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Enhanced biodegradation of transformer oil in soils with cyclodextrin – from the laboratory to the field

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Abstract

The use cyclodextrins for the intensification of bioremediation by improving the mobility and bioavailability of contaminants has recently been studied. In this work, the role of randomly methylated ß-cyclodextrin in the bioremediation of soils contaminated with transformer oil was studied both in bench scale bioreactors and through field experiments. The aims of this research were to (a) establish the scientific background of a cyclodextrin-based soil bioremediation technology, (b) demonstrate its feasibility and effectiveness in the field, and (c) develop an integrated methodology, consisting of a combination of physical, chemical, biological and ecotoxicological analytical methods, for efficiently monitoring the technology performances. The stepwise increasing scale of the experiments and the application of the integrated analytical methodology supported the development of a scientifically established new technology and the identification of the advantages and the limitations of its application in the field. At each phase of the study, randomly methylated ß-cyclodextrin was found to significantly enhance the bioremediation and detoxification of the transformer oil-contaminated soils employed by increasing the bioavailability of the pollutants and the activity of indigenous microorganisms.

Abbreviations: CD – cyclodextrin; CDT – cyclodextrin-based soil bioremediation technology; EPH – extractable petroleum hydrocarbons; RAMEB – randomly methylated ß-cyclodextrin

Introduction

Biodegradation of hydrocarbons in the environment or natural system may be limited by a large number of factors. An important rate-limiting factor in the bioremediation of historically contaminated soils is often the low bioavailability of the hydrocarbon-type contaminants. Synthetic surfactants have been used in many cases to enhance the solubilisation process, but inconclusive results have often been reported, and in some cases the applied surfactant proved to be toxic (Rouse et al. 1994). An important requirement is that the enhancing agent should have low environmental risk. On the basis of previous findings, cyclodextrins (CDs) can meet these requirements. CDs, in fact, are proved to be useful in physical and chemical treatment technologies (McCray & Brusseau 1998; Ko et al. 2000; Sheremata & Hawari 2000) as well as in soil and wastewater bioremediation due to their solubilising effects (Bardi et al. 2000; Fava et al. 1998; Gruiz et al. 1996; Olah et al. 1988).

CDs are non-toxic and biodegradable compounds and in comparison to surfactants they are less prone to form emulsions. These cyclic oligosaccharides due to their favourable structural character can form inclusion complexes with a wide variety of organic compounds (Szejtli 1996). A very large number of CDs and derivatives are known and some of them have been already applied for the enhancement of biodegradation of hardly biodegradable contaminants, like PAHs, polychlorinated biphenyls (PCBs) (Fava et al. 2002; Wang et al. 1998). Randomly methylated ß-cyclodextrin (RAMEB) is produced industrially, and has excellent solubilising ability (Fenyvesi et al. 1996). It has been shown that RAMEB is able to intensify the biodegradation of hydrocarbons in soils by also mitigating the soil toxicity towards soil microbes, plants and animals (Gruiz et al. 1996).

The biodegradation of the highly persistent PCBs was found to be significantly enhanced in the presence of RAMEB (Fava & Ciccotosto 2002; Fava et al. 2003). RAMEB also enhanced the biodegradation of mazout (distillation residue of oil refinery, formerly used as heating fuel) in occurring in a historically contaminated soil (Molnar et al. 2003). RAMEB effect is attributed partly to its impact on the surface properties and porosity of the soils (Jozefaciuk et al. 2003). RAMEB was found to be slowly biodegradable in soils, displaying about 1-year half-life time (Fenyvesi et al. 2002).

Transformer oil contamination is a frequent and very serious problem all over the world, also in Hungary, mainly at the transformer stations. At present times most of the contamination at transformer-stations derives from PCB-free transformer oils. The possibility of bioremediating transformer oil-contaminated soils has recently been demonstrated (Molnar et al. 2003). It was found that the process is adversely affected by the poor bioavailability of the transformer oil hydrocarbons. The use of CD may increase the bioavailability of oily pollutants and in turn intensify soil bioremediation. This study was undertaken to validate this hypothesis and a research approach moving from microcosms experiments to field *ex situ* and *in situ* experiments was adopted for this purpose. To better follow and understand interactions between toxic chemicals, soil matrix and biota an integrated chemical, biological and ecotoxicological analytical methodology was also developed, and used to assess the cyclodextrin-based soil bioremediation technology (CDT) and to monitor the site selected for the field experiment before, throughout and after the treatment.

