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Mineralization of hexachlorocyclohexane in soil during solid-phase bioremediation

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Abstract Soil containing hexachlorocyclohexane (HCH) was spiked with ^{14}C-γ-HCH and then subjected to bioremediation in bench-scale microcosms to determine the rate and extent of mineralization of the ^{14}C-labeled HCH to $^{14}CO_2$. The soil was treated using two different DARAMEND amendments, D6386 and D6390. The amendments were previously found to enhance natural HCH bioremediation as determined by measuring the disappearance of parent compounds under either strictly oxic conditions (D6386), or cycled anoxic/oxic conditions (D6390). Within 80 days of the initiation of treatment, mineralization was observed in all of the strictly oxic microcosms. However, mineralization was negligible in the cycled anoxic/oxic microcosms throughout the 275-day study, even after cycling was ceased at 84 days and although significant removal (up to 51%) of indigenous γ-HCH (146 mg/kg) was detected by GC with electron capture detector. Of the amended, strictly oxic treatments, only one, in which 47% of the spiked ^{14}C-HCH was recovered as $^{14}CO_2$, enhanced mineralization compared with an unamended treatment (in which 34% recovery was measured). Other oxic treatments involving higher amendment application rates or auxiliary carbon sources were inhibitory to mineralization. Thus, although HCH degradation occurs during the application of either oxic or cycled anoxic/oxic DARAMEND treatments, mineralization of γ-HCH may be inhibited depending on the amendment and treatment protocol.

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Introduction

The gamma isomer of hexachlorocyclohexane (γ-HCH, lindane) was used worldwide as an insecticide until it was banned or restricted in many developed countries. Commercial formulations of lindane contain a mixture of additional isomers, predominantly α, β and δ. Each of these isomers may be detected in soils at the site of lindane manufacture or in agricultural soils to which the commercial product was applied. The stability and low water solubility of these compounds make them persistent pollutants, while their toxicity creates environmental and human exposure concerns.

Microbial degradation of all four HCH isomers has been observed in soil under anaerobic conditions [12, 16, 26]. Aerobic biodegradation has been observed either in soil and soil slurries [3, 11, 23] or in liquid media and pure cultures [4, 13, 28]. Most studies, however, measure HCH degradation by monitoring the disappearance of parent compounds only. It is important, from a bioremediation standpoint, to have knowledge of the end-products of biodegradation, as some intermediary metabolites may be more toxic than the original contaminants. Monitoring for mineralization using radiolabeled tracers is an effective means of determining whether complete degradation has occurred. Mineralization of γ-HCH has received little attention, particularly in soil-phase, uninoculated soil, or soil containing HCH concentrations as high as those described in this study (1,736 mg/kg total HCHs).

In one of the few studies where ^{14}C-γ-HCH was used to monitor mineralization, Mougin et al. [19] demonstrated mineralization in a soil that contained only 0.8 mg/kg lindane and was moistened using culture media. When the soil was inoculated with *Phanerochaete chrysosporium* BKM-F-1767, 49.1% of the added

radiolabel was mineralized in 9 weeks, compared with uninoculated soil in which 21.6% was mineralized. Mineralization of ^{14}C-γ-HCH by *P. chrysosporium* BKM-F-1767 was also demonstrated in a pure culture [7] and an inoculated soil–corncob mixture [14]. Kohnen et al. [15] compared mineralization in a submerged soil and an aerated, moist soil, each spiked with only 10–20 mg/kg ^{14}C-γ-HCH. Mineralization was negligible in the moist soil (3% recovery of ^{14}C as $^{14}CO_2$ in 105 days) unless the soil was inoculated with a mixture of organic amendments (16.7% recovery of ^{14}C as $^{14}CO_2$ in 133 days). Mineralization of ^{14}C-γ-HCH has also been reported in flooded soil columns containing up to 60 mg/kg HCHs [16] and using pure cultures of *Sphingomonas paucimobilis* UT26 [20] and *Pseudomonas* sp. [24].

