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Development of an innovative soil remediation: “Cyclodextrin-enhanced combined technology”

Laura Leitgib^{a,*}, Katalin Gruiz^a, Éva Fenyvesi^b, Gábor Balogh^a, Attila Murányi^c

^aBudapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest, Gellért tér 4, H-1111 Hungary

^bCyclolab Cyclodextrin R&D Laboratory Ltd., Budapest, Illatos u. 7, H-1097 Hungary

^cResearch Institute for Soil Science and Agricultural Chemistry of Hungarian Academy of Sciences, Budapest, Hermann O. út 15., H-1022 Hungary

ARTICLE INFO

Article history:

Received 25 November 2006

Received in revised form

9 October 2007

Accepted 28 October 2007

Available online 21 December 2007

Keywords:

Cyclodextrin

Enhanced natural attenuation

Hydrocarbon-contaminated soil

Technology monitoring

ABSTRACT

This paper introduces an *in situ* “Cyclodextrin-enhanced soil bioremediation technology” which is a combination of 1. *in situ* bioventilation for biodegradation in the unsaturated soil zone; 2. physico-chemical treatment of the pumped ground water; 3. impulsive flushing for the three-phase soil. For enhancement of biodegradation and solubilization randomly methylated β -cyclodextrin (RAMEB) was used.

An additional aim of this study was to prove the importance of the technology monitoring which was used for characterisation of the soil processes by an integrated methodology. It consists of physico-chemical, biological and ecotoxicological methods specific for the contaminants. For technology monitoring the mobile soil phases – soil gas and ground water – were analysed. Sampling of the whole soil was carried out at the start and end of the technology application.

RAMEB resulted in the enhanced removal of pollutants both from the saturated and unsaturated soil zones. Moreover, the biodegradation was more effective than the pump and treat technology, proved by the establishment of the carbon material balance in all soil phases.

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1. Introduction

Remediation techniques using physical and chemical intervention are quite widespread in Hungary. The environmental friendly biotechnologies using living organisms for remediation have not entered the Hungarian market yet. However, the “time-consuming” bioremediation of hydrocarbon-polluted soils can be improved by the increase of hydrocarbon availability.

Due to their solubility enhancing and “microencapsulating” effect cyclodextrin are favourable to application in reme-

diation technologies. Ring-shaped cyclodextrins increase the mobility of the non-polar pollutants in contaminated soils and ground waters. They interact with organic compounds and form inclusion complexes, which are more soluble than the single molecule in aqueous phase (Szejtli, 1996). This favourable character makes some cyclodextrin-derivates especially useful for enhanced decontamination of not only the hydrocarbon-contaminated ground water but also the unsaturated soil.

Cyclodextrin-enhanced “pump and treat” technology has been developed and applied to remove dense, non-aqueous

Abbreviations: CFU, colony forming unit; ED, effect dose; EOM, extractable organic material; EPH, extractable petroleum hydrocarbon; LD, lethal dose; n.m., not measured; PCB, Polychlorinated biphenyl; RAMEB, randomly methylated β -cyclodextrin.

* Corresponding author. Vértesszőlő Petőfi u. 39. H-8085 Hungary. Tel./fax: +36 1 463 2347.

E-mail address: lleitgib@mail.bme.hu (L. Leitgib).

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doi:10.1016/j.scitotenv.2007.10.055

phase liquid from the saturated, “two-phase” soil. Hydroxypropyl- β -cyclodextrin solution was injected into the source zone and pumped out successfully with contaminants on the subsurface (McCray and Brusseau, 1998). Cyclodextrin solution increases the *in situ* removal of the hydrocarbon contaminants from aquifers (Boving et al., 2003). The cyclodextrin-technology reduced significantly the remediation time compared with the conventional pump and treat technology. Moreover, it has been observed that the cyclodextrin enhanced technology is more efficient when the injection and the extraction of the solubility enhancing agent are used through the same well in contrast with the system applying separate wells.

