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Randomly Methylated β-Cyclodextrins (RAMEB) Enhance the Aerobic Biodegradation of Polychlorinated Biphenyl in Aged-Contaminated Soils

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Abstract

The biological removal of polychlorinated biphenyls (PCBs) from contaminated soils is adversely affected by the poor bioavailability of these pollutants. Two different aged-contaminated soils containing about 890 and 8500 mg/kg of PCBs were supplemented with biphenyl and treated in slurry- and solid-phase aerobic 0.5 litre-microcosms in the presence of RAMEB at 0, 0.5, 1.0, 3.0% (w/w). RAMEB, which was non-toxic and slowly biodegraded by the soil microorganisms, significantly enhanced the bioavailability (from 30 to 50%) and the biological degradation (from 15 to 55%) and dechlorination of PCBs in both soils and treatment conditions. RAMEB effects were dependent on the concentration at which it was applied, the soil features and the treatment conditions. RAMEB enhanced PCB biodegradation by increasing both the PCB bioavailability and the availability of biodegrading bacteria in the microcosms. RAMEB appears a promising bioavailability enhancing agent for the treatment of PCB-contaminated soils, not only for its positive effects on the PCBs biodegradation, but also for its biodegradability, non-toxicity and relatively low cost.

Introduction

Lab-scale and pilot-scale studies have shown that the aerobic bioremediation of polychlorinated biphenyl (PCB)-contaminated soils is a feasible option [1]. It is known since 1985 that CD derivatives (as insoluble CD polymers) react with polychlorinated biphenyls and are used to remove these toxic compounds from wastewater [2]. The photodechlorination of chlorinated biphenyls in aqueous solution was accelerated by CDs [3]. The biodegradation of 4-chlorobiphenyl by a mixed culture of *Arthrobacter* and *Pseudomonas* strains was greatly enhanced by the presence of CDs [4]. Soil PCBs are aerobically biodegraded by the concerted action of indigenous PCB-co-metabolising bacteria and chlorobenzoic acid (CBA)-degrading bacteria [1]. In general, the biological removal of PCBs from the soil is stimulated by soil supplementation with biphenyl, O_2 and inorganic nutrients [1, 5] as well as by treatment conditions able to provide a high degree of soil mixing and homogeneity [6]. However, the biological decontamination of aged PCB-contaminated soils is often adversely affected by the low aqueous solubility of the xenobiotics, which, for this reason, are strongly adsorbed onto organic matter and therefore poorly available in the soil water phase, where the PCB-degrading microorganisms are located [7]. One possible approach to improve the PCB bioavailability in such soils may consist in their supplementation with commercially available surface-active agents [8, 9]; synthetic surfactants have been found to be effective but also often toxic and recalcitrant in the amended soil [8, 9]. Instead, phytogenic surfactants [10, 11] and cyclodextrins (CDs) [12] have proved to be very promising additives for the bioremediation of such matrixes, in particular for their biodegradability and low or absent toxicity. A relatively cheap industrial mixture of randomly methylated-β-cyclodextrins (RAMEB), has been recently tested in this field. Due to the lack of information on the effects of RAMEB on the PCB biodegradation and considering that these effects could be significantly dependent on the type of contaminated soil and treatment conditions employed, RAMEB was assayed at four different initial concentrations in the aerobic treatment of three different pristine soils (a loamy-, a humic- and a sandy-soil) inoculated with exogenous PCB-degrading bacteria and spiked with two different PCB-containing transformer oil in slurry- and solid-phase conditions. The data obtained in this study showed that RAMEB enhanced strongly (from 54 to 350%) the PCB biodegradation in all the spiked soils in both treatment conditions. RAMEB effects were dose-dependent (the highest effects were observed when it was used at 3% w/w), slightly dependent on the initial concentration of PCBs in the soil and significantly influenced by the soil type (the highest effects were observed in the sandy soil) and treatment conditions (the best results were observed in slurry phase conditions) [13]. RAMEB, which was slowly biodegraded by the soil and the inoculated bacteria, enhanced the growth of the PCB-co-metabolising biomass and the PCB availability, thus suggesting that they enhanced PCB biode-

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gradation by increasing both the PCB bioavailability and the availability of specialised bacteria in the soil microcosms [13]. On the basis of these findings, it was of special interest to extend the application of RAMEB to the aerobic bioremediation of actual site, aged PCB contaminated soils, where the problem of the low bioavailability of PCBs is often responsible for their unsatisfactory biotreatability [1, 7].

