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Biodegradation of cyclodextrins in soil

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Abstract

Cyclodextrins, especially random methylated βCD (RAMEB) and hydroxypropyl βCD (HPβCD), are becoming common enhancing additives in the bioremediation of soils formerly contaminated by hydrocarbons and/or other poorly bioavailable organic pollutants. Therefore, their degradation in the soil, particularly the most persistent RAMEB, has been of great concern. Like oil contaminants, these additives should be biodegradable via an environmentally safe technology. Hence, in this paper, the biodegradability of eight different cyclodextrins (CDs) in four different soils was examined under various treatment conditions in laboratory and pilot scale field experiments.

This paper is the first report on the potential biological fate of CDs studied under a large variety of environmental conditions and in different soil ecosystems. Data on the potential relationship between CD biodegradation and the biological removal of hydrocarbons in the CD-amended contaminated soils are also given.

All CDs were found to be more or less biodegradable; even the most persistent RAMEB was depleted from soils under favourable conditions. In the field experiments, the depletion of RAMEB to about 40% of its initial level was observed for a period of 2 years in hydrocarbon-contaminated soils of high organic matter and cell concentration.

Keywords: Contaminated soil; Remediation; Randomly methylated β-cyclodextrin

1. Introduction

Cyclodextrins (CDs) are resistant to common starch hydrolysing enzymes, to β-amylases and to most of the α-type amylases as well (French, 1957).

The CD-degrading enzymes (CDase enzymes) are responsible for the cleavage of the CD ring, which is the rate-limiting step of the hydrolysis. CD glycosyl transferase (CGTase) enzymes are able to catalyse the cyclisation of maltooligosacharides to yield CDs. Most of the CGTase-producing bacteria (e.g. *B. macerans*, *B. subtilis*, *B. coagulans*) show CDase activity as well (DePinto and Campbell, 1968; Bender, 1978): the former enzymes transform starch into CDs, the latter ones catalyse the transfer of CDs to appropriate linear maltooligosacharides. It was shown that in these bacteria the degradation of CDs is one of the consecutive reactions in the metabolism of starch. The formation, uptake and intracellular degradation of CDs is a beneficial

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starch-degradation pathway for bacteria harbouring both CD-forming and CD-degrading enzymes (Usanov et al., 1990; Pocsi, 1999).

On the other hand, some CGTase negative bacteria, like *Flavobacterium* possess CDase activity and grow well on CDs (Bender, 1993). A CD-degrading glucoamylase producing glucose as the final degradation product from all CD substrates was isolated from a *Flavobacterium* species living in soil (Bender, 1981).

Several commonly occurring strains of plant associated bacteria (*Agrobacterium*, *Bradyrhizobium*, *Xanthomonas*, *Corynebacterium*) as well as soil fungi, *Trichoderma* were found to metabolise CDs as sole carbon source (Oros et al., 1990, 2001) in the order of γCD > αCD > βCD. The CD derivatives were more resistant than native CDs, and in the case of βCD the following order of biodegradability was obtained: unsubstituted > carboxymethyl > hydroxypropyl > trimethyl > polymer (crosslinked with ethyleneglycol diepoxypropyl ether) > dimethyl.

In a controlled composting biodegradation test at 58 °C, three naturally occurring CDs (α-, β- and γCD) were completely and readily biodegradable (Verstichel et al., 2004). Chemical modification of these CDs reduced biodegradability significantly. Partially acetylated and hydroxypropyl βCDs (AcBCD and HPβCD) showed slower biodegradation rates, while no sign of degradation was observed for fully acetylated α- and βCD and randomly methylated βCD (RAMEB) during 45 d of controlled composting (Verstichel et al., 2004).

