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Effects of RAMEB on Bioremediation of Different Soils Contaminated with Hydrocarbons

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Abstract

The limiting factor of soil remediation is often the low accessibility of the pollutants. Laboratory experiments have been carried out to investigate the effect of the randomly methylated cyclodextrin (RAMEB) on bioremediation of various types of soils spiked with Diesel and transformer oil and also on actual site soils contaminated with poorly degradable mazout. The contaminated soil in the aerobic solid phase microcosm-experiments was amended with nutrients and supplemented with different amounts of RAMEB. An integrated chemical-biological-ecotoxicological methodology was applied to follow the bioremediation. The laboratory study proved the bioremediation enhancing effect of RAMEB both on artificially contaminated soils and on actual site mazout contaminated soils. RAMEB activated soil microbes by improving the bioavailability of the contaminants and accelerating biodegradation. Efficacy of RAMEB was influenced both by contaminant and environment related factors, such as the type and concentration of the polluting hydrocarbons and characteristics of the soil.

Introduction

Inherited contaminated sites still present high concentration of pollutants like petroleum hydrocarbons, chlorinated solvents, pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls. These chemicals represent serious environmental and health risk. For the remediation of contaminated soil different physical, chemical and biological technologies can be applied. The most up-to date and promising remediation technologies are based on biodegradation. Bioremediation is an inexpensive, safe and environmental friendly technology. The end-product of bioremediation is the harmless, decontaminated soil. In comparison with chemical, physical and thermal treatment technologies, however, bioremediation is time consuming. Months or years may be required to reduce the contamination to the acceptable level.

A large number of factors may have an effect on the biodegradation of a chemical pollutant in an environmental system. The role of biotechnologies in soil remediation is to upgrade biodegradation of organic contaminants by optimising environmental factors, such as nutrient and oxygen supply, humidity, pH, temperature and the bioavailability of contaminant [1].

It is of economical interest to develop technologies, which apply additives and agents to improve the efficacy of the technology without increasing environmental risk. The mobilising agent must enhance the desorption of the contaminants from soil without causing further contamination, e.g. in the subsurface waters.

Surfactants have been used to improve the biodegradability of contaminants, but mixed results of either enhancement or inhibition have been reported [2].

Due to the solubilising effect of cyclodextrins they are useful in physical and chemical treatment technologies as well as in bioremediation [3]. Favourable characteristics make cyclodextrin a tempting technological additive: it is not toxic, biodegradable and in comparison to surfactants they are less prone to form emulsions. The environmental use of cyclodextrins has recently become feasible economically, following their price decrease on the market.

The results of laboratory experiments showed that cyclodextrin can be used to reduce the sorption and enhance the transport of organic contaminants such as anthracene, pyrene and trichlorobiphenyl in soil [4]. In another study it was found that hydroxypropyl β-cyclodextrin (HPBCD) enhanced the rate of phenanthrene biodegradation [5]. The biodegradation of polychlorinated biphenyls (PCBs) was also significantly enhanced both by HPBCD and RAMEB [6, 7]. Furthermore, it has been shown that "molecular encapsulation" by RAMEB increased the bioavailability of the contaminants during biodegradation and also modified

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toxicity on soil microbes, plants and soil living animals in ecotoxicity tests [8, 9].

To characterise and follow biodegradation in soil needs and integrated approach: biological and ecotoxicological characterisation of contaminated soil gives additional information to physico-chemical data during the remediation process from the design phase through the application of the remediation technology, until the monitoring of the remedied site [10].

The aim of the present study was to investigate the application of RAMEB for different soil types and for highly persistent contaminants, like mazout. The experiments were followed by the integrated methodology: the results of physical, chemical data accomplished by the biological and ecotoxicological tests gave a detailed picture on soil, contamination, biodegrading microbes and cyclodextrins, as well as their interactions in the soil during remediation.

Experimental

Randomly methylated β-cyclodextrin (RAMEB), degree of substitution (DS):12.6 (CAVAMAX W 7), was obtained from Wacker-Chemie.

Effect of RAMEB on three soil types

Solid phase laboratory experiments were carried out in small scale reactors (250 g) with three different soils (sandy, clay and humic-loamy) for 4 weeks. Soil properties (pH-value, electric conductivity, particle size distribution) and initial nutrient status (humus, nitrogen, phosphorous and organic carbon content) were analysed.

