Reductive Dechlorination of α−, β−, γ−, and δ−Hexachlorocyclohexane Isomers with Hydroxocobalamin, in Soil Slurry Systems

ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · SEPTEMBER 2010

Impact Factor: 5.33 · DOI: 10.1021/es1012438 · Source: PubMed

Reductive Dechlorination of α-, β-, γ-, and δ-Hexachlorocyclohexane Isomers with Hydroxocobalamin, in Soil Slurry Systems

B. RODRÍGUEZ-GARRIDO,*,† T. A. LÚ-CHAU,‡ G. FEIJOO,‡ F. MACÍAS,§ AND M.C. MONTERRROSO§

Instituto de Investigaciones Agrobiológicas de Galicia, CSIC. Apdo. 122. Santiago de Compostela, E-15780, Spain, Department of Chemical Engineering, Institute of Technology, University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain, and Departamento de Edafoloxía e Química Agrícola, Facultade de Bioloxía, Universidade de Santiago de Compostela, E-15782 Santiago de Compostela, Spain

Received April 23, 2010. Revised manuscript received July 23, 2010. Accepted July 27, 2010.

The present study was carried out to test the viability of a method of reductive dehalogenation of α-, β-, γ-, and δ-hexachlorocyclohexane (HCH) in soil slurry systems. The soil slurries were maintained under anaerobic conditions, with titanium(III) citrate as a reducing agent and hydroxocobalamin (vitamin B_{12a}) as a catalyzing agent. Experiments were carried out with two soil samples with markedly different characteristics (particularly regarding organic matter content), at a small scale and larger reactor scale. HCH concentration was monitored throughout the 24 h duration of the tests. In the low organic matter soil HCH isomers degraded rapidly, in both the small scale and reactor systems, and undetectable levels (<0.5%) were reached within 5 h. However, complete degradation of HCH isomers was not achieved in soil with high organic matter content, and there were differences between the results obtained in the small scale and reactor systems. In the small scale system, the levels of degradation reached 93, 88, 94, and 91%, for α-, β-, γ-, and δ-HCH, respectively, and the nondegraded HCH was sorbed in the soil. In the reactor system, the reaction stopped after two hours (no more than 65% of any of the isomers was degraded).

Introduction

Hexachlorocyclohexane (HCH) is one of the most widely used chlorinated pesticides. There are eight geometric isomers of HCH, which are differentiated by the axial or equatorial positions of the chlorine atoms around the cyclohexane ring (α, β, γ, θ, η, ε, δ). HCH is commercially produced by the photochemical chloration of benzene and the resulting product is a mixture of different isomers known as "technical HCH" (55−80% α-HCH, 5−14% β-HCH, 8−15% γ-HCH, and 2−16% δ-HCH (*1*)). Technical HCH was used for many years as an insecticide until it was discovered that only the γ-HCH isomer possesses insecticidal properties, at which it began to be refined and commercialized under the name lindane (which contains at least 99% of the γ-HCH isomer (*2*)). During production of lindane, the other isomers were dumped uncontrolledly in areas close to the manufacturing sites, thus generating a highly toxic and persistent waste (*3*). HCH has been detected in all environmental reservoirs and there are several strongly contaminated sites worldwide, some of the most important of which are in China, India, U.S., Holland, and Spain (*1, 4*). This has led to the development of site decontamination methods in which the complete degradation of the contaminant is the final aim.

Early studies by MacRae et al. (*5*) already suggested that degradation of HCH was faster under anaerobic conditions than under aerobic conditions, and that microbial degradation was the main route of disappearance of HCH from soil. Subsequent studies coincide in that under anaerobic conditions, γ-HCH is the most readily degradable isomer, followed by α-HCH, with δ-HCH and particularly β-HCH being the most recalcitrant isomers (*6*−*9*).

Degradation of HCH (mainly γ-HCH) catalyzed by single chemical compounds, has also been reported. Under abiotic conditions degradation is influenced by both pH and Eh (*10*). Wang et al. (*11*) investigated dechlorination of γ-HCH by granular zerovalent iron under different pH, iron dosage and temperature conditions and found that γ-HCH was rapidly reduced, principally to benzene but also to CB. The photocatalytic degradation of γ-HCH was studied in aqueous solutions in the presence of the polyoxometalate $PW_{12}O_{40}^{3-}$ (*12*). Lindane was fully decomposed to CO_2, Cl^-, and H_2O, and a variety of intermediates were detected. The authors concluded that the number and nature of the intermediates identified suggest that the mechanism of lindane decomposition is based on both oxidative and reductive processes. Marks et al. (*13*) demonstrated reductive dechloration of γ-HCH with tetrapyrroles, using dithiothreitol (DTT) as a reducing agent. In a previous study carried out in our laboratory (*14*), dehalogenation of all of the HCH isomers was demonstrated with vitamin B_{12a} as a catalyzing agent and DTT or Ti (III) citrate as reducing agent, although the degradation was much more rapid with the latter agent.

Under natural conditions, hydrophobic compounds accumulate in the soil solid phase (mainly in the organic matter) and spend a short time in solution, and therefore in situ decontamination treatments (chemical or biological) are limited by a low mass transfer rate (*15, 16*). The size of particles and aggregates, and soil structure, also contribute to making the desorption rate (mass transfer rate) the limiting stage in their degradation (*17*). Ex situ reactors, in which the soil is maintained in suspension in an aqueous medium and vigorously shaken, can shorten the treatment time (*18, 19*).