We previously described bioremediation of an industrial soil containing HCHs in bench-scale laboratory microcosms using DARAMEND technology [22] under both oxic and anoxic/oxic cycled conditions. This solid-phase bioremediation technology employs organic and inorganic amendments to stimulate the decomposition of organic contaminants by indigenous soil microorganisms. The proprietary DARAMEND products are manufactured from natural plant fibers. The strictly oxic treatment involves the use of a particular amendment chosen for its capacity to facilitate aeration and promote biodegradation of organic contaminants. The cycled anoxic/oxic treatment protocol requires the addition of a different amendment combined with water and trace quantities of powdered metals, primarily zero-valent iron, to generate strong reducing (anoxic) conditions in the soil. The strongly anoxic environment enhances the rate of reductive dechlorination of organochlorine pesticides. A return to oxic conditions, through aeration and passive drying of the soil, promotes further degradation of the dechlorination products.

In our previous work, the removal of HCHs was monitored only by the disappearance of indigenous HCH compounds from the soil and both the cycled anoxic/oxic and strictly oxic treatments were comparable in their ability to enhance HCH removal in soil microcosms [22]. The present research expands upon the previous study in that soil microcosms treated using the most effective amendment protocols from the above-referenced work were spiked with ^{14}C-γ-HCH in order to determine whether complete degradation (mineralization) of HCHs occurred and to compare rates of mineralization under both strictly oxic and cycled anoxic/oxic conditions.

Materials and methods

Soil preparation and characterization

Soil from a former lindane-manufacturing facility in the mid-western United States was air-dried at 22°C, sieved (4.75 mm, number 4 mesh, US standard testing sieve) and homogenized using a rotary mixer (Blakeslee, Scarborough, Canada). The prepared soil was stored at 4°C in sealed plastic pails until needed. Triplicate samples of the prepared soil were analyzed for HCH concentrations as follows. Subsamples of 1 g of soil were extracted using US EPA extraction method 3541 for automated Soxhlet extraction and analyzed for HCH concentrations using US EPA analytical method 8081 and a Hewlett Packard 5890 series 2 gas chromatograph with electron capture detector. One soil sample was also analyzed for pH and plant-available carbon and nitrogen by the University of Guelph Soil and Nutrient Laboratory (Guelph, Canada), using standard methods [8]. Soil moisture content and water-holding capacity (WHC) were determined using standard methods [6].

Soil microcosm studies

Microcosms were prepared using United States quart-size glass mason jars, each containing 95 g of prepared air-dried soil. Prior to amendment application, the soil in each microcosm was spiked with 1×10^6 disintegrations/min (dpm) of ^{14}C-UL-γ-HCH (specific activity = 10.1 mCi/mmol; Sigma-Aldrich Canada, Oakville, Canada), applied dropwise in 24 µl of toluene using a Hamilton syringe. Amendments were then added with stirring, followed by adequate amounts of water to yield a soil moisture content of the desired percent WHC (60% or 90%). A glass test tube containing 15 ml of NaOH (2 N), to trap $^{14}CO_2$ resulting from mineralization of the ^{14}C-γ-HCH, was placed in each microcosm jar. The jars were sealed and stored in the dark at 22°C.

Two different DARAMEND products (D6386, D6390; US patents 5,411,664, 5,480,579, 6,083,394, Adventus Remediation Technologies, Mississauga, Canada, http://www.adventusremediation.com) were used to treat the microcosm soils. The two products were plant-derived organic amendments, manufactured from natural plant fibers and differing by the type of plant material used. The main difference between the two amendments was their carbon:nitrogen (C:N) ratio. D6390 contained 42% total organic carbon and 2.8% total nitrogen, for a C:N ratio of 1:15. D6386 contained 40% total organic carbon and 0.5% total nitrogen, for a C:N ratio of 1:80. The chloride concentrations were 1,928 mg/kg for D6390 and 885 mg/kg for D6386. Product D6390 was applied to all microcosms subjected to anoxic/oxic cycling. This amendment generates strong reducing conditions in soil [22]. Microcosms treated with product D6386 were incubated under strictly oxic conditions, with regular stirring and aeration.