Low bioavailability of hydrocarbon pollutants may limit the biodegradation by the indigenous micro-communities in soils. Cyclodextrins promote desorption of the non-polar contaminants from the solid surface and mobilize them to the water-phase biofilms, where the hydrocarbon-degrading microbes work (Olah et al., 1988; Steffan et al., 2001). Many publications have recently focused on hydroxypropyl β -cyclodextrin derivatives for the enhanced removal of phenols (Kawasaki et al., 2001; Chatain et al., 2004; Hanna et al., 2005, Allan et al., 2007) and polycyclic aromatic hydrocarbons (Wang et al., 1998; Garon et al., 2004; Reddy et al., 2006, Papadopoulou et al., 2007, Bardi et al., 2007; Allan et al., 2007). It has been demonstrated that randomly methylated β -cyclodextrin (RAMEB) can decrease the toxic effects of contaminants on soil microbes, plants and animals (Gruiz et al., 1996). RAMEB as bioavailability enhancing agent was successfully used for diesel-oil in unsaturated soil by Molnár et al. (2002), for PCB in slurry phase by Fava and Ciccotosto (2002); Fava et al. (2003), for transformer oil both in saturated and unsaturated soil zones by Leitgib et al. (2003); Molnár et al. (2005). It was found that RAMEB with one-year half-life time is slowly biodegradable in three-phase soil (Fenyvesi et al., 2005).

The efficiency of the technology can be assessed by some measured data depended on the class of the contaminants and the contaminated soil phases. For overall characterisation of the processes developing in contaminated soils, it is necessary to integrate the physico-chemical analyses with biological and ecotoxicological methods (Dorn and Salanitro, 2000; Juvonen et al., 2000; Margesin et al., 2000; van Gestel et al., 2001; Gruiz et al., 2001; Molnár et al., 2005) and to apply this integrated methodology in all steps of the remediation (Dott et al., 1995; Gruiz, 2005).

The aim of this study was to develop the “RAMEB-enhanced combined technology” for remediation of a site polluted with less biodegradable mixture of aged diesel and engine oils. The aim was to demonstrate all steps of the real remediation procedure beginning from the site assessment, following the lab-scale experiments, the planned and designed technology, after all to calculate the material balance from the technology monitoring data, evaluate the risk of the technology and characterise its advantages and disadvantages. The technology monitoring is an important point of this paper. It is strongly recommended to choose the appropriate methods, which are sensitive on the contaminants, and show the progress and the effectiveness of the technology.

2. Description of the technology phases

All steps of the remediation are introduced in this part of the paper. Firstly the actual site is demonstrated then the laboratory experiments with the contaminated soils originated from the site can be read. On the basis of the results of the laboratory experiments and the detailed assessment of the contaminated site the technology was planned and designed. During remediation the technology monitoring including suitable methods has responsibility for characterisation of the processes at the whole treated site.

2.1. Site assessment

The former tank station in an agricultural site in Hungary where both the soil zone and the ground water were polluted with a mixture of old diesel and engine oils (the hydrocarbon was in the range of C15–C25) was selected for demonstration of the technology. The volume of the subsurface soil contaminated with an average concentration of 1000 mg EPH kg⁻¹ was 280 m³. The highly contaminated central core was approx. 20 m³ in volume, and the average of the EPH content was 10000 mg kg⁻¹ soil (the maximum EPH content was 28800 mg kg⁻¹ at the 1.5 m depth of the unsaturated soil zone). The concentration of the aerobic heterotrophic cells was 0.4–4.6 10⁷ CFU g⁻¹ soil and of the oil-degrading cells was 1–10 10³ cell g⁻¹ soil. The unsaturated soil zone was a heterogeneous refill. The ground water level was 3 ± 1 m. The EPH content was 0.1–145 mg l⁻¹ in the ground water, the oil-degrading cell concentration was 1–100 cell ml⁻¹. The volume of the contaminated ground water was about 600 m³.

2.2. Small-scale laboratory experiments

Although the concentration of the cell factory could be enough to decontaminate the pollutant, it is a lack of the optimal conditions for the biodegradation.