Experimental

Soil

Two dump-site PCB contaminated soils, one containing 890 mg/kg of PCBs, S1, and the other about 8,500 mg/kg of PCBs, S2, (in both soils, PCB contamination was referred to Aroclor1260) were used. The two soils also contained significant amounts of mineral oil, BTEX and heavy metals, such as Pb, Cr and Cu. S1 and S2 were air-dried, sieved through a 0.2 cm sieve and analysed for their content of organic carbon (C), total nitrogen (N), total phosphorous (P), moisture, pH and granulometry as reported by Fava *et al.* [6, 12]. They were also analysed for their content of indigenous heterotrophic, PCB- and CBA-degrading aerobic bacteria (through agar plate count technique) as reported previously [11, 12].

Microcosm preparation and management

S1 and S2 were supplemented with biphenyl (4 g/kg); a set of 7 slurry-phase microcosms and a set of 7 solid-phase microcosms were developed for each soil by suspending 70 g of soil in 280 ml distilled water in 0.5 l baffled bottles and dispensing 70 g of soil in 0.5 l bottles, respectively, which were then closed with Teflon liner-screw caps. One microcosm for each set was subjected to autoclave sterilisation, whereas a second one for each set was subjected to chemical sterilization with $HgCl_2$, according to Fava *et al.* [12], to prepare sterile controls. Three microcosms for each set were then supplemented with RAMEB to have a final concentration of 0.5, 1.0, 3.0% (w/w). One of the two RAMEB-free microcosms of each set was sacrificed after 3 days to determine the main treatment parameters at the beginning of the experiment [time 0 (t_0) microcosm], whereas the remaining one was used as RAMEB-free control. Additional slurry- and solid-phase RAMEB-free microcosms were developed for each soil by using biphenyl free-S1 or S2, in order to study the effects of biphenyl addition on the soil bioremediation process. The slurry microcosms were located on a rotary shaker at 130 rpm, whereas the solid-phase ones were statically incubated; all microcosms were kept at 20 $\pm$ 2 °C in the dark. The slurry- and solid-phase t_0 microcosms and all the other microcosms were opened (after 3 and 130 days, respectively) and samples of their soil were analysed for the concentration of PCBs (via GC-ECD), CBAs (via HPLC-Diode Array Detector), chloride ions (via IC-CD), bacterial heterotrophic, PCB-co-metabolising and CBA-degrading biomass (via plate count on rich and minimal selective agar plate media) and ecotoxicity (by using the Collembola – *Folsomia candida* – mortality test) according to the procedures and by using the equipments described by Fava *et al.* [6, 12, 13]. The analyses of CBAs, chloride ion and biomass occurring in the solid-phase microcosms were performed on dedicated soil aqueous suspensions (25% w/v) prepared from soil collected from each microcosm after their mixing at 130 rpm for 2 days. RAMEB effects on PCB bioavailability in the soil microcosms and RAMEB biodegradability by the soil washed microorganisms were determined according to Fava *et al.* [11] by using RAMEB at the same concentrations at which they were applied in the soil microcosms and according to the analytical approach mentioned above.

Results

Soil characteristics and amendments

The main physico-chemical and microbiological characteristics of S1 and S2 are summarised in Table 1. The soils were amended with biphenyl (4 g/kg)(where indicated), but were not inoculated with exogenous specialised biomass, as they contained a significant concentration of indigenous PCB- and CBA-degrading aerobic bacteria (Table 1). The two soils presented a content of C, N, and P, as well as a pH suitable for a biological remediation, and therefore no additional soil manipulations were adopted. RAMEB was applied (where indicated) in the range 0–3% (w/w), as it was found to be very effective at these concentrations in the bio-treatment of the 3 PCB-spiked soils employed in a previous study [13].