Each of six treatments (three anoxic/oxic cycled treatments, three oxic treatments) was prepared in triplicate as described in Table 1. A set of three microcosms, containing only air-dried soil, was maintained as the control while unamended, moistened, stirred soil was designated treatment 7. Treatments 1–3 were amended with DARAMEND D6390 and powdered zero-valent

Table 1 Treatment protocol for bioremediation of soil containing HCH, in laboratory microcosms. D6390 and D6386 are DARAMEND organic soil amendment designations for specific proprietary formulations. Application rates are given as percentages (w/w). *Sieved* sieved to particle size >500 μm

Treatment	Amendment 1	Amendment 2	Amendment 3	Percentage of WHC	Cycled anoxic/oxic or oxic
Control	None	None	None	As is	Oxic
1	2% D6390	0.2% Fe	None	90	Cycled anoxic/oxic
2	2% D6390	0.2% Fe	CaOH to pH 8.4	90	Cycled anoxic/oxic
3	2% D6390	0.2% Fe	1% corn syrup	90	Cycled anoxic/oxic
4	3% D6386	None	None	60	Oxic
5	6% D6386 sieved	None	None	60	Oxic
6	6% D6386 sieved	None	1% corn syrup	60	Oxic
7	None	None	None	60	Oxic

iron, to create anoxic conditions in the microcosm soil. Treatments 4–6 were maintained under oxic conditions with DARAMEND D6386, either in its original form or sieved to remove particles smaller than 500 μm (Table 1). Additional amendments (for supplemental carbon sources or pH adjustments) were added to certain treatments as indicated in Table 1. Corn syrup (Bee Hive, Best Foods Canada, Etobicoke, Canada) and hydrated lime (CaOH) were purchased from local retail stores. Powdered reduced iron (product 286024P) was purchased from VWR Canlab (Mississauga, Canada). Subsequent amendment applications in the cycled anoxic/oxic treatments were calculated with consideration given to the amount of soil removed from the microcosms during sampling. Estimates of the decomposition of organic matter in soil [1, 27, 29] were used to determine that any dilution effect from the addition of the amendments could be considered negligible.

Soil pH was measured for adjustment of treatment 2 using a flat surface combination pH reference electrode (Canadawide, Ottawa, Canada) applied directly to the soil in the microcosm and a Barnant 20 digital pH/mV/ORP meter (Barnant Co., Barrington, Ill.).

Soil receiving oxic treatments was hydrated to 60% of the WHC and were stirred with a spatula and passively aerated weekly by removal of the lids for 15 min. Although the soil redox potential was not measured, past studies indicated that weekly stirring and aeration resulted in positive soil redox values. Soils receiving cycled anoxic/oxic treatments were maintained under anoxic conditions for 1 week prior to initiation of the oxic portion of the cycle. After 7 days, the soil redox was measured, to confirm that anoxic conditions had been achieved, using a Cole Parmer G-27001-62 disposable industrial ORP double-junction electrode redox probe (Labcor, Concord, Canada) and the Barnant ORP meter. Then the soil was stirred and the microcosms were left open for 1 day to allow the soil to dry to approximately 60% of WHC. On the second day, the soil was stirred again and the jars were resealed and stored for the remainder of the week. Although the soil redox potential was not measured during the oxic phase, preliminary experiments with the cycled treatment indicated this protocol of drying overnight, stirring and aeration resulted in positive soil redox potentials. After 7 days, the cycle was repeated.

Microcosms were maintained for 84 days, during which time cycled treatments underwent six complete anoxic/oxic cycles. After this period, all microcosms were maintained under oxic conditions and stirred and aerated weekly until 275 days had elapsed. On days 28, 84, 134 and 275, individual soil samples (10 g or 20 g) were removed from each microcosm and analyzed for HCHs and pH. The NaOH traps in each microcosm were replaced biweekly throughout the study and the evolution of $^{14}CO_2$ was monitored as follows. Aliquots (1 ml) of the NaOH were neutralized with 1 ml acetic acid (2 N) and stirred with 15 ml of Scintisafe scintillation cocktail (Fisher Scientific, Whitby, Canada). The samples were analyzed using a Beckman LS6500 liquid scintillation counter (Beckman Coulter Canada, Mississauga, Canada). Least significant difference (LSD) analysis was used to compare the cumulative ^{14}C recovery as $^{14}CO_2$ or the soil extraction data for all microcosms at once, while comparisons between the mean values of two individual treatments were performed using Student's *t*-test [10].