Small-scale laboratory experiments were performed to find the optimal technological parameters for the field experiment. Three small-scale solid phase reactors were set up for modelling of the bioventilation. The aim was to investigate the effect of the aeration and the nutrient supply on the degradation activity of the indigenous microflora. The aerated bioreactors were 1 l glass columns filled with 500 g of soil. Two traps filled with NaOH ensured the CO₂-free atmospheric air. A gravel bed covered by textile layer was applied to avoid the clogging due to the fine soil particles and to ensure free way for the incoming CO₂-free air. The reactors were amended with inorganic nutrients to reach a final C:N:P ratio of 100:10:1 and were incubated for 2 weeks at 25 ± 2 °C. The different aeration rates were: 10 min d⁻¹, 2 h d⁻¹ and 6 h d⁻¹. CO₂ gases produced by the microorganisms were trapped in 1 N sodium hydroxide solution and measured by HCl-titration. After two weeks the EPH content, the aerobic heterotrophic cell concentration and the hydrocarbon-degrading cell concentration of the soils were measured.

On the other hand, many laboratory tests have been performed in our laboratory to find the optimal RAMEB concentration for the efficient biodegradation of poorly degradable

pollutants. Its application was success in enhancement of biodegradation of hydrocarbon pollutants in unsaturated soil zones (Molnár et al., 2002).

2.3. New innovative complex technology using RAMEB as a bioavailability and solubility enhancing agent

At that time when we designed and performed the combined technology *ex situ* ground water treatment functioned at the demonstration site. At the beginning of the field experiment the *ex situ* “pump and treat” technology was completed with bioventilation for decontamination of the unsaturated soil zone as well as an impulsive introduction of RAMEB (50% w/v RAMEB Cawasol W7 M TL, Wacker Chemie, Munich, Germany) and nutrient solutions (NITROSOL garden chemical fertilizer containing 30% N and MAP containing 53% P₂O₅). This Cyclodextrin-enhanced combined technology functioned for 85 weeks.

For not only the saturated but also the unsaturated soil phases, one active and five passive wells supplied with perforated tubes were used for the *in situ* bioventilation. The passive wells ensured the atmospheric airflow into the subsoil. The active well was a combined one for exhaustion of the soil air and the ground water. The exhausted air production was 180 m³ d⁻¹. The contaminated water was pumped out (4–5 m³ d⁻¹) and treated on the surface by adsorption technology using activated carbon filter. Due to the pump the ground water level continuously fell and hereby restricted the moving of the soil contaminants. Moreover, we precluded the possibility of the natural evaporation because of the age and the quality of the hydrocarbon pollutant (C15–C25).

We accepted the recommendation of Molnár et al (2005) and our laboratory results and we used RAMEB solution with 0.1 g RAMEB kg⁻¹ soil concentration for enhancement of biodegradation of hydrocarbons. The 20 m³ volume of the high-polluted central soil core was treated with 1 g RAMEB kg⁻¹ soil. It was injected through the one of the passive wells placing close to the core. The first addition of 3.0 (w/v)% RAMEB solution happened on the 19th week. The second injection of 1.5 (w/v)% RAMEB solution and nutrients (25 kg N; and 2,5 kg P) was carried out on the 64th week.

To assess the effect of the additives on the activity of facultative anaerobic microorganisms, 25 kg of nutrient solution (without RAMEB) was added to the soil on the 75th week.

2.4. Technology monitoring

To characterise the soil processes at the RAMEB-treated site an integrated technology monitoring was developed and used. The technology monitoring consisted of the analyses of the mobile phases of the soil: soil gas and ground water. During remediation whole soil sampling was limited to provide an optimal function of the technology.

2.4.1. Technology monitoring for the mobile soil phases

Chemical analyses of the ground water can give information about the effect of the treatment on the contaminant removal. The CO₂ content in the soil gas characterised jointly the mi-

crobiological status and the degradative activity of the cell factory of the soil.

The Extractable Petroleum Hydrocarbon content was analysed according to EPA 8270C. The RAMEB content, the nitrite and nitrate content, the pH and the electric conductivity of the ground water were analysed according to Hungarian Standards (HS 448–12, 1982, HS 448–22, 1985, HS EN 27888, 1998). The concentration of the hydrocarbon-degrading cell was measured by the most probable number technique (Lorch et al., 1995). The CO₂ content of the soil gas was measured by the mobile Anagas CD 98 Carbon Dioxide Analyzer.

2.4.2. Statistical evaluation of the results

The CO₂ content and all data of chemical and biological analyses of the ground water were compared by correlation analysis ($p < 0.05$) by StatSoft® Statistica 6.0 program.