The main objective of this study was to investigate the degradation of α-, β-, γ-, and δ-HCH isomers in a soil slurry system under anaerobic conditions, with vitamin B_{12a} as a catalyzing agent and Ti (III) citrate as a reducing agent. The study was carried out at small scale and at a larger scale in a reactor, with two soils with very different organic matter contents; the soil samples were artificially contaminated with HCH prior to the tests.

Material and Methods

Soil Samples. Two samples of soil from Ah and Bw horizons were used for the degradation studies (hereafter named A and B horizons). The soil samples had similar properties except for the organic matter, exchangeable Al and Fe oxyhydroxides contents (Table 1). The soil was developed on granite in an area located in the region of Pontevea

* Corresponding author phone: +34-981-590958; fax: +34-981-592504; e-mail: beatriz@iiag.csic.es.
† Instituto de Investigaciones Agrobiológicas de Galicia (CSIC).
‡ Department of Chemical Engineering.
§ Departamento de Edafoloxía e Química Agrícola.

10.1021/es1012438
Published on Web 08/17/2010

TABLE 1. Properties of the Soil Samples Used in the Degradation Studies

	A horizon	B horizon
pH (H_2O)	4.86	5.12
pH (KCl)	3.78	3.99
C (g kg^{-1})	42.6	3.3
N (g kg^{-1})	2.89	1.1
C:N ratio	14.7	2.9
[a]eCEC (cmol(+) kg^{-})	4.02	1.68
Ca^{2+} (cmol(+) kg^{-})	0.13	0.09
Mg^{2+} (cmol(+) kg^{-})	0.14	0.04
Na^{+} (cmol(+) kg^{-})	0.42	0.41
K^{+} (cmol(+) kg^{-})	0.11	0.11
Al^{3+} (cmol(+) kg^{-})	3.22	1.03
sand (%)	80.8	84.2
silt (%)	15.1	13.6
clay(%)	4.1	2.2
[b]Fe_o (g kg^{-1})	0.13	0.01
[c]Al_o (g kg^{-1})	0.04	0.01
[d]Fe_d (g kg^{-1})	0.39	0.05
[e]Al_d (g kg^{-1})	0.04	0.01

[a] eCEC = Effective cation exchange capacity (Σ Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Al^{3+}, extracted with 1 M NH_4Cl). [b] Fe_o = ammonium oxalate extractable Fe. [c] Al_o = ammonium oxalate extractable Al. [d] Fe_d = dithionite-citrate extractable Fe. [e] Al_d = dithionite-citrate extractable Al.

(Pontevedra, NW Spain) and was classified as Cambic umbrisol (Humic, Alumic) (*20*), and as Humic Dystrudept (*21*). The soil had a sandy loam texture and was acid, had low clay content and a very low cation exchange capacity; the exchange complex was predominated by aluminum. The organic matter content of the A horizon was relatively high (4.26% C), but the B horizon contains almost no organic matter. In addition, the levels of exchangeable Al and of Fe oxyhydroxides are significantly higher in the A horizon than in the B horizon (Table 1).

The soil samples were dried at room temperature, sieved (2 mm), homogenized and ground. They were then spiked with a solution of acetone containing α-, β-, γ-, and δ-HCH isomers in order to achieve a final concentration of approximately 50 mg kg^{-1} for each HCH isomer. The mixture was left for 24 h under a fume extraction hood to facilitate the complete evaporation of the acetone. α, β-, δ- HCH (purity, 98.7, 98.1, and 98.6%, respectively) were obtained from Riedel-de-Haën AG and γ-HCH (purity 99%) from Sigma.

Study of Degradation in a Small Scale System. The dehalogenation tests with contaminated soils (horizons A and B) in suspension were carried out in triplicate in 50 mL glass vials with Teflon lined silicone septums (Supelco). One gram of contaminated soil was weighed into each vial, and tris-HCl buffer (400 mM, pH 8.9) and Ti (III) citrate (500 mM solution) were added to final concentrations of 200 mM and 5 mM, respectively. Ti (III) citrate was prepared in 200 mM Tris buffer from citric acid and Ti (III) chloride with a final molar ratio of citrate:Ti(III) of 2:1 inside a glovebox (N_2 atmosphere). The soil suspensions were purged with N_2 for 5 min and shaken for 24 h to equilibrate them. Samples were removed for analysis at the end of the 24 h period, which was considered the zero reaction time ($t = 0$). At this point, an additional 200 μL of 500 mM Ti citrate and 200 μL of 73 μM vitamin B_{12a} (hydroxocobalamin hydrochloride, purity 96%, Fluka Chemical Corp.) were added to the soil suspension, which were again purged with N_2 and left under continuous shaking. Samples were removed for analysis after 5 min, 1 h, 2 h, 3 h, and 5 h. To ensure that the reducing conditions were maintained, the addition of Ti citrate was repeated after 5 h, the mixture was again purged with N_2 and further samples were taken after 10 h and 24 h. To evaluate the effect of the reducing agent (Ti citrate) and the catalytic effect of the vitamin B_{12a} on HCH degradation, controls were carried out in two manners: (i) adding Tris-HCl buffer (without reducing agent or vitamin B_{12a}, Cont.A_{NR} and Cont.B_{NR}) to contaminated soil, and (ii) adding Tris-HCl buffer and reducing agent (without vitamin B_{12a}, Cont.A_R and Cont.B_R). Control vials were maintained for the same period of time as experimental mixtures (i.e., 24 h equilibration, followed by 24 h of reaction), but samples were only withdrawn for analysis at zero reaction time ($t = 0$) and at the end of the 24 h reaction period ($t = 24$).