Recently bioremediation technologies based on the application of methyl CDs as bioavailability-enhancing additives were reported (Molnár et al., 2002; Fava and Ciccotosto, 2002). βCD, γCD, HPβCD and maltosyl βCD were also found to enhance the biodegradation of certain soil contaminants (Fava et al., 1998; Wang et al., 1998; Steffan et al., 2001; Garon et al., 2004). The increasing application of CDs in soil remediation requires a deeper understanding of their biodegradability in soil. The microbial population present in polycyclic aromatic hydrocarbon (PAH)-contaminated soil was found to utilize βCD (Bardi et al., 2000), while the indigenous microflora in a polychlorinated biphenyl (PCB)-contaminated soil can use of γCD and HPβCD as sole carbon source (Fava et al., 1998). RAMEB was slowly metabolised by aerobic microorganisms isolated from PCBs-contaminated soil, when RAMEB was then used as sole carbon and energy source (Fava et al., 2003). However, no data on the biodegradation of acetyl CDs by the soil microflora have been found in the literature. To our knowledge, no studies on the fate and behaviour of CDs within the soil have been published so far.

This is the first work in which the biological fate of several CDs of industrial interest is studied in different soils and under various treatment conditions. A comparative study was performed on the biodegradability of 8 CDs in uncontaminated soil using a standard biodegradation test. The degradability of βCD by the microflora of soil historically contaminated with transformer oil was compared to that of its derivatives: RAMEB, AcBCD in laboratory-scale experiments. RAMEB was then used as a bioavailability enhancing additive to intensify the ex situ bioremediation of soils contaminated with motor oil and transformer oil. The soil samples were analysed for RAMEB content to determine the degradation of RAMEB under field conditions. The correlation between the bioremediation-intensifying effect and the degree of RAMEB depletion was also investigated.

2. Experimental

2.1. Materials

αCD, βCD, γCD (CAVAMAX® W6, W7 and W8), HPβCD (=(2-hydroxy)propyl-β-CD; degree of substitution (DS) defined as number of substituents on a CD molecule DS = 4, CAVASOL® W7 HP), RAMEB (random methylated βCD; DS = 13, CAVASOL® W7 M TL) were obtained from Wacker Chemie (Munich, Germany). Fully acetylated αCD (DS = 18, lot number CY-1002.0), fully acetylated βCD (DS = 21, lot number CY-2002.0), partially acetylated βCD (DS = 7, lot number CY-2002.1) were obtained from Cyclolab (Hungary).

2.2. Soils

Table 1 represents the characteristics of the soils used in the biodegradability test, in the laboratory bioremediation experiment and in the two ex situ field experiments (S1, S2, S3 and S4).

The soil (SI) used in the biodegradability test was a mixture of sandy soil derived from a field in Lokeren and four woodland soils: two from Moerbeke, and one from Zwijnaarde and Gontrode, all in Belgium. Before use, the soils were sieved through a 2 mm sieve to remove stones, and plant debris, and thoroughly mixed. The soil mixture showed a total solids content of 77.6% with a water holding capacity (WHC) of 28.8%, which corresponds to 41.2% of its total capacity (WHC_{tot} = 69.9%). The soil having water content between 40% and 60% of the total water holding capacity can be used as standard soil for biodegradability tests (ISO 17556, 2003). A pH of 7.6 and a C/N ratio of 16 ensure sufficient conditions for biodegradation.

The S2 soil used in the laboratory scale bioremediation experiment is a sandy soil derived from a transformer station (Nepliget, Budapest, Hungary). It was historically contaminated with transformer oil (with no PCB content). The soil was homogenised before use.

Table 1
Soil characteristics

Characteristics	S1	S2	S3	S4
Total solids (TS, %)	77.6	81.3	86.9	87.4
pH	7.6	8.4	8.0	8.1
Electric conductivity (mS cm^{-1})	0.14	n.m.	0.91	0.67
Total N (g kg^{-1} TS)	2.5	n.m.	1.14	0.95
C/N ratio	16	n.m.	42.9	63.0
Aerobic heterotrophic cells (CFU g^{-1} related to dry soil)	1.7×10^7	0.76×10^7	2.3×10^7	5.2×10^7
Oil degrading cells (CFU g^{-1})	n.m.	46×10^3	2.9×10^3	120×10^3
Extractable material content (mg kg^{-1})	n.m.	22000	24000	35900
EPH content (mg kg^{-1})	n.m.	20000	20000	12400

n.m. = not measured.