2.4.3. The integrated methodology for the soil

As part of the integrated methodology physical, chemical, biological and ecotoxicological methods were used for characterisation of the solid phase of soil before and after remediation. Soil samples were taken at every half metre of the central core and close to the passive wells. For the physical-chemical and the ecotoxicological analyses soils were homogenised, air-dried and sieved (<2 mm), while the biological methods need naturally moist soils collected from the site.

Physico-chemical parameters of the soil were measured according to Forster (1995). The extractable organic material (EOM) content of the soil was measured gravimetrically after extraction with hexane:acetone (2:1) in an ultrasonic bath. The (EPH) content of the soil was analysed by gas chromatography with flame ionisation detector (GC-FID) according to Hungarian Standard: HS 21470-94 (2001) but with modified Temperature Program. The temperature of the injector raised to 340 °C in order that the concentration of the aged hydrocarbon components can be measured.

To characterise the general microbial status of the soil heterotrophic bacterial cell concentration was measured according to HS 21470/77 (1988). The concentration was given as colony forming unit (CFU) g⁻¹ soil. The concentration of the hydrocarbon-degrading cells of the contaminated soil was measured by the most probable number technique, using a basal mineral medium that was completed with the hydrocarbon as the only carbon and energy source and with 1.5 g l⁻¹ of the 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium-chlorid (INT) as an electron acceptor. The dilution series in triplicate were incubated for 14 days at 28 ± 2 °C in the dark. After incubation formazan was evaluated as proposed by Lorch et al. (1995).

The harmful effect of the hydrocarbon-contaminated soil on the soil ecosystem was characterised by direct contact environmental toxicity tests developed or modified from the standard proposals. The *Vibrio fischeri* bioluminescence inhibition, the nitrification activity inhibition, the *Tetrahymena pyriformis* reproduction inhibition, the *Folsomia candida* mortality and the *Sinapis alba* root and shoot elongation inhibition tests were applied. The detailed procedure of these tests has been described by Leitgib et al. (2007).

2.4.4. Evaluation and interpretation of the environmental toxicological results

The results of the environmental toxicity tests were calculated and given as percentage. In most cases inhibition data were plotted against the mass of the contaminated soils. For interpretation of the results endpoint values of the *V. fischeri*, the *F. candida* and the *S. alba* were determined from the dose-response curve by using of ORIGIN 6.0 software and presented as ED₂₀ (LD₂₀) value, which mean soil dose causing 20% inhibition (lethality). The results of the bioassays with different sensitivity were characterised as: “non-toxic”, “slightly toxic”, “toxic” and “very toxic”.

3. Results

3.1. Results of the laboratory experiment

The indigenous hydrocarbon-degrading microflora was significantly activated by aeration in all bioreactors filled with the soil derived from the actual site.

The concentration of the hydrocarbon-degrading cells increased with three orders of magnitude in the bioreactor where the airflow was set 2 or 6 h d⁻¹, and two orders of magnitude where the airflow was adjusted to 10 min d⁻¹. The maximal reduction of the EPH-content was observed in the bioreactor aerated 6 h d⁻¹, where the initial value reduced to 64.5%.

Results of the laboratory experiment are given in Table 1.

Biological characterisation of the soils showed that the enhanced indigenous microflora are able to biodegrade the contaminant, so the technology could be based upon their activity. For the enhancement of bioavailability and as a consequence of biodegradation, cyclodextrin was applied in addition to the Enhanced Natural Attenuation and the *ex situ* groundwater treatment by phase separation and sorption on activated carbon filter.

3.2. Results of the field experiment

3.2.1. Results of the soil gas and groundwater analyses

Due to the combined technology optimal conditions were set and kept for aerobic biodegradation of the pollutant in the site.

During remediation the change of the CO₂ content [mg m⁻³ soil gas] can be seen in Fig. 1a.

The CO₂ content increased from 255 mg m⁻³ to 780 mg m⁻³ in the soil gas within 19 weeks. RAMEB addition was carried out on the 19th and the 64th weeks, which resulted in fast increase of the CO₂ content from 780 mg m⁻³ and 650 mg m⁻³ to 1000 mg m⁻³ in the soil gas. After both cases the CO₂ production decreased slightly. These results proved the bioavailability enhancing effect of RAMEB in the unsaturated soil zone.