Materials and methods

Experimental: bioremediation experiments

The development of the new technology for intensifying the bioremediation of soil contaminated with transformer oil (type: TO40A, National Power Line Company LTD., Hungary) using RAMEB was done in more steps, using small and large-scale laboratory experiments, followed by pilot and full-scale field experiments. A 50% (w/v) aqueous solution of RAMEB (Cawasol W7 MTL, Wacker Chemie, Munich, Germany) was used in all experiments.

Small-scale laboratory experiments on the remediation of transformer oil-spiked soils

A large number of experiments (70 experiment-series) were done in static solid phase and in mixed slurry-phase aerobic reactors to determine the influence of soil characteristics and of some technological parameters, such as way and extent of RAMEB addition, on the effects of RAMEB on the remediation of selected soils through 4–9 weeks-experiment. Two hundred and fifty grams of soil were spiked with transformer oil in the range of 10,000–30,000 mg kg^{-1}, and amended with inorganic nutrients (N – $(NH_4)_2SO_4$, P – KH_2PO_4) to reach a final C:N:P ratio of 100:10:1and RAMEB in a concentration range of 0–1% (w/w). The role of soil properties on the process was also studied by employing three different types of pristine soils (sandy, clay, humic-loamy).

Pilot scale laboratory experiments on remediation of transformer oil-spiked soils

The 10-month-long pilot scale laboratory experiment was planned on the basis of the results collected in the previous small-scale experiments. The remediation has been performed in 60 l fixed-phase soil reactors. Brown forest soil (40 kg) was spiked with 30,000 mg kg^{-1} of transformer oil. RAMEB was applied in the concentration of 0.0, 0.5 and 1.0% (w/w). The soil was amended with inorganic nutrients as described above and treated under aerobic conditions at 25 °C. Optimal humidity (10–15% w/w) was ensured and maintained throughout the whole experiment.

Field experiments
On the basis of the results collected from the previous lab-scale studies, we designed and performed field experiments for the remediation of actual-site transformer oil-contaminated soils. The new technology was tested through both *ex situ* (Site I.) and *in situ* (Site II.) experiments; the results obtained were evaluated, interpreted and compared. Site I was a former military site recently contaminated through an illegal disposal of transformer oil (about 20,000 mg kg^{-1}). Only a small volume (10 m^3) of soil of the unsaturated zone was found to be contaminated; however, due to the high risk of the potential transfer of the oil from the vadose zone to the groundwater, the soil had been excavated and placed on a confined area. Two isolated biopiles (one with RAMEB and the other one without this additive, CD-treated and untreated pile, respectively) were constructed with 0.5 m^3 of the contaminated soil occurring excavated from the site. The applied RAMEB concentration was 1.0 g kg^{-1} provided as 50% (w/v) aqueous solution. Nutrient supply and humidity control was applied. Manual mechanical mixing was used every 2 weeks to guarantee aerobic conditions and homogeneity; the experiment was maintained for 30 weeks.

Site II was an inherited contaminated site with a long-term pollution derived from a leaking spare-transformer of a transformer station in Budapest. The oil from the point source had already reached the groundwater. The average concentration of the transformer oil contaminant in the soil was about 20,000 mg kg^{-1} in the soil and close to 1 mg l^{-1} in the groundwater. Both the saturated and the unsaturated zones have to be decontaminated. A complex methodology was applied for the remediation of the 50 m^3 of contaminated soil occurring at the site. The *in situ* bioventing of the unsaturated zone was combined with the *ex situ* physico-chemical treatment of the groundwater. Nutrients (40 kg garden chemical fertiliser containing 15% P_2O_5, 15% N and 15% K_2O; Pétisó, Agrolinz Chemikalien GmbH, Linz) were added three times, i.e., at the 9th, 13th and 21st week, along the 47 weeks-experiment. RAMEB was applied to improve desorption and the solubilisation of contaminants into the aqueous soil phases (biofilms, capillary water, pore water, and groundwater), where biodegradation by soil microbes was going on. Aqueous RAMEB solution (containing 50% (w/v) RAMEB) was added in three equal details (10 kg RAMEB each) together with the nutrients dissolved in 2 m^3 water.