Bioremediation of the soils

The occurrence of aerobic conditions (about 4 mg/l of dissolved O_2 in slurry-phase conditions) and a pH close to 7 was observed in all the soil microcosms. No aerobic bacteria were detected in the autoclave-sterilised microcosms, whereas a low concentration of heterotrophic bacteria was detected in the chemically sterilised microcosms at the end of the experiment. Autoclave-sterilized and t_0 microcosms were therefore used to quantify changes of the main microcosm parameters throughout the experiment. RAMEB was found to be non toxic towards the soil bacteria, which were found to be capable of slowly metabolising these compounds in shake flask cultures containing RAMEB in the range 1.25–7.5 g/l as the sole carbon and energy source (data not shown). RAMEB was found to be also capable of significantly increasing the PCB bioavailability in both soils; the occurrence of this agent in slurry-phase microcosms resulted in increments of the PCB recovery in the microcosm water-phase of about 3–10%, when applied at 0.5%, and of about 50%, when applied at 3%.

The average disappearance percentage of the PCBs initially occurring in S1 and S2 (54 and 57 GC peaks ascribed to soil PCBs in the organic extracts of S1 an S2, respectively, were chosen and used for PCB depletion studies) is summarised in Table 2. These values were calculated

Table 1. Main characteristics of the soils S1 and S2

Parameters	Soil S1 (PCBs: 890 mg/kg)	Soil S2 (PCBs: 8,500 mg/kg)
Chemical composition		
Total organic carbon (g/kg)	21.0	66.2
Total nitrogen (g/kg)	1.2	2.82
Total phosphorous (g/kg)	0.816	0.532
Chloride ions (mg/L, soil + H_2O 25% w/v)	10.1	95.1
PH	7	7.45
Chemical-Physical properties		
Moisture of the air-dried soil (g/kg)	7.8	18.1
Field capacity (% w/w)	25.7	40.95
Granulometry:		
Sandy fraction (0.053–2 mm) %w/w	88	72
Loamy fraction (0.002–0.0053 mm) %w/w	11	22
Clay fraction (<0.002 mm) %w/w	1	6
Microbiological properties		
Heterotrophic aerobic bacteria (CFU/g)	1×10^6	6×10^7
PCB-co-metabolising bacteria (CFU/g)	1×10^6	1×10^6
CBA-biodegrading bacteria (CFU/g)	9×10^5	1×10^7

Table 2. Average removal of total PCBs of S1 and S2 in slurry- and solid-phase conditions

Microcosms	S1 PCB removal %		S2 PCB removal %	
	Slurry-phase	Solid-phase	Slurry-phase	Solid-phase
No Biphenyl	13.4	14.7	23.6	8.1
Biphenyl + RAMEB 0%	23.6	18.8	22.8	7.5
Biphenyl + RAMEB 0.5%	2.9	19.9	21.5	6.9
Biphenyl + RAMEB 1.0%	−0.9	18.8	10.8	11.6
Biphenyl + RAMEB 3.0%	−4.8	21.6	32.9	10.4

by comparing the total area of the chosen PCB peaks detected in the organic extracts of the soil samples of the biologically active microcosms with that of the same peaks revealed in the corresponding sterile microcosms after 130 days of treatment, and by using Aroclor1260 as the standard. The data summarised in Table 2 indicate that: (a) S1 and S2 PCBs were only slightly depleted in the biologically active microcosms; (b) in the absence of RAMEB, comparable biphenyl-enhanced PCB depletion values were observed in S1 in the two treatment conditions; on the contrary, PCB depletion was not influenced by the presence of biphenyl and was higher in slurry-phase conditions in S2; (c) RAMEB influenced appreciably the PCB depletion in both soils and treatment conditions: an increase of PCB concentration was observed in S1 slurry-phase microcosms, probably because the PCB-solubilising effects of RAMEB prevailed on PCBs biodegradation; (d) RAMEB effects were often dose-dependent.