When the combined technology was performed on the site, the EPH content had decreased to a mean value of 0.7 mg l⁻¹ in the ground water due to the function of the conventional pump and treat technology. The first RAMEB treatment caused 131 mg l⁻¹ EPH concentration in the pumped-out water. In a few days later the mean of the EPH concentration decreased to 0.25 mg l⁻¹ ground water. Due to the second addition of RAMEB and nutrient solutions on the 64th week the EPH-content increased from 0.2 mg l⁻¹ to 2.5 mg l⁻¹ as it is shown in Fig. 1a. In both cases the rise of the pollutant concentration was attributed to desorption from the soil particles to the ground water by phenomena of the solubilization. At the end of the remediation the oil content decreased to 0.1 mg l⁻¹ ground water which value conforms to the requirements of the Hungarian environmental quality criteria.

Not only the aerobic but also the anaerobic biodegradation processes could be observed. The added nutrients stimulated the facultative anaerobic microorganisms. The change of the nitrate concentration in the ground water is presented in Fig. 1b.

The integrated methodology showed appearance of an alien contaminant (probably an artificial fertilizer) in the subsurface soil on the 23rd week. The combined remediation technology eliminated the alien contaminant within a short time.

3.2.2. Correlation analyses

Correlation analyses helped us to find the relations between changes of all measured parameters of the mobile phases during remediation. A correlation matrix can be seen in Table 2.

EPH content strongly correlated to the RAMEB content and the electric conductivity, also with the CO₂ content, the ability to UV absorption, the nitrite and the nitrate content at *p* < 0.05 significance levels. Significant correlation between the RAMEB content and the oil content, the CO₂ content, the electric conductivity, the nitrate content and the ability to UV absorption are reported.

These results proved the positive effect of the RAMEB on the biodegradation and solubilization of the hydrocarbon pollutants.

3.2.3. Results of the integrated methodology for soil samples

The physico-chemical properties of the high-polluted soil core (20 m³) at the beginning and at the end of the remediation are summarised in Table 3.

Increase of the redox potential in the subsurface was observed due to the bioventilation. Not only the pollutant concentration but also the carbon bounded to humus was reduced in the deeper layer of the soil, which suggests a high

Table 1 – The effect of aeration on the removal of the EPH and the concentration of the indigenous microorganisms in the soils originated from the contaminated site

	Start	After 2 weeks		
		10 min aeration a day	2 h aeration a day	6 h aeration a day
EPH reduction [%]	0	17.5	26.8	35.5
Aerobic heterotrophic cell concentration [CFU g ⁻¹ soil]	1.1 10 ⁶	7.7 10 ⁶	18.9 10 ⁶	18.7 0 ⁶
Oil-degrading cell concentration [cell g ⁻¹ soil]	2 10 ⁴	5 10 ⁶	15 10 ⁶	15 10 ⁶