The integrated methodology for soil characterisation and technology monitoring

The integrated analytical methodology was applied to follow up of the laboratory and field experiments, and also for the initial and final assessment of the CDT-treated sites. We developed test batteries including specific combinations of suitable chemical, physical, biological and ecotoxicological methods depending on the aim of testing. The applied test-sets included standardised methods as well as newly developed tests. With the growing scale of the experiments, a more detailed analytical program was created. Soil properties (pH-value, electric conductivity, mechanical composition) and nutrient status (humus, nitrogen, phosphorous and organic carbon content) were analysed before starting the remediation experiments according to Forster (1995). Extractable organic material content was measured after hexane–acetone (2:1) extraction by gravimetry (HS 21470-94, 2001). The so-called Extractable Petroleum Hydrocarbon (EPH) content was analysed from the same extract by gas chromatography with flame ionisation detector (GC-FID) according to the Hungarian Standard (HS 21470-94, 2001). Hydrocarbon content was also analysed by the more selective Fourier transform infrared spectroscopy (FT-IR). Aerobic heterotrophic bacterial cell concentration was determined by colony counting after cultivation of microorganisms occurring in soil suspensions in water on peptone–glucose–meat extract (PGM) agar plates in Petri-dishes. The population density of the hydrocarbon-degrading cells was measured after cultivation in tubes of liquid nutrient medium. For growing the oil-degrading cells a dilution series of contaminated soils were used in three replicates, containing transformer oil as the only carbon source supplemented with inorganic salts and trace elements. The most probable number (MPN) was calculated from the red colour (+/−) in the tubes by using probability tables (Lorch et al. 1995). The CO_2 production by the soil microbes in laboratory scale was measured by a self-designed respirometer. The CO_2 was absorbed in NaOH and determined by HCl titration. In the field experiments CO_2 and O_2 content of the soil gas was measured by an Oldham Mx21 Gas Analysator (Oldham Gas Detection Ltd., UK). During the technology monitoring the CO_2 production and O_2 consumption were proportional with the microbiological activity. For direct contact ecotoxicity testing testorganisms of three different trophic levels were used. The interactive ecotoxicity tests ensure the contact between the soil and the testorganism, showing the actual toxicity and ensuring higher environmental reality. These are self-developed tests based on similar Hungarian, German and European standard methods for wastewaters or hazardous waste materials. *Vibrio fischeri* bioluminescence test (DIN 38412, 1991), *Sinapis alba* root and shoot elongation test (HS 21976-17, 1994) and *Collembola* (*Folsomia candida)* (ISO/FDIS 11267, 1998) mortality test were modified for soil and used in all experiments. *Vibrio fischeri* is a marine-living bacterium very commonly used for ecotoxicity testing. *Sinapis alba* is also widely used test plant. The Collembolans, commonly known as springtails, are the most numerous and widely occurring insects in terrestrial ecosystems.

The endpoints used for the plant and animal tests were ED_{20} (LD_{20}) or ED_{50} (LD_{50}) values, where ED_{20} (LD_{20}) and ED_{50} (LD_{50}) mean soil doses that caused 20% and 50% inhibition (lethality). ED_{20} (LD_{20}) and ED_{50} (LD_{50}) values were determined from dose-response curve (inhibition percent values of different dilutions) after sigmoidal fitting of data by ORIGIN 6.0 software.

In case of *Vibrio fischeri* test, the inhibition of samples was given in Cu-equivalent by comparing the measured results to a Cu-calibration curve: ΣCu20 and ΣCu50 (mg Cu kg^{-1} soil). These Cu-equivalent values can be compared with the effect based soil quality guidelines. Data evaluation of small-scale laboratory experiment series was processed by correlation analyses by the use of StatSoft® Statistica 6 program.