Soils were spiked with 30 000 ppm Diesel oil and 30 000 ppm transformer oil, then amended with inorganic nutrients (N, P) and supplemented with RAMEB in a concentration of 0; 0.1; 0.3; 0.5 and 0.7 (w/w)%. Soil samples were taken after 2 and 4 weeks and analysed by chemical, biological and ecotoxicological methods.

Effect of RAMEB on the biodegradation of mazout

Biodegradation experiments were carried out in small scale (200 g) solid phase reactors for 4 weeks. The soils originated from different points of a heterogeneously contaminated actual site: the initial mazout concentration was: 12 780 (Site I), 21 040 (Site II), 30 900 (Site III) and 100 000 (Site IV) mg mazout/kg soil. The soil reactors were supplemented with nutrients and treated with RAMEB in a concentration of 0; 0.3; 0.5; 0.7 and 1.0%. Integrated chemical-biological-ecotoxicological methodology was applied to follow the bioremediation process.

Chemical analyses: Extractable Petroleum Hydrocarbon (EPH) content was measured by gas chromatography with flame ionization detector (GC-FID) after hexane-acetone (2:1) ultrasonic extraction of air-dried soil samples according to the standards of EPA 8270 and HS 21470-94 and by Fourier Transform Infrared Spectroscopy (FT-IR) after CCl4 Soxhlet extraction. Chemical data were evaluated by STATISTICA 5 for WINDOWS.

Biological characterisation: general microbial activity of the soil was characterized by the number of aerobic heterotrophic cells (HS 21470/77, 1988) determined by plating and colony counting. The hydrocarbon-degrading cell number was measured by the Most Probable Number method (MPN), detecting the hydrocarbon utilising enzyme activity and evaluating the counts statistically [11].

Direct contact ecotoxicological tests were developed for the investigation of the toxic effect of soil samples. These interactive tests ensure the contact between the soil and the testorganism. These are self-developed tests based on Hungarian, German and European standard methods for testing waste waters or hazardous waste materials [11]. *Photobacterium phosphoreum* bioluminescence test (DIN 38412, 1991; ISO/CD 11348, 1994), *Azotobacter agile* dehydrogenase enzyme activity test (HS 21978/30, 1989) and *Sinapis alba* root and shoot elongation test (OECD No. 208, 1984; HS 22902-4, 1991; HS 21976-17, 1994) were modified for whole soil and applied for measuring toxicity during soil treatment.

Results and discussion

The effect of RAMEB on biodegradation in different soil types

Soil properties have an influence on the soil life and also the biodegradation processes in the soil. In our laboratory experiments the effect of soil type on the biodegradation of contaminants and on the efficacy of RAMEB was studied using artificially contaminated soil. Unpolluted soils were selected to represent typical Hungarian soils and a wide range of soil properties. The physico-chemical characteristics of the applied soils are presented in Table 1. The artificially contaminated sandy, clay and humic-loamy soils were sampled and analysed after 2 and 4 weeks after RAMEB treatment. An enhanced degradation of Diesel oil and transformer oil was observed as an effect of RAMEB mainly in the beginning of the process (after 2 weeks) when bioavailabaility is the limiting factor of biodegradation, as shown in Table 2. Removed Diesel oil in sandy soil was 40% in case 0.3% RAMEB treatment compared to the 25% removal from untreated soil. Clay soil needs higher CD concentration: 0.5% RAMEB increased the oil removal from 3% to 21% after 2 weeks. Even higher effect on transformer oil biodegradation was measured on the effect of RAMEB treatment: 1% removal from untreated soil increased to 41% in sandy soil, 16% to 46% in clay soil, and 30% to 62% in humic-loamy soil. The highest oil degradation rate was found in humic-loamy soil. Humic-loamy soil is the “best-quality” habitat for microorganisms due to its good physico-chemical properties (high humus, nitrogen and phosphorous content; pH; mechanical composition).

The correlation analyses by the use of software STATISTICA showed very good correlation between RAMEB-concentration and the removed oil content particularly in

Table 1. Physico-chemical characteristics of the three types of soil

Type of the soil	pH_{H_2O}	EC 1:2.5 mS/cm	Humus content % (w/w)	Nitrogen content g/kg	Phosphor. content g/kg	Org. carbon content g/kg	Mechanical composition Sand % (w/w)	Silt % (w/w)	Clay % (w/w)
Sandy	5.12	0.07	0.45	0.49	0.299	2.65	87.12	9.60	3.28
Clay	7.40	0.31	3.91	1.81	0.326	23.01	4.33	46.80	48.87
Humic-loamy	7.30	1.38	4.18	2.10	0.462	26.15	18.98	56.31	24.71

EC = electric conductivity.