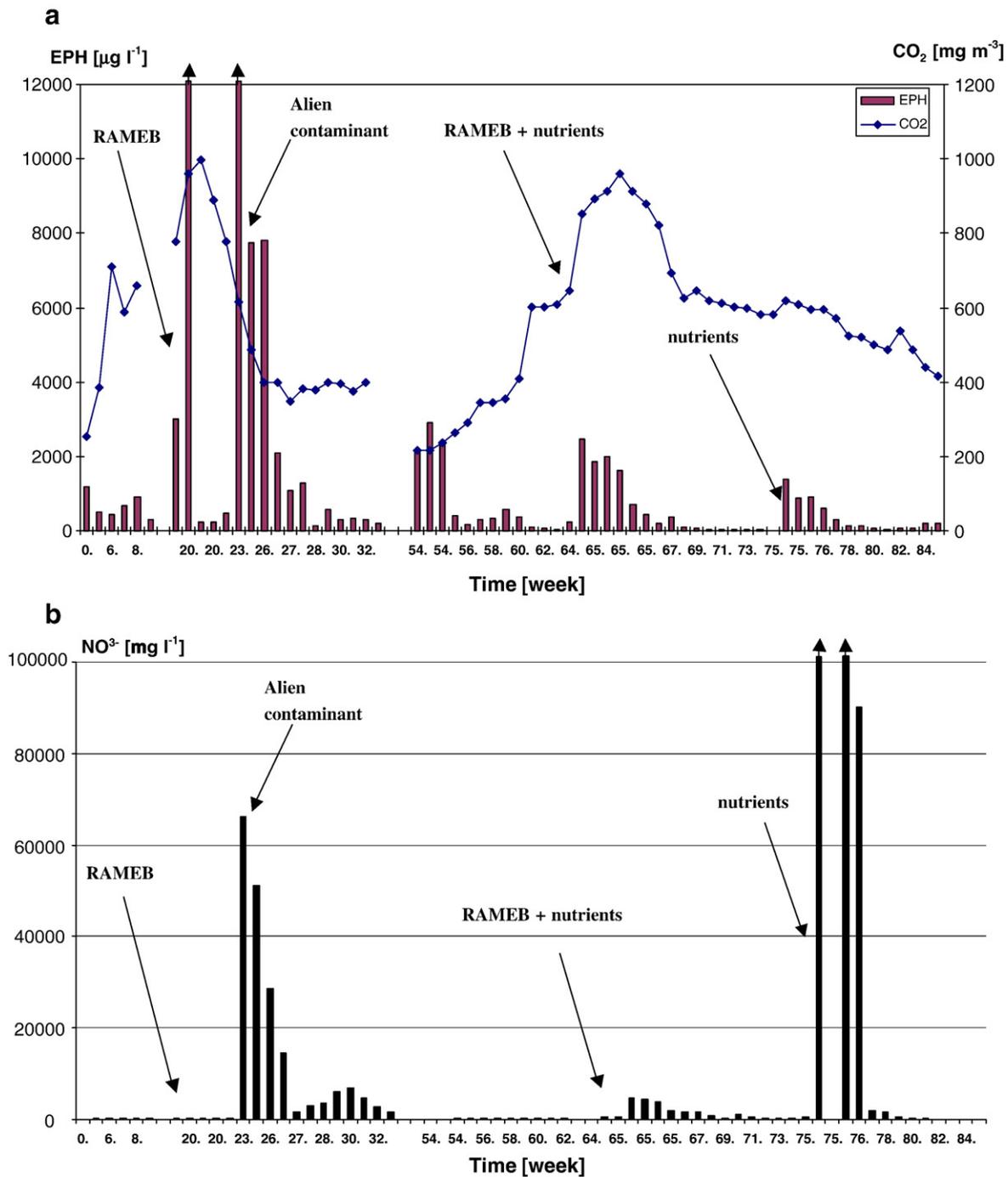


Fig. 1 – a. Change of the EPH content [$\mu\text{g l}^{-1}$] and the CO_2 content [mg m^{-3}] during bioremediation. b. Change of the nitrate content [mg l^{-1}] during bioremediation.

biodegradation activity of the indigenous soil microflora. At the end of the remediation there was a fall in the total nitrogenous content as well.

Results of the chemical, biological and ecotoxicological analyses at the end of the remediation are presented in Table 4.

According to the relevant governmental regulation based on site-specific risk assessment $3000 \text{ mg EPH kg}^{-1}$ soil was the target value at our field experiment. At the end of the

remediation the EPH-content achieved the site-specific target value in the subsurface soil except at the depth of 0.5–2.0 m in the central core (see Table 4). The concentration of the indigenous oil-degrading bacteria was still high. It predicts a further capability on the biodegradation. All environmental test organisms especially the bacteria and the mustard seeds were very sensitive to the contaminated soil and indicated toxic effect on the soil layers where the high hydrocarbon concentrations were measured.

Table 2 – Correlations between chemical properties of the ground water — hydrocarbon content, RAMEB content, nitrite content, nitrate content, sulfate content, pH, electric conductivity, ability to UV absorption — and the CO₂ content of the soil gas

	EPH content	Oil-degrading cell conc.	RAMEB content	Nitrite content	Nitrate content	Sulfate content	pH	Electric conductivity	UV-absorption	CO ₂ content
EPH content	1									
Oil-degrading cell conc.	-0.22	1								
RAMEB content	0.83	-0.26	1							
Nitrite content	0.62	-0.10	0.51	1						
Nitrate content	0.58	0.01	0.58	0.44	1					
Sulfate content	0.31	-0.23	0.40	0.43	-0.30	1				
pH	0.33	0.26	0.00	-0.18	-0.01	-0.15	1			
Electric conductivity	0.83	-0.10	0.87	0.60	0.83	0.24	0.01	1		
UV-absorption	0.68	0.11	0.70	0.45	0.94	-0.07	0.12	0.92	1	
CO ₂ content	0.67	-0.28	0.88	0.34	0.30	0.66	-0.03	0.74	0.50	1

Bold values are significant at $p < 0.05$.