Results and discussion

Small-scale laboratory experiments on the remediation of transformer oil-spiked soils

A large number of laboratory experiments were performed with spiked soils in order to find differences, trends and optimal parameters in the use of RAMEB for the enhancement of transformer oil biodegradation. The experiments proved that RAMEB significantly increase the apparent water solubility of transformer oil constituents (data not shown). The biodegradation of transformer oil was faster in solid-phase than in slurry-phase bioreactors. In one of the biodegradation experiments, the transformer oil content decreased from the initial 30,000 to 11,600 mg kg^{-1} and to 7700 mg kg^{-1} in solid-phase reactor with 0.1% RAMEB and 0.5% RAMEB, respectively, at the end of the 5-weeks-experiment. In the parallel RAMEB free solid-phase reactor EPH was removed to 17,300 mg kg^{-1} at the end of the 5 weeks. In slurry-phase conditions, the transformer oil content decreased from the initial 30,000 to 13,500 mg kg^{-1} and to 11,000 mg kg^{-1} in the presence of 0.1% and 0.5% of RAMEB, respectively, after 5 weeks. The EPH-depletion in the parallel slurry-phase untreated soil was 15,400 mg kg^{-1} after the same treatment time. Usually the increments of the transformer oil degradation percentage in the presence of RAMEB were 50–80% in solid-phase conditions, and 15–35% in slurry conditions. In solid-phase experiments carried out on sandy, clay and humic-loamy soils, an enhanced degradation was observed in the presence of RAMEB. The physico-chemical characteristics of different soil types used in the experiments are shown in Table 1.

RAMEB effects were observed in particular at the beginning of the remediation process, when a scarce bioavailability was probably the main limiting factor for the pollutant biodegradation (Figure 1). RAMEB significantly shortened the lag-phase of the bioremediation, especially in the humic-loamy soil and when it was applied at concentration higher than 0.3–0.5%.

The highest oil degradation rate and extent was found in the humic-loamy soil (Figure 1). The statistical analyses by the use of STATISTICA showed very good correlation between RAMEB-concentration and removed oil content in most

Table 1. Physico-chemical characteristics of different soils

Type of the soil	pH_{H2O}	EC 1:2.5	Humus content	Nitrogen content	Phosphor content	Org. carbon content	Mechanical composition		
							Sand	Silt	Clay
		mS/cm	% (w/w)	g kg^{-1}	g kg^{-1}	g kg^{-1}	% (w/w)	% (w/w)	% (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
Hum-loamy	7.30	1.38	4.18	2.10	0.462	26.15	18.98	56.31	24.71

EC = electric conductivity; Hum-loamy = humic-loamy.

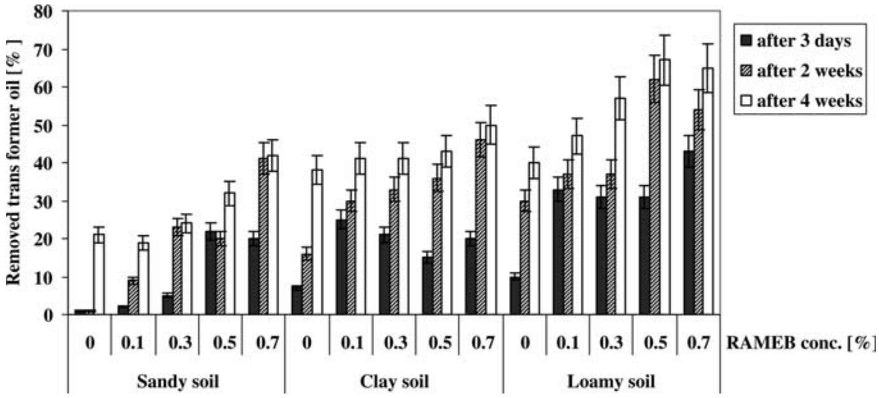

Figure 1. Effect of RAMEB on the transformer oil degradation in different types of soils in small-scale laboratory experiment. Error bars represent standard deviations (SD ± 10% or less).

cases. Correlation factors were 0.80–0.97 at $p < 0.05$ significance level. The number of transformer oil-degrading bacteria increased on the effect of RAMEB, especially in humic-loamy and clay soil, where the oil degradation rate was higher than in sandy soils (Table 2).