Under reducing conditions, the Co (III) of vitamin B_{12a} may be reduced to Co (II) (B_{12r}) or to Co (I) (B_{12s}). The reduction is easily visible by the change in color from red (B_{12a}) to amber (B_{12r}) and to blue (B_{12s}). B_{12s} is the predominant form present in a solution with excess Ti citrate (*14*).

Study of Degradation in Reactor System. The same process as described above was carried out at a larger scale in a stirred tank reactor (2 L capacity) fitted with a turbine-type impeller. In order to retain any volatile compounds and to minimize loss of contaminants during N_2 purging, one of the upper exit tubes was fitted with a condenser through which water circulated at 5 °C. The tests were carried out at room temperature and at a shaking speed of 250 rpm.

For each type of contaminated soil (horizon A and B), a reactor test was carried out with a suspension of 5% soil (90 g of contaminated soil and 1.8 L of solution) in a reducing solution of Ti citrate in Tris-HCl buffer. The mixture was shaken for 24 h and a sample was removed for analysis (considered the zero reaction time ($t = 0$)). The vitamin B_{12a} was then added to the reactor, the mixture was purged with N_2, maintained under shaking, and samples were removed at different times: 5 min, 2, 3, 5, 10, and 24 h. As in the small scale system, Ti citrate was added after removing samples at 0 and 5 h. The concentrations of buffer, Ti citrate and vitamin B_{12a} were the same as in the small scale tests. After adding all of the reagents and removing each sample, the test mixture was again purged with N_2. In addition, two controls were prepared for each horizon in separate reactors: (i) with Tris-HCl (without reducing agent or vitamin B_{12a}, Cont.A_{NR}, and Cont.B_{NR}), and (ii) with Tris-HCl and Ti citrate (without vitamin B_{12a}, Cont.A_R, and Cont.B_R).

Sample Preparation and Analytical Methods. The soil suspensions were centrifuged to separate liquid and solid phases. HCH in the liquid phase was extracted with hexane (ratio 1:1, v:v) in an ultrasound bath for 60 min. The organic phase was then dried with anhydrous sodium sulfate (Na_2SO_4). The solid phase of the suspensions was mixed with pulverized anhydrous sodium sulfate, placed in a stainless steel cell (11 mL) and extracted under pressure in an ASE200 accelerated solvent extractor, with hexane:acetone (1:1) and a single extraction cycle, at 2000 psi and 100 °C for 5 min. The preheating time required to reach the desired pressure and temperature values was 1 min. The spiked soil samples (A and B) used for the experiments were analyzed in the same way. All of the extracts obtained were stored at −18 °C for posterior analysis by gas chromatography.

For detection of degradation compounds (e.g., pentachlorocyclohexene (PCCH), tetrachlorociyclohexene (TCCH) and chlorobenzene (CB)), and detection and quantification of HCH isomers, extracts were analyzed in a gas chromatograph system (model 3400, Varian Inc., Palo Alto, CA) equipped with a 0.25 mm × 30 m column (CP-Sil 8 CB Low Bleed/MS) (Varian Inc.), with helium as the carrier gas (80 KPa) and a mass spectrometer detector with ionic trap (Varian Inc.). The GC/MS operating conditions were as follows: splitless injection; oven temperature program 35 °C (6 min), increased by 3 °C min^{-1} to 100 °C, and then by 8 °C min^{-1} to 270 °C. Interface and ion source temperatures were 280 and 220 °C, respectively, and the ionization energy was 70

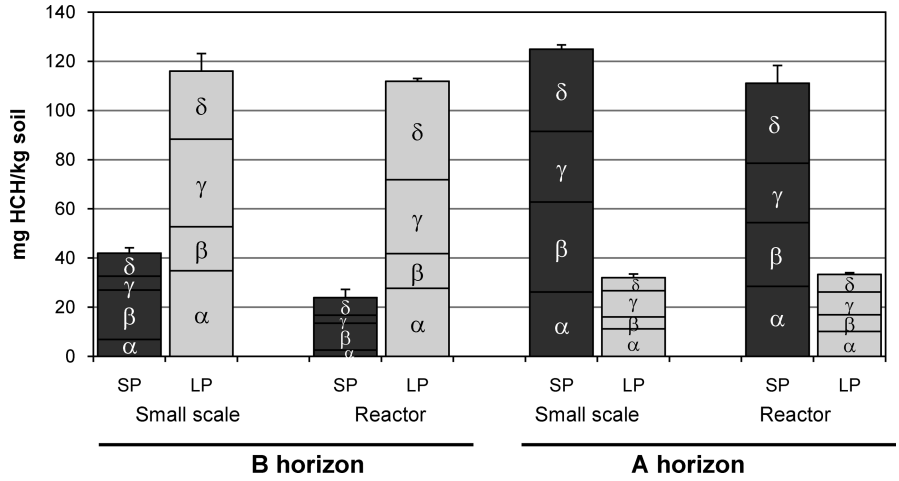

FIGURE 1. Distribution of α-, *β*-, *γ*-, and *δ*-HCH isomers in the solid (SP, dark gray bars) and liquid phases (LP, light gray bars) of the small scale and reactor systems.

eV. HCH concentration was quantified with external standard calibration, which was linear in the range 0.01−5.0 mg L^{-1} (C.V. ($n = 5$) ≤ 7.2%). HCH isomers, PCCH, and CB were identified by injection of the reference compounds and comparison of their retention times and mass spectra with those recorder in the samples. TCCH was identified by comparing the mass spectra with those of the NIST-02 library. The recoveries of the HCH isomers for the contaminated soils were 86.6, 88.7, 87.9, and 95.7% for α, *β*-, *γ*-, and *δ*-HCH, respectively.

