removing the readily desorbable CB would result in significantly decreased rates and extent of mineralization. As described above, the isopropanol extraction is designed to remove an easily desorbed fraction of the contaminant from the soil. The isopropanol extraction has resulted in observed partition coefficients similar to those obtained after

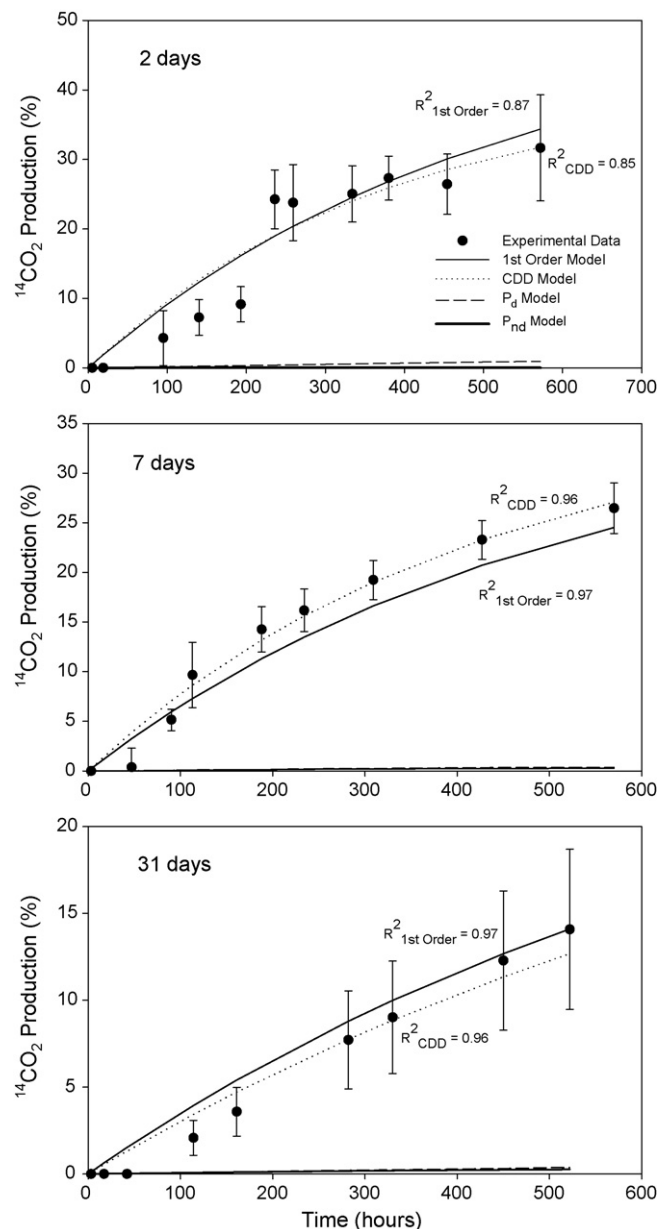


Fig. 4. Patterns of mineralization in PPI soil containing only desorption-resistant chlorobenzene.

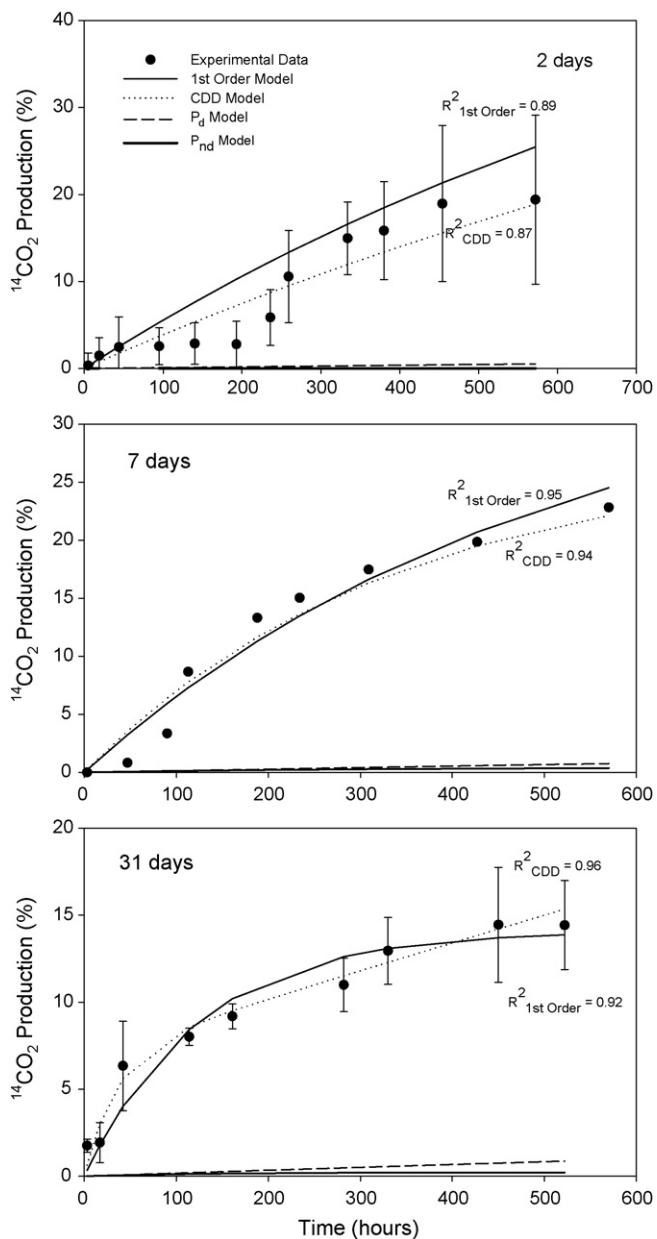


Fig. 5. Patterns of mineralization in marsh soil containing only desorption-resistant chlorobenzene.

long-term multiple desorption steps [30]. These experiments were performed with both soils because they yielded various fractions of desorption-resistant CB with different contact times. The behavior of the marsh soil indicates that equilibrium with this phase may be faster in highly organic soils.