The concentration of specialised biomass decreased by increasing RAMEB concentration to values higher than 0.3–0.5%. This was probably due to the increase of pollutant toxicity probably due, in turn, to the growing pollutant bioavailability in the reactor waster-phase. Humic-loamy soil is the 'best-quality' habitat for microorganisms probably for its favourable physico-chemical properties (high humus, nitrogen and phosphorous content; high pH; good mechanical composition). A slower growing microflora was detected in clay soil; this was probably due to the lower porosity, and lower redox potential of this soil with respect to the sandy and humic-loamy soils. Higher oil-degrading cell concentration was in agreement with lower EPH content of soils. During the experiments performed on different types of soil toxicity slightly increased, probably as a result of the increased pollutant bioavailability. The lowest toxicity was measured in humic-loamy soil, where the hydrocarbon biodegradation was larger than in the other two soil types (data not shown).

Pilot scale laboratory experiments on remediation of transformer oil-spiked soils

The laboratory experiment resembling *in situ* bioremediation conditions showed the benefits of RAMEB on the rate and the extent of transformer oil biodegradation. RAMEB enhanced the availability of both the aerobic heterotrophic and oil-degrading bacteria in the humic-loamy forest soil (data not shown) and CO_2 production (Figure 2). The presence of more specialised microbial

Table 2. Effect of RAMEB on oil-degrading cell concentration in different soils after 4 weeks bioremediation in small-scale laboratory experiments

RAMEB [w/w]	Transformer oil-degrading cell concentration [* 10^3 cell g^{-1} soil]		
	Sandy soil	Clay soil	Humic-loamy soil
0%	5 (1–23)	210 (45–983)	15 (3–70)
0.1%	24 (5–112)	240 (52–1123)	110 (24–515)
0.3%	8 (2–37)	1100 (235–5148)	150 (32–702)
0.5%	8 (2–37)	1100 (235–5148)	46 (10–215)
0.7%	1 (0–5)	460 (98–2153)	24 (5–112)

Values are the MPN of transformer oil-degrading cells after statistical evaluation. Numbers in parentheses represent the lower and

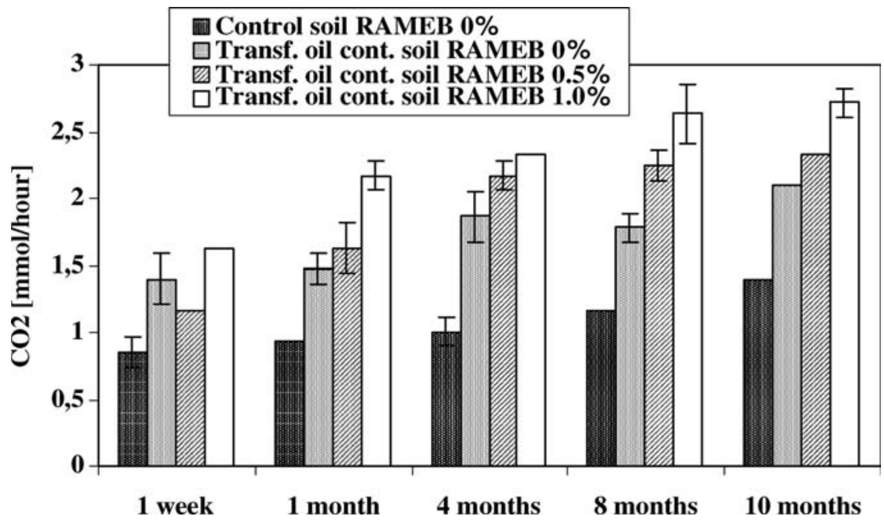

Figure 2. Changes of the microbial activity throughout the pilot scale experiment estimated as CO_2 production rates. Values are the means of three replicates. Error bars represent standard deviations.

biomass is in agreement with the enhanced biodegradation rates observed in the presence of RAMEB (Table 3 and Figure 2).

The soil toxicity (detected by jointly using microbial, plant and animal testorganisms) increased during the first 3 weeks of treatment, as a result of the increased pollutant bioavailability; later, in the presence of RAMEB, it decreased significantly, parallel with the decreased contaminant concentration. Figures 3 and 4 show the results of the toxicity measured by *Vibrio fisheri* bioluminescence and *Sinapis alba* root elongation test.