Results and Discussion

Distribution of HCH Isomers between Phases in the Slurry System. The concentration of total HCH in the contaminated soil ranged between 161.3 and 194.0 mg HCH kg dry $soil^{-1}$, with roughly equal proportions of the four isomers. After equilibration of the suspensions, by shaking the spiked soil in a Tris-HCl buffered aqueus solution (Cont.A_{NR} and Cont.B_{NR}, $t = 0$), the contaminant transferred to the liquid phase. The extent of the transfer dependend on soil type, the isomer considered and the scale of work (Figure 1). In the suspension of Cont.B_{NR} the HCH isomers passed readily to the liquid phase (84, 47, 86, and 75% of α-, *β*-, *γ*-, and *δ*-HCH, respectively, passed into solution in the small scale system), whereas HCHs remained bound to the soil in Cont.A_{NR}, and a lower concentration was detected in the liquid phase (<25% of HCH added in the small scale system) than in the suspension of Cont.B_{NR}. This transfer was slightly more pronounced in the reactor than in small scale system.

Disappearance of HCH Isomers in B Horizon. The results of the kinetic studies in the B horizon are shown in Figure 2a (small scale) and 2b (reactor scale). The results obtained after the analysis of the solid and liquid phases are presented as stacked bars for each of different sampling times throughout the reaction period. Lines are used to indicate the evolution of the controls during this period.

Low or no HCH degradation was observed after soil incubation in the presence of tris-HCl and absence of both the reductant and the vitamin B_{12a} (see Cont.B_{NR} in Figure 2a and b). Under these conditions a significant reduction was only observed in α-HCH in the small scale system. However, disappearance of *γ*- and α-HCH isomers was observed when Ti citrate was used, thus providing evidence of the influence of the reducing power on the reductive degradation of these isomers. Percentages of disappearance in Cont.B_R of the small scale test at $t = 0$ were approximately 34% and 45% for α- and *γ*-HCH, respectively. After addition of vitamin B_{12a}, the 4 isomers disappeared rapidly, and only 7% of the original HCH was retained in the soil after 5 min. Five hours after adding the vitamin B_{12a} to the reactive mixture, none of the HCH isomers were detected in either the liquid phase of the mixture, or in the soil (Figure 2a), and therefore degradation of all of the HCH was considered complete. Slight differences in the degradation rate of the different isomers were detected, with the alpha and gamma isomers being the most rapidly degraded.

The results obtained in reactor system are consistent with those of the small scale system. Incubation in a reducing medium and in the absence of vitamin B_{12a} produced an intense degradation of α- (81%) and *γ*-HCH (100% was eliminated at the end of the reaction period) but had no effect on the *β*- and *δ*-HCH isomers. After the addition of vitamin B_{12a}, the four isomers disappeared rapidly from the B horizon, within 5 min (more than 95% of α-, *γ*-, and *δ*-HCH and 79% of *β*-HCH). Five hours after addition of vitamin B_{12a}, none of the HCH isomers were detected in either liquid or solid phases (Figure 2b). The higher velocity at which the degradation occurred in the larger scale system (both in the presence and the absence of the catalizer) is in agreement with a higher transfer rate of the contaminant to the aqueous phase in the reactor. In the presence of vitamin B_{12a} degradation was so rapid that HCH was not detected in the liquid phase at any time, indicating that the degradation rate was greater than the speed of re-establishment of the equilibrium between the soil and the liquid phase.

The order of degradation obtained for the different HCH isomers ($\gamma > \alpha > \delta > \beta$) coincides with that previously observed in the degradation test in water (*14*) and also with that reported by various authors (*6, 7, 22*). This order in the degradation rate may be explained by the orientation of Cl atoms around the cyclohexane ring in each HCH isomer, as the axially orientated atoms are more reactive than the equatorially orientated atoms; *γ*-HCH contains three atoms of Cl in the axial 1 position, α-HCH contains two atoms of Cl in this position, *δ*-HCH 1axial atom of Cl and *β*-HCH isomer none (*3, 7*). Comparison of the results obtained in the slurry system with those obtained in liquid medium under the same conditions (*14*) shows that the reaction rate was slower in the former, probably because of restrictions associated with the mass transfer between the soil and the liquid (*19, 22*). Quintero Diaz (*23*) also found in HCH degradation studies with water and soil samples and suspensions that the degradation rate was greater in water samples, followed by suspensions, and was slowest in soil samples, demonstrating that mass transfer is a key step in the degradation process.