Several HPLC/Diode-Array detectable aromatic compounds accumulated in the S1 and S2-biologically active microcosms, especially in slurry-phase conditions. Some of these products were detected at higher concentrations along with new compounds in the RAMEB-supplemented microcosms with respect to the RAMEB-free ones. On the basis of their UV-absorption spectra and retention times, 3 of the peaks detected in the S1 RAMEB-supplemented slurry-phase microcosms were characterised as 2-, 4- and 2,5-chlorobenzoic acids. On the contrary, the occurrence of 3,4-chlorobenzoic acid was revealed in the S2-RAMEB amended microcosms.

A very small release of chloride ions was detected in the solid-phase biologically active S1 and S2-microcosms, whereas significantly higher dechlorination yields were observed in the corresponding slurry ones. Table 3 reports the net production of chloride ions in the S1 and S2 microcosms; the data reported in Table 3 were obtained by subtracting to the chloride ion concentration measured in the active microcosms that detected in the t_0 microcosms and that due to the added RAMEB (which contained 8.93 g/kg of chloride ions). The data in Table 3 indicate that: (a) the average PCB-dechlorination yields were generally higher in S2 with respect to S1, and markedly higher in slurry-phase conditions than in solid-phase ones; (b) the presence of RAMEB enhanced strongly the PCB dechlorination yields (about 190% for S1 to more than 250% for S2) with effects which were dose-dependent in S2.

In general, important changes of the concentration of heterotrophic and specialised aerobic cultivable bacterial biomass were observed in all the biologically active microcosms, in particular in the presence of RAMEB (Table

Table 3. Net production of chloride ions in the S1 and S2 microcosms at the 130th day of treatment

Microcosms	Chloride ions in S1 (mg/L)		Chloride ions in S2 (mg/L)	
	Slurry-phase	Solid-phase	Slurry-phase	Solid-phase
No Biphenyl	1.4	1.3	11.9	1.9
Biphenyl + RAMEB 0%	2.1	1.4	12.1	1.4
Biphenyl + RAMEB 0.5%	4.7	1.5	26.0	6.9
Biphenyl + RAMEB 1.0%	4.3	1.3	28.1	11.5
Biphenyl + RAMEB 3.0%	6.2	4.1	41.8	15.6

Table 4. Indigenous bacteria in S1 and S2 at the beginning and at the end of the treatment

Microcosms	Type of bacterial biomass	S1 bacteria concentration (CFU/mL)		S2 bacteria concentration (CFU/mL)	
		Slurry-phase	Solid-phase	Slurry-phase	Solid-phase
Time 0 control	Heterotrophic	1×10^6	1×10^6	6×10^7	6×10^7
(after 3 days)	PCB degrading	1×10^6	1×10^6	1×10^6	1×10^6
	CBA degrading	3×10^5	3×10^5	2×10^6	2×10^6
No Biphenyl	Heterotrophic	8×10^5	3×10^6	2×10^8	3×10^8
(after 130 days)	PCB degrading	6×10^4	2×10^6	8×10^7	1×10^8
	CBA degrading	4×10^3	8×10^5	1×10^8	1×10^8
Biphenyl +	Heterotrophic	1×10^7	8×10^6	4×10^7	3×10^8
RAMEB 0%	PCB degrading	2×10^6	5×10^6	6×10^6	1×10^8
(after 130 days)	CBA degrading	9×10^6	6×10^6	6×10^6	5×10^7
Biphenyl +	Heterotrophic	6×10^6	1×10^7	1×10^8	1×10^8
RAMEB 0.5%	PCB degrading	4×10^6	1×10^7	4×10^7	2×10^8
(after 130 days)	CBA degrading	3×10^6	2×10^7	3×10^7	1×10^8
Biphenyl +	Heterotrophic	6×10^7	6×10^6	5×10^7	2×10^8
RAMEB 1.0%	PCB degrading	5×10^7	3×10^6	2×10^7	2×10^8
(after 130 days)	CBA degrading	4×10^7	2×10^6	1×10^7	1×10^8
Biphenyl +	Heterotrophic	2×10^7	2×10^7	1×10^8	2×10^8
RAMEB 3.0%	PCB degrading	2×10^7	1×10^7	3×10^7	2×10^8
(after 130 days)	CBA degrading	7×10^6	1×10^7	2×10^7	7×10^7

4). Data in Table 4 indicate that: (a) a larger amount of indigenous aerobic bacteria occurred in S2 with respect to S1; (b) in the absence of RAMEB, an important increase and persistence of the heterotrophic and specialized bacteria were observed in both soils and in both treatment conditions; c) the presence of RAMEB caused a strong increase of the concentration and the persistence of the heterotrophic and specialised bacteria in both soils and treatment conditions, in particular in S2 in solid-phase microcosms.