Ex situ *pilot field experiment*

RAMEB was found to enhance the hydrocarbon degradation also in the *ex situ* field biotreatment. The enhancing effects of RAMEB on oil removal were remarkable at the beginning (Table 4). Later, however, the rate of biodegradation of the transformer oil in the soil treated with RAMEB was only about 10–20% higher than in the parallel RAMEB untreated soil.

Figure 5 shows the oil-degrading cell concentration during *ex situ* treatment.

In the freshly contaminated soil, the biodegradation became effective in a short time thanks to the good quality soil with an abundant soil microflora. The number of oil-degrading cells decreased in parallel with the consumption of the contaminant (data not shown).

Based on the chemical and biological results we can state that the efficacy of RAMEB was lower (10–20%) than it was expected on the basis of the

Table 3. Oil removal percentages throughout the pilot scale experiment

	Time				
	1 week	1 month	4 months	8 months	10 months
Removed oil [%]					
RAMEB 0%	22	52	62	71	75
RAMEB 0.5%	20	65	68	79	81
RAMEB 1%	23	63	69	80	85

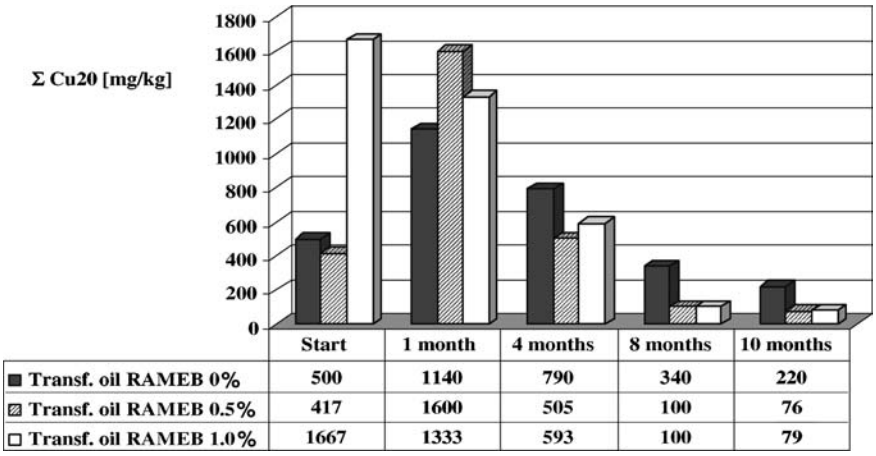

Figure 3. Soil toxicity measured by bioluminescence test in the pilot scale laboratory experiment.

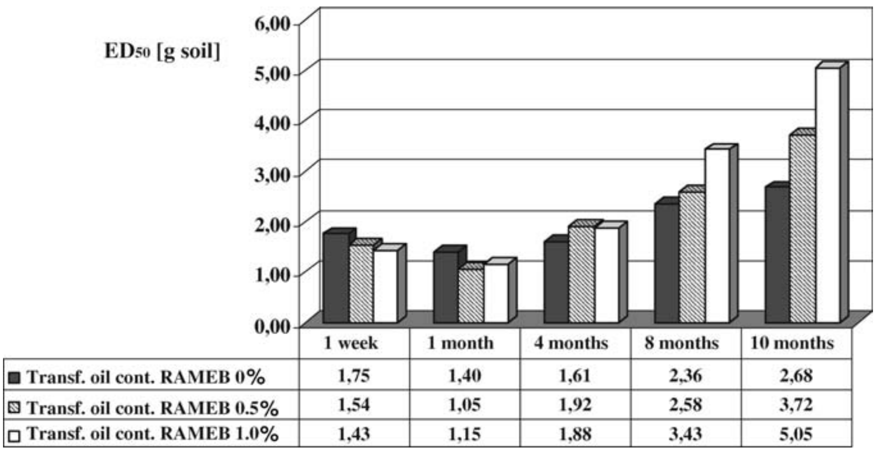

Figure 4. Soil toxicity measured by *Sinapis alba* root elongation test in the pilot scale laboratory experiment.

laboratory results, where 50–60% increase of pollutant removal was measured. The lower effects observed in the field experiment may be ascribed to a lower and probably insufficient oxygen supply achieved under these conditions.