Results and discussion

Soil characterization

The prepared soil contained HCHs at initial concentrations of 1,360, 196, 34 and 146 mg/kg for α-, β-, δ- and γ-isomers, respectively (Table 2). Total nitrogen and total organic carbon contents were 0.02% and 0.13%, respectively. The soil pH was 6.3 and the moisture content was 7.4%, at the start of the study.

Microcosm maintenance

Maintenance of the soil microcosms included measurement of the soil redox potential during the anoxic phases to ensure that anoxic conditions were achieved. During the anoxic phases, redox potentials in the soil subjected to cycled treatments ranged from -300 mV to -500 mV. Due to the relatively low soil moisture content, the redox potential was not measured in the strictly oxic soil microcosms. However, in similar studies using the same soil at 60% of WHC, the application of 3%

Table 2 Influence of soil treatments on concentrations of alpha, beta, delta, gamma and total HCH in soil following 84 days of treatment. Initial values are time-zero concentrations and all values given are the average of triplicate soil microcosms. *LSD* Least significant difference ($\alpha = 0.05$)

Treatment	Isomer concentration [mg/kg (± standard deviation)]				
	Alpha	Beta	Delta	Gamma	Total
Initial	1,360 (104)	196 (14)	34 (0)	146 (82)	1,736 (74)
Control	1,795 (341)	238 (25)	42 (8)	132 (44)	2,208 (397)
1	1,747 (227)	323 (41)	56 (22)	71 (42)	2,197 (227)
2	1,721 (419)	291 (53)	44 (10)	80 (61)	2,135 (411)
3	1,985 (106)	297 (44)	52 (3)	107 (64)	2,441 (224)
4	365 (76)	180 (40)	4 (1)	47 (29)	596 (90)
5	295 (179)	156 (38)	4 (2)	24 (14)	479 (204)
6	1,065 (643)	257 (95)	6 (0)	51 (30)	1,378 (760)
7	2,062 (352)	310 (36)	13 (4)	40 (7)	2,426 (398)
LSD	608	86	16	67	688

D6386 and weekly stirring resulted in consistently positive redox values ($> +100$ mV).

The soil pH in treatment 2 returned to neutral values by the end of the oxic phase and thus re-application of CaOH at the initiation of the next anoxic cycle was required to raise the pH to 8.4. The drop in pH was presumably due to the accumulation of volatile fatty acids or protons produced by microbial processes.

HCH degradation and mineralization of ^{14}C-γ-HCH

Mineralization data, showing the cumulative percent recovery of $^{14}CO_2$ from ^{14}C-γ-HCH, are shown in Fig. 1. Mineralization in the unstirred control was negligible ($< 1\%$ cumulative recovery). Over 20% cumulative recovery of $^{14}CO_2$ was observed in each of the oxic treatments. In the stirred, unamended treatment 7, 34% of the radiolabeled γ-HCH was mineralized to CO_2 by day 78. This level of recovery exceeded those reported by Kohnen et al. [15] and Mougin et al. [19], who described lower percent recoveries (16.7%, 21.6%, respectively) from comparable spikes added to soils that had been amended with additional sources of carbon and nutrients. The application of 3% D6386 (treatment 4) was the only treatment in which cumulative mineralization exceeded that of treatment 7. The cumulative percent recovery of $^{14}CO_2$ for treatment 4 reached 47% by day 78 and did not change appreciably for the remainder of the experiment. Kohnen et al. [15] also reported enhanced mineralization of ^{14}C-γ-HCH in soil amended with organic amendments, compared with an unamended, aerated soil in which mineralization was negligible ($< 10\%$ cumulative recovery).