The S3 and S4 soils used in the ex situ field experiment are sandy soils derived from a former military site in Dunaujvaros (Hungary) recently contaminated through an unlawful disposal of transformer oil (about 20000 mg kg^{-1}) and diesel and motor oil (about 12 000 mg 10 kg^{-1}), respectively. The high extractable material content compared to the extractable petroleum hydrocarbon (EPH) content in the S4 soil suggests that there is a large fraction of high boiling point contaminants, which cannot be measured by gas chromatography. The C/N ratio was too high, additional N was applied in the form of NH_4NO_3 fertilizer to optimise it.

2.3. Methods

2.3.1. Soil analysis

Total solids were calculated from the weight loss upon drying at 105 °C; total N is the sum of the organic nitrogen and the ammonia nitrogen measured by Kjeltec apparatus (ISO 5563, 1984); C/N ratio was determined by elementary analysis. The pH and salt content of the soil sample diluted with distilled water at a ratio of 5:1 (v/v) was measured with a pH-electrode (ISO 10390, 1994) and a conductivity meter (EN 13038, 2000), respectively.

The concentration of the aerobic heterotrophic cells was determined by plating on agar gel and colony counting (Hung. St. 21470/77, 1988) and the concentration of the oil degrading cells was measured by the Most Probable Number method (Hung. St. 21470/77, 1988).

The soil solvent extractable material content (SEM) was determined by extracting the soil samples (5 g) with hexane–acetone (2:1 v/v, 2×10 ml) in an ultrasonic bath (10 min), followed by weighing of extract residue after drying. The EPH content was measured by gas chromatography with flame ionisation detection of the above extracts according to EPA 8270 and HS 21470-94 (2001). The gravimetric analysis of the extract gives the weight of all material (SEM content), which can be extracted from the soil by the hexane–acetone mixture, while EPH is the hydrocarbon fraction in it, which can be volatilised under the conditions of the gas chromatographic measurement (boiling point < ≈600 °C).

A 'one peak' size exclusion HPLC method was used to determine the CD content in the soil (Fenyvesi et al., 2002). The soil extracts obtained by ultrasonication of soil suspensions in diluted hydrochloric acid (pH 3) were analysed after solid phase microextraction (SPME) on RP18 SPME columns (LiChrolut® 200 mg, Merck) using TSK-GEL G 2000 SW column (TosoHaas). CDs are retained on the SPME column via complex formation, while linear dextrins pass through. The method is therefore specific for the intact rings of CDs, and CDs opened by microbial activity are not detected. In the case of βCD-containing samples, diluted hydrochloric acid-ethanol (1:1, v/v) was used for the extraction. Ethanol as a competitive guest can displace included soil hydrocarbon contaminants and the resulting βCD fraction contains CD that would otherwise precipitate in the form of insoluble contaminant CD complexes.

2.4. Biodegradation experiments

The biodegradability test was executed according to ISO 17556 (2001). The test was carried out in duplicate using 500 g SI soil and 0.2% of test or reference material (cellulose or CD as carbon source) in each reactor. A technical control (empty reactor) and a soil control (the soil without the added carbon source) was applied. The reactors were incubated at 20 °C ± 2 °C in the dark. During the aerobic biodegradation the solid carbon content of organic materials is converted into CO_2 by microbial activity. The produced carbon dioxide was captured in 1 N or M KOH traps, placed inside the tightly closed container above the soil. The amount of CO_2 absorbed in the 1 N KOH solution (with the formation of K_2CO_3), was determined titrimetrically with 1 N HCl. The titration was done in two steps with an automatic titrator 5 (Metrohm 702 SM Titrino). The first step involves the conversion of the excess of KOH to KCl and of K_2CO_3 to $KHCO_3$ (pH 8.0). The second step involves the conversion of $KHCO_3$ to KCl and CO_2 (pH

3.9). The amount of HCl used during the second titration step is a direct measure for the amount of CO_2 that is captured. The percent biodegradation was calculated as the percentage of elemental carbon in the test substrate that was mineralized to form CO_2. Sufficient O_2 supply was provided by the air in the headspace.