The positive effect of RAMEB was marked during the first period. RAMEB shortened the lag-phase of the bioremediation process, when the bioavailability of the contaminants was probably the main limiting factor.

Table 4. Transformer oil removal during the *ex situ* pilot bioremediation experiment

	Start	2 weeks	6 weeks	9 weeks	24 weeks	30 weeks
Removed transformer oil [%]						
RAMEB-untreated	0	4	18	33	54	61
RAMEB-treated	0	16	34	45	57	67

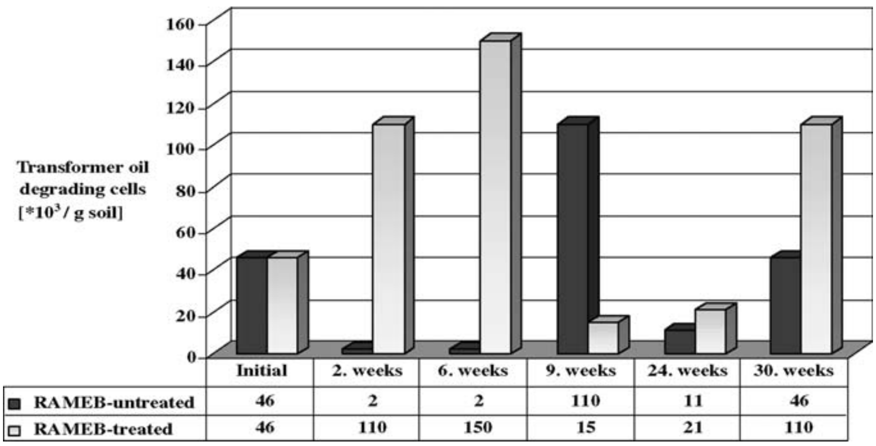

Figure 5. Effect of RAMEB on the concentration of transformer oil-degrading bacteria during bioremediation in the *ex situ* field experiment.

In situ *field experiment*

According to the previous experiences from laboratory and *ex situ* field trials *in situ* field experiment was also performed on a site historically contaminated with transformer oil. The combined technology (the bioventing of the unsaturated soil zone with the application of RAMEB and nutrients and the *ex situ* physico-chemical treatment of the pumped groundwater) had a significant effect by causing a marked reduction of EPH-content of the groundwater (Figure 6).

The fast decrease of the EPH-content indicated that microflora became resistant and active towards pollution, as demonstrated by the increasing CO_2 and decreasing O_2 content of the soil gas, after the first and second RAMEB and nutrient addition. The second addition caused slighter re-

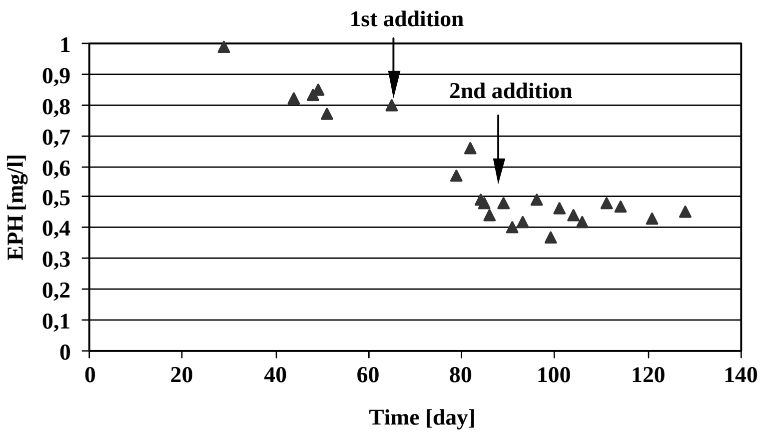

Figure 6. EPH-content of the groundwater from June to November in the *in situ* field experiment.