Table 2. Effect of RAMEB on oil degradation in different soils contaminated with 30 000 ppm of Diesel and transformer oils

RAMEB concentration [w/w]	Removed Diesel oil [%] After 2 weeks Sandy soil	Clay soil	Humic-loamy soil	After 4 weeks Sandy soil	Clay soil	Humic-loamy soil
RAMEB 0%	25	3	18	41	30	55
RAMEB 0.1%	24	3	5	40	20	44
RAMEB 0.3%	40	8	28	46	27	46
RAMEB 0.5%	36	21	31	34	25	60
RAMEB 0.7%	32	18	37	47	41	58
	Removed transformer oil [%] After 2 weeks Sandy soil	Clay soil	Humic-loamy soil	After 4 weeks Sandy soil	Clay soil	Humic-loamy soil
RAMEB 0%	1	16	30	21	38	40
RAMEB 0.1%	9	30	37	19	51	47
RAMEB 0.3%	23	33	37	24	41	57
RAMEB 0.5%	20	36	62	32	43	57
RAMEB 0.7%	41	46	44	37	50	61

case of soils contaminated with transformer oil. Good correlation occurs when the correlation coefficient is close to +1. The correlation factors in case of transformer oil contaminated soil are in most cases close to 0.9.

Results of cell concentrations correspond well with EPH content measured by GC-FID. Sandy soil was a poor, humic-loamy soil was a good habitat for soil microbes. In clay soil a different type of microflora has been developed due to low porosity, and low redox potential, compared to the sandy and humic-loamy soils. The concentration of aerobic heterotrophic and oil-degrading bacteria increased to a higher value on the effect of RAMEB especially in humic-loamy and clay soil, where the oil degradation rate was higher than in sandy soils (Figure .). An optimal cyclodextrin concentration is shown by the maximal cell concentrations.

Toxicity test result show the increased bioavailabilty of the contaminants during the first 4 weeks. Toxicity measured by *Photobacterium phosphoreum* bacterial and *Sinapis alba* plant test correlates with the availability of the contaminants and with the properties of the soil, but not with the EPH content. Among the two tests *Photobacterium phosphoreum* bioluminescence test was the most sensitive to oily contaminants. The results of *Photobacterium phosphoreum* luminescence test are summarised in Table 3. Inhibition of samples is given in Cu equivalent (ΣCu_{20}). $\Sigma Cu_{20} = ED_{20\,Cu}/ED_{20\,sample} * 10^6$, where ED_{20} means soil (or Cu) doses caused 20% inhibition.

During the 4 weeks experiment toxicity showed an increasing tendency due to growing bio-availability. Ecotoxicity testing is useful to prove that the end-product of the remediation fulfils the target quality, representing an acceptable risk level (not toxic for soil living testorganisms of three trophic levels). The lowest toxicity was measured in humic-loamy soils, where the biodegradation of hydrocarbons is more advanced compared to sandy and clay soils.

Bioremediation of soil contaminated by mazout using RAMEB

Actual site mazout-contaminated soil was applied to study the effect of RAMEB addition on the biodegradation of this poorly degradable hydrocarbon. Mazout, the residual product of petroleum refining contains heavy, high molecular weight hydrocarbons. The mazout removal after 4 weeks calculated from the GC-FID results are summarised in Table 4.