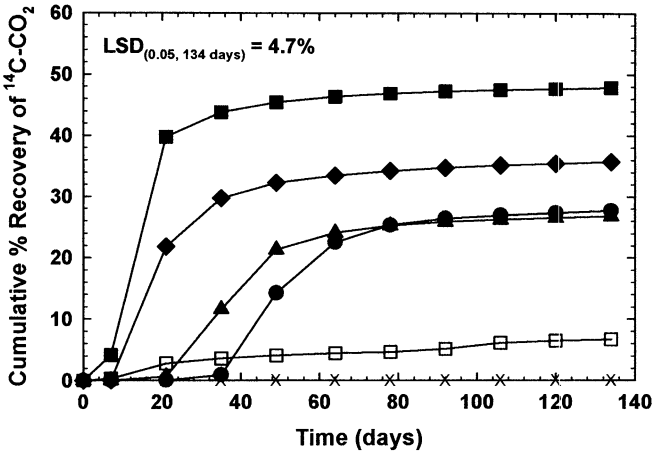

Fig. 1 Cumulative percent recovery of $^{14}CO_2$ during mineralization of ^{14}C-HCH in soil during 134 days of bioremediation in microcosms. Each data point is the average of triplicate samples. *Crosses* Unstirred control, *open squares* treatment 1, *filled squares* treatment 4, *filled triangles* treatment 5, *filled circles* treatment 6, *filled diamonds* treatment 7

Amendment of soil with sieved D6386 (treatments 5, 6) was intended to create a more open soil matrix with an increased number of air-filled macropores. However, both of these oxic treatments inhibited γ-HCH mineralization, as demonstrated by an increased lag time prior to the onset of mineralization and lower recovery of $^{14}CO_2$. Doubling the amendment application to 6% and addition of a supplemental carbon source (corn syrup) might have negated the benefit of increased aeration. It was intended to provide additional carbon to compensate for reduced bioavailability (due to large particle size) of carbon in the sieved amendment. This might not have been necessary and the indigenous microorganisms might have failed to induce enzymes for the mineralization of xenobiotic compounds such as HCH, because more readily metabolized carbon sources were present. Repression of HCH biodegradation by glucose and other carbon amendments has been reported for aerobic soil slurries [2] and stirred anaerobic cultures [18]. However, other reports have indicated that carbon amendment might enhance HCH removal, depending on the nutrient requirements of the degrading microorganisms and the initial carbon content of the soil [9, 12, 17], or by promoting lower redox potentials and enhancing anaerobic degradation rates [26]. In this study, the extraction data demonstrated that HCH removal (without mineralization) was equal in treatments 4 and 6 by day 275 (Fig. 2, Table 3), suggesting that, although mineralization was inhibited, the ability of indigenous microorganisms to dechlorinate HCHs was not impaired by the additional carbon.

Mineralization was not detected at significant levels ($< 10\%$ cumulative recovery) in any cycled anoxic/oxic

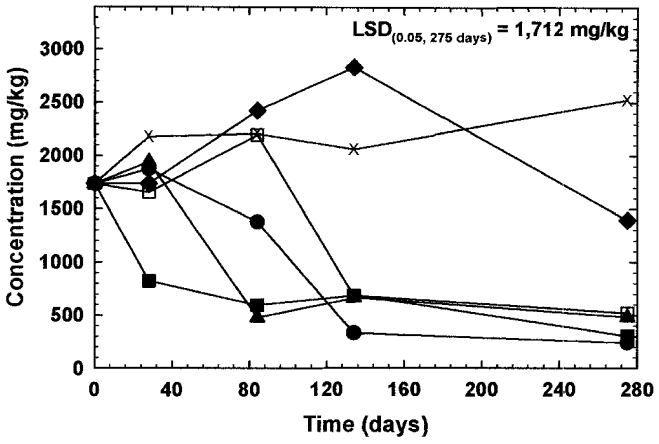

Fig. 2 Total HCH concentrations in soil following 275 days of bioremediation in microcosms. Each data point is the average of triplicate samples. *Crosses* Unstirred control, *open squares* treatment 1 *filled squares* treatment 4, *filled triangles* treatment 5, *filled circles* treatment 6, *filled diamonds* treatment 7