The biodegradation of the CDs in contaminated soil was monitored using laboratory scale bioremediation experiments. S2 soil (440 g) was mixed with 40 ml water containing 0, 2.25 or 4.5 g CD, resulting in soils with 0%, 0.6% and 1.2% CD content relative to dry soil. The soils were supplemented with water and nutrients (mineral salt medium containing 10 mg kg^{-1} elemental N) on the 14th and 21st day. The reactors were incubated at 25 ± 1 °C and soil samples were taken at defined time intervals for analysis of both contaminant and CD content.

The biodegradation of RAMEB was also investigated in two field experiments performed with S3 and S4 soils, respectively. Two isolated piles (one with RAMEB and the other without this additive) were formed from both soils with a volume of 0.5 and 2 m^3, respectively. Tents were erected over the piles to exclude rainwater and reduce the loss of soil moisture by evaporation. RAMEB was applied as 10% w/v aqueous solution to reach 0.2% and 0.8% RAMEB content relative to the dry soil for S3 and S4, respectively. Inorganic nutrients (N, P) were supplied by adding aqueous solution of NH_4NO_3 and K_3PO_4 and the humidity was controlled by watering the piles regularly. The piles were mixed by hand every 2 weeks to guarantee aerobic conditions. The experiments were conducted for 30 and 68 weeks, for pile S3 and S4, respectively, with experiment S3 started 9 weeks later than experiment S4. The treatment was intermitted from November to March (15–35 weeks) because of winter season. Soil samples were taken at defined intervals and analysed for RAMEB, SEM and the extractable petroleum hydrocarbon (EPH) content.

3. Results

3.1. Biodegradability test

Table 2 shows the percentage of the biodegraded fractions and the half-life ($t_{1/2}$) of the test items studied in the standard biodegradability test using SI soil. The biodegradation percentages of cellulose, αCD, βCD, peracetylated α- and βCD and partially acetylated βCD as a function of time are shown in Fig. 1a. The biodegradation rate of RAMEB, HPβCD and γCD is shown in Fig. 1b.

After 178 d of incubation, a biodegradation of 89%, 94%, 97%, 96% and 89% was obtained for α-, β-, γCD and acetylated α- and βCD, respectively. The reference item cellulose had reached a biodegradation percentage of 102%. The test is considered valid when the degree of biodegradation of the reference material is more than 60% at the plateau phase or at the end of the test. The results show that this was easily obtained.

The test was extended to 280 d for partially acetylated βCD and HPβCD. After 280 d a biodegradation percentage of 103% and 98% was found for partially acetylated βCD and HPβCD, respectively. They were also completely biodegraded.

Table 2
Percent biodegradation (mass basis) and half-lives of test substrates in soil S1 after 178 and 280 d

Test item	Biodegradation (%)		Half-life time $t_{1/2}$ (d)
	After 178 d	After 280 d	
Cellulose	102 ± 11	108 ± 12	35
αCD	89 ± 3	n.m.	17.5
βCD	94 ± 6	n.m.	17.5
γCD	89 ± 2	n.m.	20
Peracetyl αCD	97 ± 4	n.m.	62
Peracetyl βCD	96 ± 1	n.m.	65
Partially acetylated βCD	85 ± 1	103 ± 5	17.5
HPβCD	72 ± 1	98 ± 6	122
RAMEB	−19 ± 3	n.m.	–