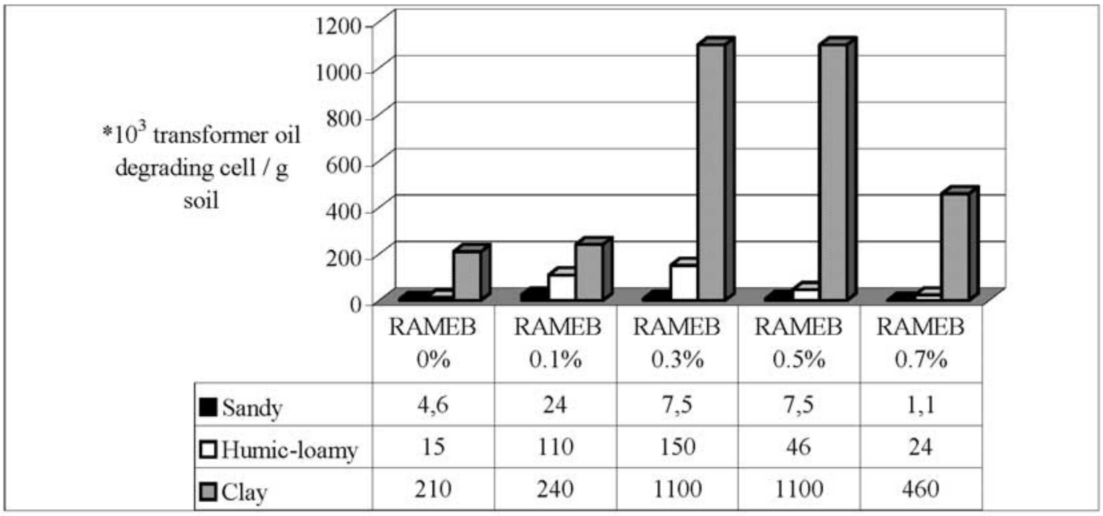

Figure 1. Effect of RAMEB on transformer oil-degrading cell number in different soils after 4 weeks.

Table 3. Effect of RAMEB on soil toxicity (*Photobact. phosph.* bioluminescence test)

RAMEB concentration [w/w]	ΣCU20* – Diesel oil contaminated soils [mg Cu/kg soil]						
	After 2 weeks				After 4 weeks		
	Sandy soil	Clay soil	Humic-loamy soil		Sandy soil	Clay soil	Humic-loamy soil
RAMEB 0%	263	771	<80		634	961	239
RAMEB 0.1%	289	208	<80		634	1150	252
RAMEB 0.3%	273	1293	<80		649	804	135
RAMEB 0.5%	225	564	<80	525	795	227	
RAMEB 0.7%	379	514	<80		591	570	164
	ΣCU20 – transformer oil contaminated soils [mg Cu/kg soil]						
	After 2 weeks				After 4 weeks		
	Sandy soil	Clay soil	Humic-loamy soil		Sandy soil	Clay soil	Humic-loamy soil
RAMEB 0%	<80	<80	131		488	447	204
RAMEB 0.1%	<80	<80	<80		1298	597	<80
RAMEB 0.3%	<80	<80	<80		488	478	<80
RAMEB 0.5%	<80	<80	<80		488	510	248
RAMEB 0.7%	<80	<80	<80		337	413	<80

$^{*}\Sigma Cu20 = ED_{20\,Cu}/ED_{20\,sample} * 10^6$, where ED_{20} means soil (or Cu) doses caused 20% inhibition.

Table 4. Effect of RAMEB on mazout removal from contaminated soils

RAMEB concentration [w/w]	Removed mazout [%] I	II	III	IV	[mg/kg] I	II	III	IV
RAMEB 0%	51	34	<10	9	6 540	7 220	<3000	8810
RAMEB 0.3%	72	20	<10	<3	9 210	4 120	<3000	<3000
RAMEB 0.5%	73	54	<10	<3	9290	11 290	<3000	<3000
RAMEB 0.7%	53	40	10	40	6760	8430	3090	40 100
RAMEB 1.0%	69	45	21	36	8770	9460	6440	36 010

Initial mazout concentrations: I: 12780 ppm; II: 21 040 ppm; III: 30090 ppm; IV: 100 000 ppm.

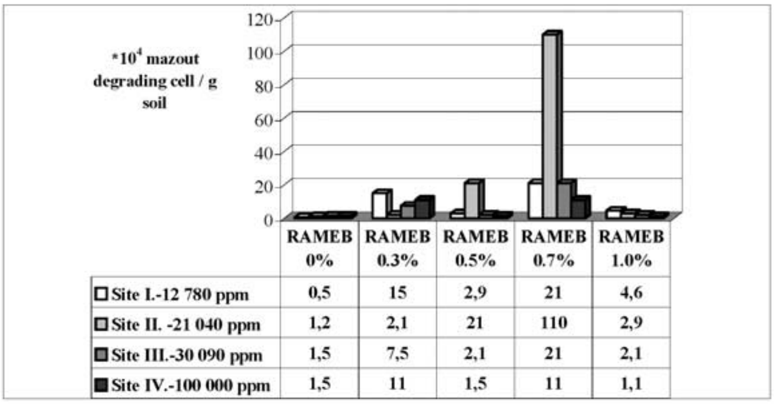

Figure 2. Effect of RAMEB on mazout-degrading cell number after 4 weeks.