treatments. A plot of the data from treatment 1 is shown in Fig. 1 to represent the cycled treatments. MacRae et al. [16] used ^{14}C-γ-HCH to demonstrate mineralization of γ-HCH in several submerged soils spiked with 15–60 mg/kg HCHs. Up to 30% recovery, from a spike of approximately 2×10^8 dpm per 20 g soil, was attained in 60 days, depending on the soil tested. Results reported by Kohnen et al. [15] were also in contrast to our study, in that γ-HCH mineralization was more rapid in a submerged soil than in a moist, aerated soil. In both studies, the assumption was made that the submerged soils were predominantly anoxic. However, redox potentials were not reported. It is possible that mineralization of γ-HCH might occur under slightly anoxic conditions but is inhibited under the highly anoxic conditions obtained using cycled anoxic/oxic DARAMEND technology. The reason for the lack of mineralization in the cycled treatments, even after cycling had ceased and oxic conditions were maintained (days 84–275) is not known. One possibility is that, following dechlorination, much of the radiolabel converted to dead-end metabolites. For example, 1,2,4-trichlorobenzene and 2,5-dichlorophenol are thought to form spontaneously from unstable intermediates of γ-HCH degradation and are not readily degraded by *S. paucimobilis* UT26 [21].

The higher chloride content of amendment D6390, compared with amendment D6386, may also have been responsible for the reduced rate of HCH removal and mineralization in cycled anoxic/oxic microcosms. A single application of D6386 at a rate of 3% added about 2.5 mg chloride to each microcosm. During cycling, seven applications of D6390 at a rate of 2% contributed approximately 27 mg chloride per microcosm, i.e., tenfold more than in the oxic treatments. Seech et al. [25] reported that chloride ions at 100 mg/l completely inhibited PCP mineralization by pure cultures of PCP-degrading bacteria. Other differences between the two amendments (i.e., nitrogen content) might also have contributed to differences in HCH degradation rates between the two treatment protocols. Extraction data verified destruction of HCH compounds, which can be accomplished by removal of a single chlorine atom. Thus, it is possible to achieve substantial degradation of HCH even when there has been little or no mineralization. Further studies are needed to determine the predominant intermediates of HCH degradation that may allow for identification of a bottleneck in HCH metabolism.

Extraction data from day 84 indicated that the concentrations of total HCHs in the oxic treatments 4, 5 and 6 were reduced significantly compared with control values. The same trend was observed for the predominant α-isomer. The β-isomer was not reduced significantly in any treatments. This was not surprising, as the β-isomer is the most recalcitrant of the HCH isomers [13, 23] and is most readily degraded anaerobically [26]. The δ- and γ-isomers were significantly reduced in all oxic treatments (4, 5, 6, 7). In treatment 4, where the most rapid mineralization of ^{14}C-γ-HCH was observed, total and γ-HCH concentrations were reduced by 66% and 68%, respectively, by day 84. Treatment 4 was the only treatment to show a significant reduction in total HCHs as early as day 28 (Fig. 2). In the same treatment, the γ-HCH concentration was reduced by 98% after 275 days, resulting in a residue of only 3 mg/kg (Table 3).

Extraction data and LSD analysis for days 84 and 275 indicated significant reductions of indigenous

Table 3 Influence of soil treatments on the concentration of alpha, beta, delta, gamma and total HCH in soil following 275 days of treatment. Initial values are time-zero concentrations and all values given are the average of triplicate soil microcosms. *LSD* Least significant difference ($\alpha = 0.05$)

Treatment	Isomer concentration [mg/kg (± standard deviation)]				
	Alpha	Beta	Delta	Gamma	Total
Initial	1,360 (104)	196 (14)	34 (0)	146 (82)	1,736 (74)
Control	2,107 (1,899)	294 (248)	42 (18)	85 (96)	2,528 (2,260)
1	344 (485)	164 (55)	5 (4)	7 (9)	521 (544)
2	26 (22)	102 (30)	2 (0)	1 (0)	131 (32)
3	123 (21)	136 (23)	3 (1)	2 (1)	265 (30)
4	162 (92)	137 (41)	3 (1)	3 (2)	305 (59)
5	381 (558)	92 (65)	6 (5)	4 (5)	482 (629)
6	160 (138)	48 (17)	2 (1)	32 (51)	241 (194)
7	1,138 (150)	235 (22)	9 (1)	12 (2)	1,394 (170)
LSD	1,255	165	12	67	1,483

γ-HCH in the cycled anoxic/oxic treatments (Tables 2, 3). Statistical analysis (t-test$_{(\alpha=0.05)}$) between individual treatments indicated that, by day 275, the removal of total HCHs was significantly greater in all of the amended treatments than in the untreated control or the unamended, stirred treatment 7. The removal of total HCHs from treatments 1–3 and the removal of γ-HCH from treatments 2 and 3 resulted in residual concentrations comparable with those in the oxic treatments (Table 3). These results agree with our previous study, in which comparable reductions in total HCH concentrations could be attained in microcosms treated under either oxic or cycled anoxic/oxic conditions [22].