Disappearance of HCH Isomers in A Horizon. No HCH degradation was observed after incubation of soil A in the presence of Tris-HCl and in the absence of both the reductant

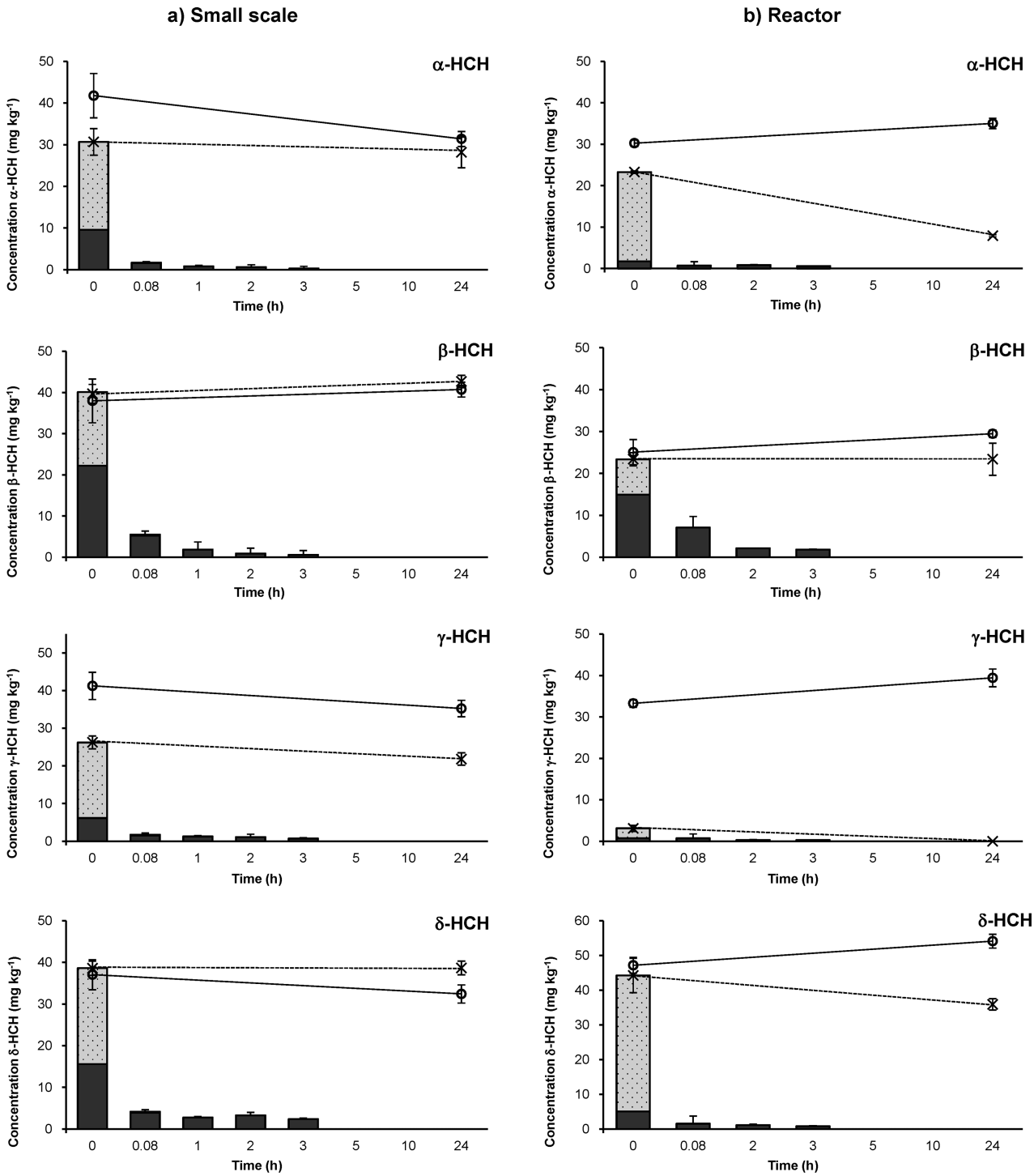

FIGURE 2. Concentrations of α-, *β*-, *γ*-, and *δ*-HCH (mg kg^{-1} dry soil) at different times in (a) small scale and (b) reactor systems prepared with B horizon. ○: Tris-HCH. ×: Tris-HCl and titanium citrate. ■: Tris-HCl, titanium citrate and B_{12a}, solid phase concentration. Dotted bars: Tris-HCl, titanium citrate and B_{12a} liquid phase concentration.

and the vitamin B_{12a} (see Cont.A_{NR} in Figure 3a and b). The addition of the reducing agent practically had no effect on the small scale system but did have an effect in the reactor, although this effect was less important than that observed in horizon B. The addition of vitamin B_{12a} promoted an important increase in the rate of degradation of all of the isomers, in the small scale system. After 5 min, only 13.8 mg kg^{-1} of α-HCH, 28.4 mg kg^{-1} of *β*-HCH, 11.3 mg kg^{-1} of *γ*-HCH and 27.3 mg kg^{-1} of *δ*-HCH remained in the suspension. The distribution of HCHs between the solid and liquid phase demonstrated that most of the residue that was not degraded was sorbed in the solid phase. The concentration remained almost constant for 5 h, after which further amounts disappeared. After 10 h HCHs isomers were still present in the solid phase but were not detectable in the liquid phase (Figure 3a). This may indicate that HCH residue was occluded in the soil organic matter and did not pass to the solution, and therefore the degradation, which took place in the liquid medium, did not proceed. At the end of the reaction, 93% of α-HCH, 88% of *β*-HCH, 94% of *γ*-HCH, and 91% of *δ*-HCH were degraded.