The effects of aging on CB mineralization were significant in both soils. Figs. 4 and 5 show mineralization experiments in both soils with theoretical curves developed from first-order, CDD, P_d , and P_{nd} models. In contrast to the freshly added CB sorption experiments, data from both soils were not well fitted with the first-order and CDD models (Table 4). Although R^2 ranged from 0.85 to 0.97, parameter estimates were often poor with standard errors exceeding the value of the parameter itself. Mineralization curves from the desorption-resistant treatments had distinctly different sigmoidal shapes when compared with the freshly added CB curves. Much of the problem in the model fit appears to result from the lag effect of degradation in most of the treatments. The extent of mineraliza-

Table 4

Parameter estimates of chlorobenzene mineralization based on the simple first-order model and B_f for the soils containing only desorption-resistant chlorobenzene and artificially "aged"

Soils	Aging (days)	B_f	First-order model		
			P_{max}	k	R^2
PPI	2	0.495	50.399 (22.214) ^a	0.002 (0.001)	0.874 (4.047)
	7	0.154	36.058 (5.983)	0.002 (0.001)	0.967 (1.642)
	31	0.093	28.624 (8.998)	0.001 (0.001)	0.969 (1.039)
Wetland	2	0.125	58.415 (55.937)	0.001 (0.001)	0.886 (2.485)
	7	0.138	29.347 (5.148)	0.003 (0.001)	0.949 (1.769)
	31	0.097	14.087 (1.021)	0.008 (0.002)	0.915 (1.310)

^a Values in parentheses are standard errors of parameter estimates.

tion (P_{max}) was different between the two soils ranging from 50.3% to 28.6% in PPI soil and from 58.4% to 14.1% in wetland soil when the soils were aged from 2 to 31 days (Table 4). Due to the standard errors, however, only the P_{max} from the marsh soil after 31 days of aging was statistically lower. No statistical difference was observed in the rates of mineralization although the rates were an order of magnitude lower than those from the previous experiment where CB was freshly added ($\sim 0.001 \text{ day}^{-1}$ versus $\sim 0.01 \text{ day}^{-1}$).

As shown in Figs. 4 and 5, theoretical mineralization curves derived from the P_d and P_{nd} models far underestimated the experimental data. This indicates that CB-degrading bacteria have an access to desorption-resistant CB in both soil types. For these calculations, the bioavailability factor, B_f , was calculated from Eq. (3).

If CB were present in the desorption-resistant fraction of soils containing high organic carbon content, the CB would be less accessible to CB-degrading microorganisms when compared with a soil with low organic carbon content. Despite large differences in organic carbon contents between soils used in the present study, few differences in B_f were observed. Results from the freshly added and desorption-resistant mineralization assays were remarkably similar. Based on these results, there is little evidence that difference in organic carbon content alone results in different mineralization behavior.

This study was in agreement with previous findings [21–23] that aging served to decrease overall mineralization rate and extent. The present study demonstrated that soil-sorbed CB was available to CB-degrading bacteria. The extent of bioavailability of soil-sorbed CB decreased with aging. However, despite large decreases in the porewater concentration CB mineralization was still a significant process influencing the fate of CB in soils. Aging may decrease bioavailability and reduce the mineralization rates, but degradation can potentially still proceed at environmentally relevant rates.

4. Conclusions

1. Differences in soil:water ratios did not affect mineralization rate and extent in the PPI soil despite aqueous phase concentrations. Differences were observed in the marsh soil particularly when the soil:water ratio was very low. In both soils, the presence of soil solids had a stimulatory effect on the rate of mineralization. Mineralization results under different soil:water ratios could not simply be explained as a function of the initial porewater concentration and the initial microbial population size.
2. CB-degrading microorganisms biodegraded sorbed CB in both wetland soils. Evidence for biodegradation of sorbed CB includes mineralization curves that plotted well above theoretical mineralization curves assuming instantaneous desorption preceding biodegradation. Other evidence was also obtained from computation of initial mineralization rates. IMRs were $0.14 \mu\text{g/L/h}$ and $1.92 \mu\text{g/L/h}$ for the marsh soil and mineral PPI soil, respectively.

IMRs plotted above a reference line defined by the mineralization rate of a soil-free control, also indicating that CB-degrading bacteria had access to sorbed CB.

- After aging CB-contaminated soil and the subsequent extraction of a readily desorbable CB fraction, microorganisms still mineralized a portion of the remaining desorption-resistant CB. Despite reductions of porewater CB concentrations of 3 orders of magnitude, mineralization of desorption-resistant CB was observed even though the rate and extent of mineralization were reduced.

Biodegradation of desorption-resistant contaminants is slower but still occurs with environmentally relevant rates in wetland soils where the contaminant was artificially aged using isopropanol. This suggests that microbial degradation could proceed in soils where the chlorobenzene was present in a desorption-resistant form with a much higher partition coefficient. Biological treatment may remain an option at sites with long exposure histories and a significant mass of contaminant in the desorption-resistant form.

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