Soil extraction data for samples taken on day 84 indicated that the concentrations of total HCHs and some individual isomers, in some cases, apparently increased. It was also observed that certain triplicate sets of samples, particularly those with high HCH concentrations, exhibited large variability (standard deviations nearly equal to the average HCH concentrations). These phenomena might be attributed to the presence of visible granules of solid HCHs in the soil. Inclusion of one of these granules in a sample for extraction would result in a "spike" of HCH in that sample. Generally, as degradation of HCHs resulted in lower total concentrations, variability in the data was also reduced. As dissolved HCHs were gradually removed, more of the pure product could become dissolved in soil pore water. Furthermore, soil homogeneity might have increased as the study proceeded, due to frequent stirring.

Based on standard calculations of biomass carbon [5], we estimated the proportion of organic material to be incorporated as biomass C (k_c) at 0.45. Using this value, we can estimate that 55% of the ^{14}C-HCH should be recovered via respiration or bound irreversibly to the soil, whereas 45% of the label would end up as biomass. Recoveries of nearly 50% of the total radiolabel as $^{14}CO_2$ suggest that degradation was nearly complete. These data have significant implications for field application, in that nearly 50% of the ^{14}C-γ-HCH spike was completely mineralized within one typical temperate-climate growing season (100–200 days) under oxic conditions and substantial removal of total HCHs was observed during the 275 days of treatment (Fig. 3).

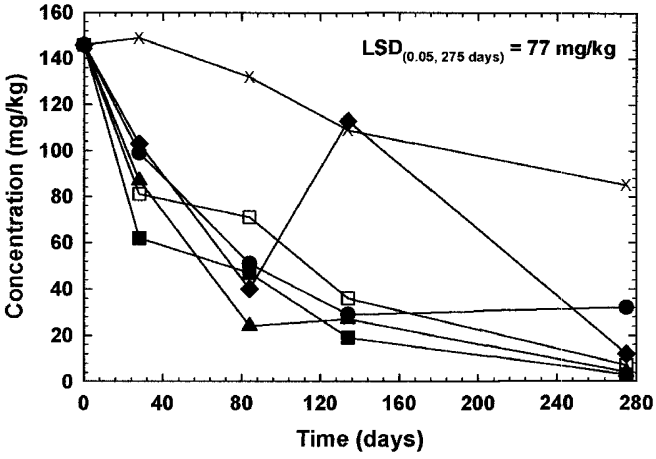

Fig. 3 Lindane concentrations in soil following 275 days of bioremediation in microcosms. Each data point is the average of triplicate samples. *Crosses* Unstirred control, *open squares* treatment 1, *filled squares* treatment 4, *filled triangles* treatment 5, *filled circles* treatment-6, *filled diamonds* treatment 7

Conclusions

Current opinion is divided on the subject of whether HCHs are more readily biodegraded under aerobic or anaerobic conditions. This study offers insight as to which treatment approach might result in more complete degradation of target HCH compounds during solid-phase bioremediation treatment, particularly using DARAMEND amendments. The results demonstrate that mineralization of γ-HCH is possible during solid-phase bioremediation treatments of a highly contaminated soil, under oxic conditions, but mineralization might be inhibited by excessive carbon amendment. In the cycled anoxic/oxic treatments, HCH removal did not appear to be accompanied by mineralization of the added radiolabel to $^{14}CO_2$, suggesting that the indigenous HCH isomers were merely biotransformed. Lack of mineralization during the cycled anoxic/oxic treatment might be due to a number of factors, including the high moisture content and extremely anoxic conditions, or different chemical makeup of the amendment used. Further investigation into each of these factors is warranted.

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