3.2.4. Material balances

Material balances let us know about the mass (element) flux in the soil phases during remediation. With full knowledge of the numerical information about the mass/element fluxes the technologists design, perform, monitor and intervene in the applied technology to ensure optimal function. Moreover, the material balance indicates the efficiency of the technology.

For mathematical description of the material balances there are some parameters to be measured depending on the quality of the pollutants and the contaminated phases of the soil. In our case a mixture of hydrocarbons has contaminated both the saturated and the unsaturated soil zones. For this reason the carbon flux was the most important parameter to be determined in all soil phases. The change of the carbon content was monitored by measurement of the total petroleum hydrocarbon in the groundwater and of the carbon dioxide in the soil gas. In the soil solid phase, the concentration of the hydrocarbon and the carbon bounded to the humus was measured before and after remediation lasting 85 weeks.

3.2.4.1. Material balance of the groundwater. Due to the 448-day active function of the combined technology, the total EPH content in the ground water was 1407 g m⁻³ (see Fig. 1a). The amount of the total hydrocarbon was 7 kg (the performance of the ground water pump worked 5 m³ d⁻¹). The most part of the removed pollutants (4.9 kg EPH) was resulted in the flushing with RAMEB solution, which exerted the solubilizing effect for 49 days.

3.2.4.2. Material balance of the solid phase of the soil. The average concentration of the EPH in the soil solid phase was: 10000 mg kg⁻¹ in the highly polluted core (approx. 20 m³) and 1000 mg kg⁻¹ in 280 m³ of soil. It means the mean value of the initial total hydrocarbon content of the site was 720 kg in the unsaturated soil (300 m³). At the end of the remediation the residual hydrocarbon content was about 312 kg (the average of the EPH contents: 3400 mg kg⁻¹ in the highly polluted 20 m³ of soil core and 500 mg kg⁻¹ in the 280 m³ of soil). The eliminated (degraded or flushed into the ground water) amount of the hydrocarbon from the whole site was 408 kg.

The difference between the eliminated (408 kg) and the pumped (7 kg) hydrocarbon contents shows the degraded hydrocarbon content. It follows from the foregoing that the indigenous soil micro-community activated by the aeration and the bioavailability-enhancing agent degraded the largest amount of the hydrocarbon pollutant (401 kg). The mixture of the old diesel and engine oils was in the range of C15–C25 that means 345 kg of the elemental carbon in the biodegraded hydrocarbon pollutant.

The initial carbon content ranged between 4.5 t and 17.1 t in the subsurface soil, which is approximately 10.8 t of organic carbon in the 300 m³ volume of contaminated soil. At the end of the remediation the organic carbon bounded to the humus decreased to 8.1 t (4.5–11.7 t) at the site. It means 2.7 t carbon elimination from the soil humus. On the base of the results not only the pollutant was degraded but also the organic carbon bounded to the humus of the deeper layers of the soil.

Consequently, the total mass of the biodegraded carbon was approx. 3.1 t (0.35 t of carbon originated from the hydrocarbon and 2.7 t carbon originated from the humus) in the unsaturated soil.

3.2.4.3. Material balance of the soil gas. The CO₂ content of the soil gas lets us know about the carbon flux in the soil gas. The exhausted soil gas was 180 m³ a day. During remediation the total mass of the exhausted CO₂ was 15.7 t, which means 4.3 t of carbon. On the basis of these facts the bioventilation as well as the RAMEB-and the nutrient-addition resulted in an effective biodegradation capacity in the unsaturated soil and the most part of the carbon (3.1 t of hydrocarbon and humus) was removed by the indigenous soil microflora.