The concentration of HCH isomers of the replicate samples from the reactor systems varied in wide ranges, especially in the intermediate extraction times and the

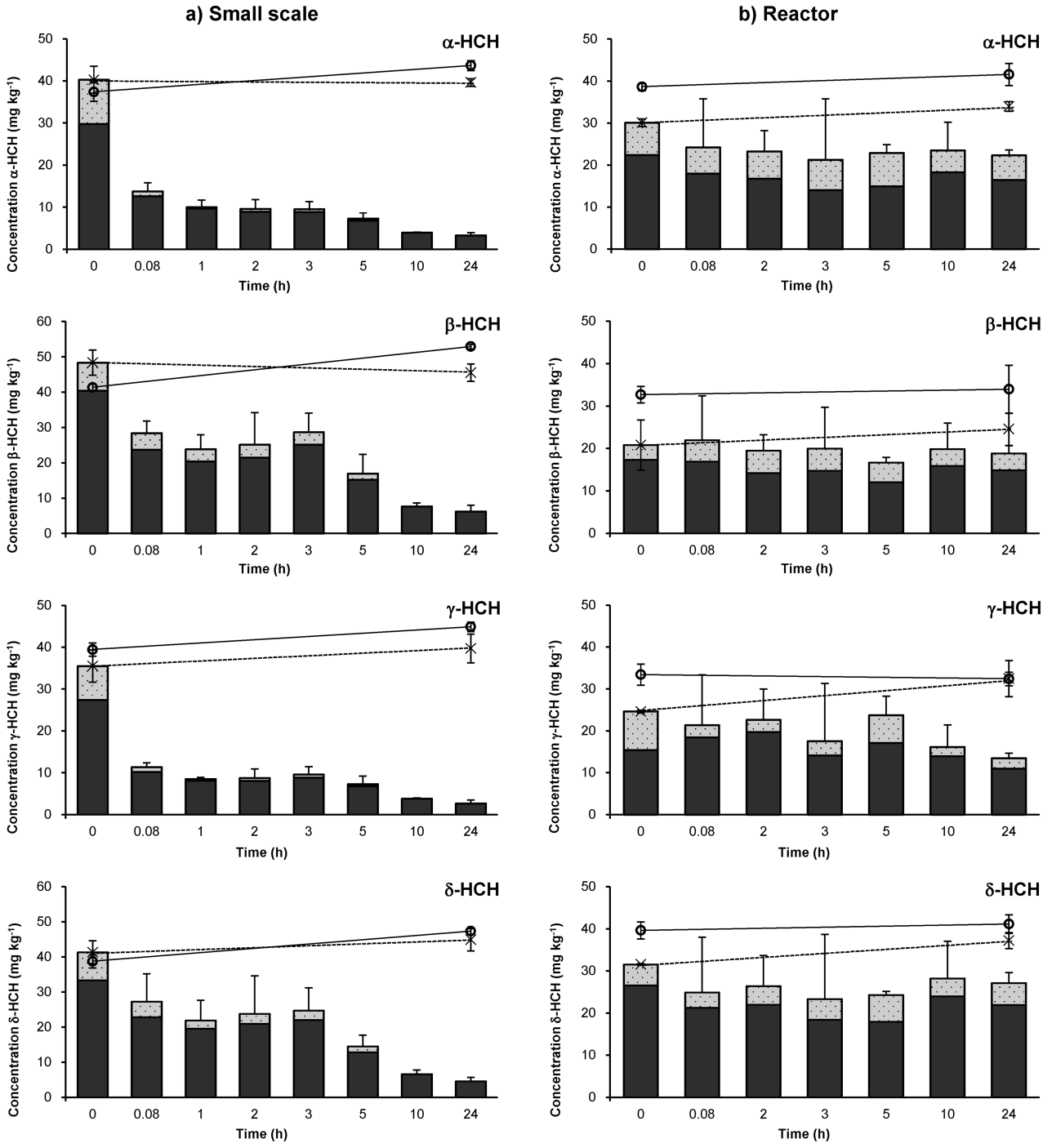

FIGURE 3. Concentrations of α-, *β*-, *γ*-, and *δ*-HCH (mg kg^{-1} dry soil) at different times in (a) small scale and b) reactor systems prepared with A horizon. ○: Tris-HCH. ×: Tris-HCl and titanium citrate. ■: Tris-HCl, titanium citrate and B_{12a}, solid phase concentration. Dotted bars: Tris-HCl, titanium citrate and B_{12a} liquid phase concentration.

catalyzer effect of the vitamin B_{12a} was not significant in this system. After 24 h of reaction, only 49% of the α-HCH, 48% of the *β*-HCH, 69% of the *γ*-HCH and 45% of the *δ*-HCH had disappeared (Figure 3b). In this case the presence of organic matter in the soil was not the only factor that caused deceleration of the reaction, as although most of the HCH was retained in the soil, it was also detected in the liquid phase. The results indicate that, although the vitamin B_{12a} catalized the dehalogenation of the HCH isomers in the small scale system with soil from horizon A, in the reactor the reaction was blocked. The increased reactivity achieved in the reactor, as a result of the shaking, may have favored other soil reactions making it difficult to maintain the pH and redox potential conditions required for the vitamin B_{12a} to act, i.e. conditions close to neutral and with a sufficiently low Eh to produce reduction of Co (III) to Co (I) (*24, 25*). The high content of exchangeable Al in the A horizon (Table 1) may have consumed a large part of the buffering capacity of the solution, provided by the Tris-Cl, whereas the iron oxyhydroxides may have consumed part of the reducing capacity of Ti citrate. Additional experiments in which pH and Eh are controlled are underway to further clarify this aspect.