Table 5. Results of Azotobacter agile dehydrogenase enzyme inhibition test after 4 weeks

Initial mazout Concentration mg/kg	RAMEB Concentration %	Soil doses caused 20% and 50% inhibition ED_{20} [g]	ED_{50} [g]	Characterisation
Site I – 12 780	0	>0.5	>0.5	Non toxic
Site I – 12 780	0.3	>0.5	>0.5	Non toxic
Site I – 12 780	0.5	>0.5	>0.5	Non toxic
Site I – 12 780	0.7	>0.5	>0.5	Non toxic
Site I – 12 780	1.0	>0.5	>0.5	Non toxic
Site II – 21 040	0	>0.5	>0.5	Non toxic
Site II – 21 040	0.3	>0.5	>0.5	Non toxic
Site II – 21 040	0.5	>0.5	>0.5	Non toxic
Site II – 21 040	0.7	>0.5	>0.5	Non toxic
Site II – 21 040	1.0	0.180	0.250	Toxic
Site III – 30 090	0	0.013	0.070	Very toxic
Site III – 30 090	0.3	0.011	0.046	Very toxic
Site III – 30 090	0.5	0.045	0.153	Toxic
Site III – 30 090	0.7	0.090	0.213	Toxic
Site III – 30 090	1.0	0.117	0.125	Toxic
Site IV – 100 000	0	0.005	0.010	Very toxic
Site IV – 100 000	0.3	0.044	0.098	Very toxic
Site IV – 100 000	0.5	0.011	0.047	Very toxic
Site IV – 100 000	0.7	0.014	0.042	Very toxic
Site IV – 100 000	1.0	0.120	0.125	Toxic

ED_{20}, ED_{50} – soil doses caused 20% and 50% inhibition of enzyme activity.

The soil used in the experiment differed not only in mazout content, but also in the quality of the contaminant. The soil of 30 090 mg/kg initial mazout concentration was highly toxic and the cell number was also extremely low (no adaptation or additional toxic pollutant).

The degradation rate percentage was higher in case of 12 780 and 21 040 mg mazout/g soil-contamination, but the absolute amount of the removed mazout was the highest in case of 100 000 mg/kg mazout contaminated-soil treated with 0.5–0.7% RAMEB. At low mazout-contamination level the 0.3 and 0.5%, at high contamination level the 0.7 and 1.0% RAMEB concentration has been found to be the most effective.

The concentration of mazout degrading cells has been increased upon RAMEB addition (Figure 2). The highest RAMEB effect was observed at 0.7% concentration. The results demonstrated the benefit of RAMEB-addition in mazout degradation in spite of the short duration of the experiment (4 weeks).

Azotobacter agile dehydrogenase enzyme inhibition test and *Sinapis alba* shoot elongation test were found to be sensitive to mazout contaminant. The results of *Azotobacter agile* test are summarised in Table 5.

The toxicity of soil samples has become acceptably low in case of 12 780 and 21 040 mg/kg initial oil-content. At high contamination level of mazout (30 090 and 100 000 mg/kg) high toxicity was observed even after 4 weeks. Being mazout very toxic in this case longer duration of experiments is required to achieve successful bioremediation.

A growing trend in toxicity in the beginning of the remediation process correlates with increasing bioavailability. Decrease of toxicity at the end of the biodegradation process is proportional with the rate of biodegradation, with the final removal of the toxic pollutants.

Conclusion

In all test systems an enhanced degradation of hydrocarbons and increased microbial activity were observed on the effect of RAMEB. Based on these experiments performed on artificially and actual site contaminated soils RAMEB can be used as additive for enhancing bioremediation of soils contaminated with hydrocarbons. RAMEB has the potential to reduce clean-up time especially in soils of inherited sites, contaminated with poorly degradable hydrocarbons.

Future plans involve pilot-scale laboratory experiments and field experiments which are necessary to progress RAMEB-enhanced bioremediation from an emerging technology to a proven technology.

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