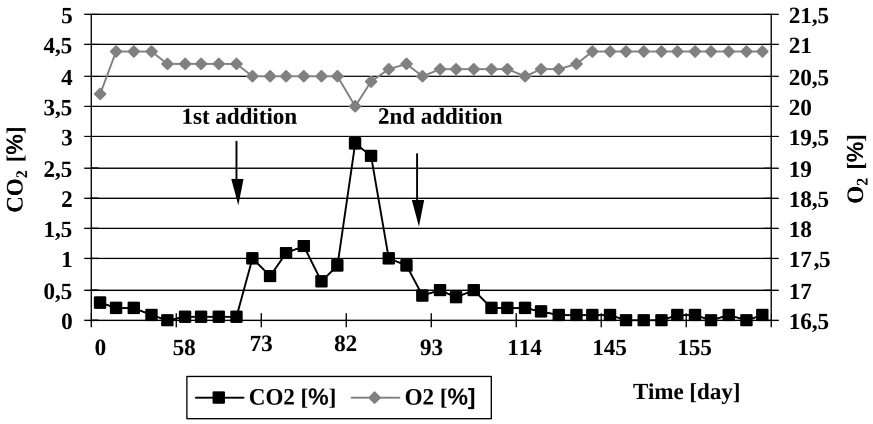

Figure 7. The results of soil gas analyses (CO_2 and O_2 content) during the first period of the *in situ* bioremediation.

sponse, probably due to the fact that only less biodegradable contaminants were left in the contaminated zone (Figure 7).

The increased soil activity due to the aeration of the soil and RAMEB and nutrient supply had a long-term effect on the process. The combination of the *in situ* bioventing with the addition of RAMEB and nutrients and the treatment of the contaminated groundwater on the surface resulted in a fast reduction of the transformer oil content of the soil (Table 5).

Table 5. EPH-content of the soil during the experiment of *in situ* bioremediation

Soil sampling depth [m]	Start	24 weeks	47 weeks (end)
EPH-content in the soil [mg kg^{-1}]			
10–30 cm	25,000	1,600	210
80–90 cm	25,000	800	260

The transformer oil removal in the soil was approximately 99% after the 47 weeks of the in situ bioremediation. The ecotoxicity tests with bacterial (*Vibrio fischeri*), plant (*Sinapis alba*) and animal (*Folsomia candida*) testorganisms showed a decrease in toxicity, with an acceptable end-value after 24 weeks treatment, before the winter break. The *Vibrio fischeri* test indicated a mild toxicity in

Table 6. Ecotoxicity of the contaminated soil before and after the *in situ* treatment

Testorganisms and endpoints of the tests	Depth of contaminated soil			
	10–30 cm		80–90 cm	
	Start	End	Start	End
V. fischeri luminescence inhibition				
ED_{50} [mg]	22	50	18	65
ΣCu20 [mg Cu kg^{-1} soil]	320	< 80	450	< 80
Interpretation	Toxic	Non-toxic	Very toxic	Non-toxic
Sinapis alba root and shoot				
Elongation inh. ED_{50} [g]	4	> 5	2	> 5
Interpretation	Slightly toxic	Non-toxic	Toxic	Non-toxic
Folsomia candida mortality				
LD_{50} [g]	12	> 20	5	> 20
Interpretation	Toxic	Non-toxic	Toxic	Non-toxic

ED_{20} and ED_{50} = soil doses (in g), which caused 20% and 50% inhibition. $\Sigma Cu20 = ED_{20\ Cu} / ED_{20\ sample} * 10^6$; toxicity given in Cu-equivalent: the Cu concentration, which would cause the same toxicity.

the upper 10–30 cm layer of the contaminated soil, but no toxicity in deeper levels, in accordance with the oil content, detected before the winter break. No toxicity was shown by the soil samples at the end of the *in situ* experiment (Table 6).

Conclusion

The cyclodextrin-enhanced bioremediation technology (CDT) was assessed and validated. RAMEB was applied as an additive in the bioremediation of different transformer oil-contaminated soils in stepwise technological experiments (from laboratory to field scale).

CDT was found to have the potential to speed up and intensify the bioremediation of transformer oil-contaminated soils, by reducing the typical treatment time of a conventional biological soil treatment technology, mainly by shortening the lag-phase, where the pollutant bioavailability is generally the limiting factor for biodegradation. Shorter treatment-time and longer utilisation of the site may make CDT cost-effective. As RAMEB itself is a biogenic, non-toxic and slowly biodegradable compound in soil, the environmental risk associated to its use of RAMEB is much lower than that generally associated to the use of synthetic surfactants or solvents for mobilising and improving the bioavailability of hydrophobic contaminants.

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