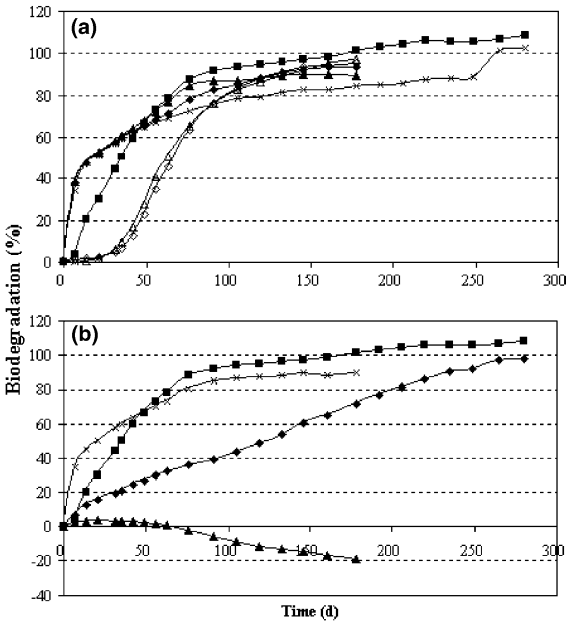

Fig. 1. (a) Time courses for % (mass basis) biodegradation (% amount at $T = 0$) for: cellulose (■), αCD (▲), βCD (◆), peracetylated αCD (△), peracetylated βCD (◇), and partially acetylated β-CD (×). (b) % degradation for cellulose (■), RAMEB (▲), HPβCD (◆) and γCD (×) in S1 soil using 0.2% of starting material relative to soil.

The initial rate of biodegradation decreased in the order of αCD ≈ βCD ≈ AcβCD > γCD > cellulose > HPβCD > peracetyl αCD ≈ peracetyl βCD ≫ RAMEB.

No biodegradation was observed for RAMEB. Even a negative biodegradation percentage was found, which can be explained by the fact that the CO_2 production in the control reactors was higher than the CO_2 production in the RAMEB reactors, resulting in a negative net CO_2 production and so in negative biodegradation percentages.

It should be noted that CDs might mobilise organic compounds, present in the soil, making them more bioavailable and therefore biodegradable by soil microorganisms producing CO_2 during biodegradation. In this experiment, however, the CO_2 production seems to originate primarily from the biodegradation of CDs. The evolution of CO_2 in case of the three natural CDs was proceeding at identical rates, although their contaminant inclusion and mobilisation capacity should be different due to the difference in the cavity size. According to earlier studies the methylated CDs have superior solubilising and mobilising ability compared to other CDs (Fenyvesi et al., 1996) including HPβCD (Boving et al., 1999) but even negative CO_2 evolution rate was obtained for RAMEB in this biodegradability test. According to another observation HPβCD can mobilise PAHs better than βCD (Badr et al., 2004) but we found much slower CO_2 production for HPβCD than for βCD. Based on these observations, it might be assumed that the mobilising effect was only limited in this standard biodegradability test.

3.2. Laboratory biodegradation experiments

In these experiments S2 soil contaminated with 20 000 mg kg^{-1} transformer oil was supplemented with βCD and its random methylated and partially acetylated derivatives (0.6% and 1.2% of dry soil), respectively. The large concentration of the heterotrophic cells and of the specialised oil-degrading bacteria shows that the soil microflora had been adapted to the contamination. The aim of the CD addition was to enhance the biodegradation of the poorly biodegradable transformer oil. In the course of the experiment, contaminant and CD additive concentrations were regularly measured to study in parallel the biodegradation of both the contaminants and of the CDs. The biodegradation of CDs in the soil was followed by determination of the CDs extracted from soil samples with diluted aqueous solution of hydrochloric acid. The applied SPME/HPLC method is specific for the intact rings of CDs, therefore CD degradation products were not detected. The extraction efficiency values measured in uncontaminated soils were 92%, 90% and 99% for βCD, AcβCD and RAMEB, respectively. In case of contaminated soil the recovery was 90% and 98% for AcβCD and RAMEB, respectively, while it was reduced to about 73–75% for βCD due to the low solubility of some hydrocarbon/βCD complexes. Therefore 1:1 mixture of ethanol and hydrochloric acid solution (pH 3) was used in this case to force the decomposition of the complexes, resulting in acceptable (91%) recovery for βCD.

In the course of the bioremediation experiment the CD concentration was found to decrease in all samples (Figs. 2a and b). The βCD was degraded very fast: only about 20–30% of the initial βCD remained after a week. AcβCD was degraded gradually to reach about 10% of its initial content after 36 d. As it was expected from the results of the standard biodegradability test, among the three CDs involved in this study RAMEB is the most resistant to the microbial attack, but even RAMEB content started to decrease slowly after about a week of adaptation. At the end of the experiment (after 36 d) 30% deficiency compared to the initial RAMEB content was observed. The order of biodegradation rate (βCD > AcβCD > RAMEB) was similar to that obtained in the biodegradability test. In this experiment, however, the RAMEB concentration was also decreased slowly as a sign of biodegradation.