The results indicate that the proposed method of reductive degradation of HCH, catalyzed by vitamin B_{12a}, in the presence of Ti citrate, applied to soils in suspension, is a viable technique for the ex situ decontamination of poorly buffered, sandy soils with a low capacity to retain the contaminant.

However, although the method is practically viable for more complex edaphic matrices, such as organic matter rich soils, with high potential acidity and/or high iron oxide contents, additional trials are needed to achieve the maintenance of the conditions for reducing degradation.

Products Formed during Degradation of HCH. Different HCH degradation products were detected, these were PCCH, TCCH, and CB (Supporting Information (SI) Figures S1 and S2). The isomers of PCCH and TCCH cannot be distinguished by the mass spectra, as they are very similar, and so they were distinguished by their retention times. In general, only small amounts of all degradation products were detected (below the limits of quantification for CB).

In horizon B degradation products were only detected in a few samples at the initial times and in small amounts. In this horizon, of the eight existing isomers of PCCH and the seven of TCCH (*7*), three of PCCH and one of TCCH were detected in the small scale system, and two of PCCH and one of TCCH were detected in reactor test (SI Table S1).

In horizon A the degradation products PCCH, TCCH and CB were found, although in this case, three isomers of PCCH and another three of TCCH were detected (SI Table S1). The peak areas of these products were higher in the A horizon than B horizon and they were detected throughout the degradation study, until $t = 24$ h. CB was only detected in the liquid phase of the suspensions, whereas PCCH and TCCH were detected in the liquid phase and particularly in the solid phase, which may indicate that the organic matter has a greater affinity for the latter two compounds.

None of the HCH degradation products were found to accumulate over time, not even CB, which is reported as the final product in many degradation routes (*13, 26*). In fact, in the present study the amount of CB decreased over time, which may indicate that (i) CB is an intermediate product, or (ii) CB was lost through volatilization during N_2 purging.

The degradative route suggested by these results may begin with vicinal dehalogenation (elimination of two Cl atoms from two adjacent C atoms, with formation of a double C=C bond and production of Cl_2) to form TCCH (*6, 13, 26*) or antiperiplanar dehydrohalogenation (elimination of 1 Cl and 1 H of adjacent C atoms, formation of a double C=C and formation of HCl) to form PCCH, although this compound is generally detected when the reaction begins with an oxidative step (*26, 27*). The beta isomer cannot react by antiperiplanar dehydrohalogenation to form PCCH as for this reaction to occur, an H and a Cl must be present in antiparallel positions, which does not occur in the beta isomer (*7*). The formation of PCCH may also be attributed to hydrolysis reactions, the velocity of which has been shown to increase in alkaline conditions (*10*). The subsequent reactions would also be dehydrohalogenation or dehalogenation, with possible formation of trichlorobenzenes (TCB), dichlorobenzenes (DCB) or chlorobenzene (CB) (*10*). Different authors have reported that formation of benzene only occurs with enzymatic mediation in biotic media (*3, 8*). Liu et al. (*10*) also proposed its formation in the presence of solid FeS in abiotic media. In the present study we were not able to confirm or rule out the presence of benzene as this compound is eluted very rapidly under the chromatographic conditions used, and if it was present it would probably elute during the solvent delay time. Further studies in which single isomers are incubated on an individual basis could help clarify the origin of the different degradation products obtained.

Acknowledgments

This research was supported by the Spanish Ministry of Education and Science and EU (FEDER) (CTM2006-13862-CO2-01 and CICYT-CTQ2007-66788/PPQ). T.A.L.-C. thanks the economical support of the Isabel Barreto Program (Galician Goverment, Spain) during the development of this work.