S1 and S2 exhibited a very strong intrinsic ecotoxicity (measured by using the Collembola acute mortality test), probably due to their high content of PCBs and of other hydrocarbons and heavy metals. No significant depletion of the initial ecotoxicity was observed in the soil samples collected from the different microcosms at the end of the treatment (after 130 days), thus indicating that S1 and S2 were not detoxified under the employed treatment conditions.

Discussion

A slight removal of the PCBs originally occurring in S1 and S2 was observed in both treatment conditions. The lack of significant PCB depletions in the sterile microcosms along with the persistence of PCB-co-metabolising and CBA-degrading bacteria, the production of CBAs and of chloride ions in the biologically active microcosms suggest, taken together, that the observed PCB depletions were mainly due to aerobic biodegradation. The biodegraded PCBs were only partially dechlorinated, especially in solid-phase conditions (the average dechlorination of the PCBs depleted in S1 and S2 was less than 5% in solid-phase conditions and less than 10% in slurry-phase). These findings combined with the absence of any significant detoxification of S1 and S2, indicate that both soils were poorly remediable under the conditions employed in this study.

Significant enhancements of the biological degradation of PCBs were observed in the presence of RAMEB both in S1-microcosms (about 15% in solid-phase conditions; whereas an increase of detected PCBs was revealed in the corresponding slurry-phase microcosms), and S2-

microcosms (up to 44%, in slurry conditions, and up to 55%, in the solid-phase ones). Comparable dechlorination yield enhancements were often observed in the RAMEB-supplemented slurry-microcosms. RAMEB effects did not change proportionally with the concentration at which this agent was applied: the highest PCB-biodegradation and dechlorination enhancements were observed in the 3%-supplemented soils, but important positive effects were also achieved with RAMEB at 1%. Among S2 microcosms, significantly higher RAMEB effects on PCB biodegradation and dechlorination were observed under slurry-phase conditions than under the solid-phase ones. This can be ascribed to the higher homogeneity and mass-transfer rates probably achieved under the former treatment conditions [6]. This could also account for the strong increase of PCB availability in S1 slurry-microcosms, where probably the process of PCB solubilisation was larger than those of PCB biodegradation occurring in the same microcosms. However, important positive RAMEB effects on the PCBs biodegradation were also observed in solid-phase conditions, which are treatment conditions of great practical relevance. Slightly higher RAMEB effects on the soil PCB bioavailability and biodegradability were observed in S2-microcosms; however, considering the similarity of the yields of PCB biodegradation and dechlorination observed in the 2 soils, it can be concluded that RAMEB effects were only slightly dependent on the PCB content and the microbiological, physical and chemical properties of the soils subjected to treatment.

RAMEB CDs, which were slowly metabolised by the indigenous soil bacteria, enhanced the growth and the persistence of the PCB-co-metabolising biomass and enhanced significantly (up to 50%) the PCB availability in the soil slurry-phases; this confirms previous evidence [13] according to which RAMEB can enhance the PCB biodegradation by increasing both the PCB bioavailability and the availability of specialised bacteria in the microcosms.

In conclusion, RAMEB was found to enhance strongly the bioavailability of PCBs of the 2 different actual site contaminated soils employed in this study. RAMEB also enhanced significantly the biological degradation and dechlorination of the pollutants of the soils, which however were poorly biotreatable. The results of this study are of great relevance as they show that RAMEB is a biodegradable, non-toxic and very effective PCB-bioavailability enhancing agent also in the case of historically PCB-contaminated soils.

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