The half-life time ($t_{1/2}$) calculated from the curves in Fig. 2a and b at 0.6% and 1.2% initial concentrations were 56.8 and 54.6 d for RAMEB, 8.6 and 8.5 d for AcβCD and 5.7 and 4.6 d for βCD, respectively.

The contaminant-content of the soil samples was also analysed to observe the effect of CDs on the biodegradation of the transformer oil. Significant reduction in SEM

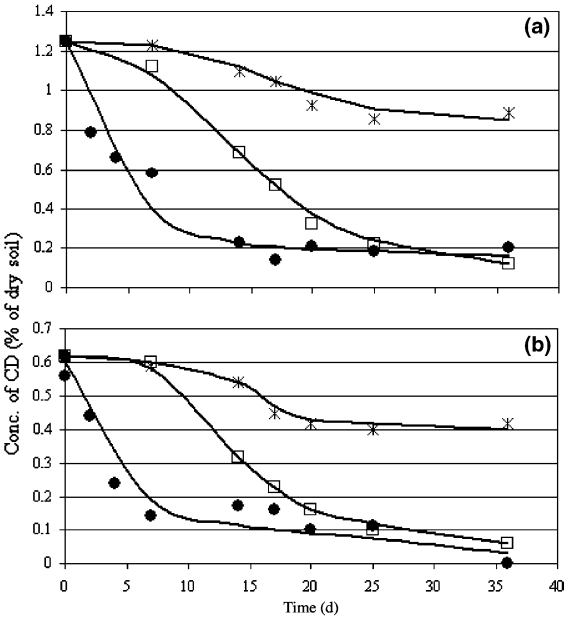

Fig. 2. The decrease of βCD (●), RAMEB (✳) and AcBCD (□) concentration in S2 soil contaminated with transformer oil. The initial CD concentration was 2a (top): 1.25%, 2b (bottom): 0.62% of dry soil.

and EPH content of the soil samples were observed only in the case of RAMEB-treated soils after 36 d of the experiment (the SEM content was 100% of its initial value, the EPH content 97% in the absence of RAMEB, while 66% and 90% SEM and 66% and 82% EPH related to the initial values were obtained for the soil samples treated with 0.6% and 1.2% RAMEB, respectively). In accordance with previous observations RAMEB was more effective in enhancing the biodegradation of hydrocarbon pollutants at 0.6% concentration than at 1.2% level (Molnár et al., 2002). The other two CDs, especially the native βCD, did not significantly improve the biodegradation of the contaminants during the short time of the experiment partly because of the lower solubility and solubilising potency of native βCD compared to that of RAMEB, partly because both βCD and acetyl βCD were probably quickly consumed by the soil microorganisms. These results justify the selection of RAMEB for intensification of bioremediation of soils contaminated with hydrocarbons.

3.3. Biodegradation in the course of field remediation

Based on the results of the laboratory experiments RAMEB was selected as a bioavailability enhancing agent to intensify the bioremediation in the ex situ field experiment. It was applied at 0.2% and 0.8% concentration relative to the dry soil in case of transformer oil (S3 soil) and motor oil (S4 soil) contamination, respectively.

The study focused primarily on the biodegradation of the contaminants in both S3 and S4 soils. The oil removal rate was higher in the RAMEB-treated soils than in the control in both experiments especially at the beginning of the treatment (Table 3). The efficacy of RAMEB in accelerating the biodegradation of the contaminants decreased parallel to its decreasing concentration in the soil. This relationship between the residual RAMEB content and the degree of hydrocarbon biodegradation is an additional proof to the beneficial effect of RAMEB on the bioremediation of soils contaminated with hydrocarbons.

The RAMEB content started to decrease at the sixth and seventh week after its addition to S3 and S4 soils, respectively (Fig. 3). (The S3 experiment started 9 weeks later than the S4 experiment.) During winter the RAMEB content remained approximately constant in both soils, and started to decrease again after the temperature rise in spring. In spite of the regular manual mixing (in every 2–3 weeks), there was some inhomogeneity in the soil (S4) reflected both by the RAMEB and contaminant content. The trend is, however, clear: at the end of the experiment the RAMEB content decreased to about 40% of its initial value. The half-life time ($t_{1/2}$) of RAMEB is about 1/2 and 1 year in S3 and S4 soils, respectively. The difference between the two soils might be in connection with the abundance of the microflora in them.