Supporting Information Available

More detailed information regarding intermediates detected in the different degradation studies is available in Figure S1, Figure S2, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Breivik, K.; Pacyna, J. M.; Münch, J. Use of α-, β- and γ-hexachlorocyclohexane in Europe, 1970−1996. *Sci. Total Environ.* **1999**, *239*, 151–163.
(2) Walker, K.; Vallero, D. A.; Lewis, R. G. Factors Influencing the Distribution of Lindane and Other Hexachlorocyclohexanes in the Environment. *Environ. Sci. Technol.* **1999**, *33*, 4373–4378.
(3) Phillips, T. M.; Seech, A. G.; Lee, H.; Trevors, J. T. Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. *Biodegradation* **2005**, *16*, 363–392.
(4) Bhatt, P.; Kumar, M. S.; Chakrabarti, T. Fate and Degradation of POP-Hexachlorocyclohexane. *Crit. Rev. Environ. Sci. Technol.* **2009**, *39*, 655–695.
(5) MacRae, I. C.; Raghu, K.; Castro, T. F. Persistence and biodegradation of four common isomers of benzene hexachloride in submerged soils. *J. Agric. Food Chem.* **1967**, *15*, 911–914.
(6) Jagnow, G.; Haider, K.; Ellwardt, P. C. Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers by anaerobic and facultative anaerobic bacteria. *Arch. Microbiol.* **1977**, *115*, 285–292.
(7) Buser, H.; Müller, M. D. Isomer and enantioselective degradation of hexachlorocyclohexane isomers in sewage sludge under anaerobic conditions. *Environ. Sci. Technol.* **1995**, *29*, 664–672.
(8) Middeldorp, P. J. M.; Jaspers, M.; Zehnder, A. J. B.; Schraa, G. Biotransformation of α-, β-, γ-, and δ-hexachlorocyclohexane under methanogenic conditions. *Environ. Sci. Technol.* **1996**, *30*, 2345–2349.
(9) van Eekert, M. H. A.; Van Ras, N. J. P.; Mentink, G. H.; Rijnaarts, H. H. M.; Stams, A. J. M.; Field, J. A.; Schraa, G. Anaerobic transformation of β-hexachlorocyclohexane by methanogenic granular sludge and soil microflora. *Environ. Sci. Technol.* **1998**, *32*, 3299–3304.
(10) Liu, X.; Peng, P.; Fu, J.; Huang, W. Effects of FeS on the transformation kinetics of γ-hexachlorocyclohexane. *Environ. Sci. Technol.* **2003**, *37*, 1822–1828.
(11) Wang, Z.; Peng, P.; Huang, W. Dechlorination of γ-hexachlorocyclohexane by zero-valent metallic iron. *J. Hazard. Mater.* **2009**, *166*, 992–997.
(12) Antonaraki, S.; Triantis, T. M.; Papaconstantinou, E.; Hiskia, A. Photocatalytic degradation of lindane by polyoxometalates: Intermediates and mechanistic aspects. *Catal. Today* **2010**, *151*, 119–124.
(13) Marks, T. S.; Allpress, J. D.; Maule, A. Dehalogenation of lindane by a variety of porphyrins and corrins. *Appl. Environ. Microbiol.* **1989**, *55*, 1258–1261.
(14) Rodriguez-Garrido, B.; Camps Arbestain, M.; Monterroso, M. C.; Macias, F. Reductive dechlorination of α-, β-, δ-, and γ-hexachlorocyclohexane isomers by hydroxocobalamin in the presence of either dithiothreitol or titanium(III) citrate as reducing agents. *Environ. Sci. Technol.* **2004**, *38*, 5046–5052.
(15) Harms, H.; Bosma, T. N. P. Mass transfer limitation of microbial growth and pollutant degradation. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 97–105.
(16) Simoni, S. F.; Schafer, A.; Harms, H.; Zehnder, A. J. B. Factors affecting mass transfer limited biodegradation in saturated porous media. *J. Contam. Hydrol.* **2001**, *50*, 99–120.
(17) Pignatello, J. J.; Xing, B. Mechanisms of slow sorption of organic chemicals to natural particles. *Environ. Sci. Technol.* **1996**, *30*, 1–11.
(18) Ogwada, R. A.; Sparks, D. L. Kinetics of ion exchange on clay minerals and soil: I. Evaluation of methods. *Soil Sci. Soc. Am. J.* **1986**, *50*, 1158–1162.
(19) Rijnaarts, H. H. M.; Bachmann, A.; Jumelet, J. C.; Zehnder, A. J. B. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of alpha -hexachlorocyclohexane in a contaminated calcareous soil. *Environ. Sci. Technol.* **1990**, *24*, 1349–1354.
(20) IUSS Working Group WRB. World Reference Base for Soil Resources 2006, World Soil Resources Reports No 103; FAO: Rome, 2006.

(21) Soil Survey Staff. *Soil Taxonomy. A Basic System of Soil Classification for Making and Interpretating Soli Surveys*, Agriculture Handbook 436 NRCS-USDA: Washinton, DC, 1999.
(22) Quintero, J. C.; Moreira, M. T.; Feijoo, G.; Lema, J. M. Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. *Chemosphere* **2005**, *61*, 528–536.
(23) Quintero Díaz, J. C. Biorrecuperación de suelos contaminados con hexaclorociclohexano. Ph.D. Thesis, University of Santiago de Compostela, Spain, 2004.
(24) Lexa, D.; Saveant, J. M. The electrochemistry of vitamin B_{12}. *Acc. Chem. Res.* **1983**, *16*, 235–243.
(25) Giorgetti, M.; Ascone, I.; Berrettoni, M.; Conti, P.; Zamponi, S.; Marassi, R. In situ X-ray absorption spectroelectrochemical study of hydroxocobalamin. *J. Biol. Inorg. Chem.* **2000**, *5*, 156−166.
(26) Bachmann, A.; Walet, P.; Wijnen, P.; De Bruin, W.; Huntjens, J. L. M.; Roelofsen, W.; Zehnder, A. J. B. Biodegradation of alpha- and beta-hexachlorocyclohexane in a soil slurry under different redox conditions. *Appl. Environ. Microbiol.* **1988**, *54*, 143–149.
(27) Sahu, S. K.; Patnaik, K. K.; Bhuyan, S.; Sreedharan, B.; Kurihara, N.; Adhya, T. K.; Sethunathan, N. Mineralization of α-, γ-, and β-isomers of hexachlorocyclohexane by a soil bacterium under aerobic conditions. *J. Agric. Food Chem.* **1995**, *43*, 833.

ES1012438