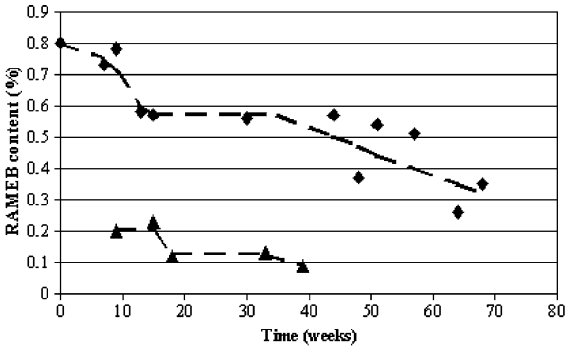

Fig. 3. Changes in the RAMEB content of soil samples contaminated with transformer oil (S3 soil, ▲) and with motor oil (S4 soil, ◆) in ex situ bioremediation experiments.

It is a new finding that RAMEB—persistent in both the composting and in the standard soil biodegradability test—slowly disappears from the soil. This result cannot come from the failure of the applied analytical method to detect RAMEB in complexes with the contaminants. The recovery of RAMEB from contaminated S4 soil was 98%.

On the other hand, it is not a surprising that RAMEB can be biodegraded by an active soil microflora. Fava et al. found that biomass increased in liquid cultures containing RAMEB as the only energy source and inoculated with microbes isolated from soils contaminated with PCBs (Fava et al., 2003). Certain *Xantomonas*, *Corynebacterium* species can live on methylated

Table 3
EPH content in percentage of initial amount and % difference (Δ%) between the EPH content of the RAMEB-untreated and RAMEB-treated soils (S3 and S4) at different sampling times

EPH content, % of the initial amount (S3 soil)				EPH content, % of the initial amount (S4 soil)			
Time (week)	Control soil	RAMEB-treated soil	Δ	Time (week)	Control soil	RAMEB-treated soil	Δ
6	82	66	16	9	100	78	22
9	67	55	12	16	96	65	29
24	46	43	3	31	82	67	15
30	39	33	6	58	38	32	6

CDs as sole carbon source (Oros et al., 1990). These bacteria are common degraders of oil contaminants (Cole, 1994; Alexander, 1994); and their appearance in the soils contaminated with hydrocarbons is conceivable.

The degradation pathway of RAMEB is not known as yet. This will be further investigated after elaboration of an appropriate analytical method for identification of the metabolites.

4. Conclusions

The CDs involved in this study were all biodegraded by soil microorganisms in the order of αCD ≈ βCD ≈ γCD ≈ AcβCD > cellulose > HPβCD > peracetyl αCD ≈ peracetyl βCD ≫ RAMEB.

RAMEB, which was found to be non-biodegradable in the standard biodegradability test in standard uncontaminated soil, disappeared slowly from real soils historically contaminated with hydrocarbons. The microflora of these soils was long adapted to the xenobiotics. The large cell concentration and their high activity as well as the favourable conditions for general biodegradation (frequent supply of oxygen, moisture and inorganic nutrients, constant optimal temperature) especially in the laboratory bioremediation experiment might have caused the fast clearance of CDs. In the ex situ field remediation experiments where the conditions were less favourable (not so frequent aeration and moisture supply, changing temperature according to the actual weather) a slower depletion both of contaminants and of RAMEB was observed. A very important finding of this study is that a correlation was found between the enhancement of contaminant depletion and the decreasing RAMEB concentration in the soil.

These observations confirm that RAMEB is a good choice for intensifying the bioremediation, not only because of its highest bioavailability enhancing effect, but also because its degradation rate is optimal in the contaminated soil: slow enough to ensure the necessary RAMEB concentration throughout the treatment, but high enough to be eliminated from the soil, after finishing its duty (after fulfilling its role).

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