4. Discussion

Before planning a remediation technology site-specific assessment has to be performed on the contaminated site. The complex information on the contaminants and the site makes possible the technology selection for remediation. Laboratory experiments with the soils originated from the actual site help to select the most appropriate technology(ies) for the

Table 3 – Physico-chemical properties of the contaminated soil before and after remediation

Depth [m]	pH		Redox potential [mV]		Humidity [%]		EC [mS cm ⁻¹]		C		Humus			
	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End		
0 m	8.03	7.60	99	268	12	21	0.48	0.33	1.15	1.37	1.98	2.37		
0.5 m	7.56	7.46	147	337	23	20	0.49	0.46	1.20	2.60	2.07	4.60		
1 m	7.02	7.22	-91	279	31	30	0.87	0.59	2.43	1.93	4.19	3.32		
1.5 m	7.20	7.62	-68	52	31	30	0.55	0.52	3.83	1.93	6.61	3.33		
2 m	7.18	7.75	-150	18	37	27	0.66	0.34	3.63	0.89	6.25	1.54		
Depth [m]	CaCO ₃ [%]		P ₂ O ₅ [mg kg ⁻¹]		K ₂ O [mg kg ⁻¹]		Ca [%]		Total N [mg kg ⁻¹]		NH ₄ ⁺ -N [mg kg ⁻¹]		NO ₃ ⁻ -N [mg kg ⁻¹]	
	Start	End	Start	End	vStart	End	Start	End	Start	End	Start	End	Start	End
0 m	4.5	4.2	622	386	535	283	2.4	1.63	1080	1400	18	18	7	25
0.5 m	4.9	0.8	20	172	166	306	3.3	0.62	1030	2000	14	11	25	7
1 m	6.5	0.4	54	20	160	231	1.0	0.42	1860	1600	32	14	7	4
1.5 m	12.2	4.6	85	29	205	160	1.3	2.04	1820	1400	78	35	7	7
2 m	11.2	15.9	87	8	219	107	1.4	5.56	1800	500	68	4	21	4

EC: Electric conductivity.

decontamination. The site assessment on our demonstration site and the laboratory experiments indicated the potential for improvement of the biodegradative activity of the indigenous microflora in the soil.

Based on these findings, the existing *ex situ* ground water remediation technique was complemented with bioventilation and an impulsive flushing of RAMEB and nutrient solutions on the site. Randomly methylated β -cyclodextrin (RAMEB) was applied to improve desorption of the pollutants adsorbed on the soil particles and increase the concentration in the aqueous biofilms.

During remediation the *technology monitoring* should give a realistic picture on the processes existing in all soil phases and the ground water. The technology monitoring has to monitor technological parameters, like temperature, pH, redox-potential, aeration, water and additive flow to control the efficient functioning of the technology, the activity of the soil microflora and the risks of the technology due to contaminant mobilisation. In this case it was strongly recommended the analysis of the mobile soil phases, like soil gas and ground water. Chemical data from analyses of the ground water indicate the effect of the treatment on the contaminant removal. The CO₂ content in the soil gas is proportional to the microbiological status and the degradative activity of the cell factory. The soil solid phase was estimated at the beginning and after remediation by the use of an integrated methodology including specific self-developed methods.

During remediation the *risk of the applied technology* should be always evaluated and (if it is necessary) intervened by the technologists. The continuously depression with the wells and pumping, followed by the treatment of the contaminated groundwater restricted the contaminant transport by the groundwater flow to the surrounded environment. This technological step resulted in low level of the groundwater.

The soil gas does not represent a risk because of the age of the hydrocarbon pollutant. The mixture of motor oil and diesel oil contaminated the soil phases for years and does not contain volatile components (the gas chromatography of the soil pollution showed that the contamination is in the range of C15–C25). RAMEB is a starch-like organic substance and has not toxic effect (Gruiz et al., 1996). The half-life-time of RAMEB is 1 year, an ideal life span for the application in soil remediation. The waste was the activated carbon filter used for the pumped water treatment.

After-monitoring was applied at the end of the remediation. It means checking of the concentration and/or the effects of the residual contaminant in the soil phases. Integrated monitoring is necessary for the after-monitoring: an adequate combination of physical–chemical methods and biological–toxicological testing. Biological tests characterise the biological status of the soil, the biodegradative activity and the potential for its improvement, the interactions between the contaminants, the soil phases and the soil biota. The environmental toxicity of the contaminated soil is proportional with the bioavailability of the contaminants and may call the attention to the “chemical time bomb phenomena” and to toxic compounds that are not measured or measurable by the chemical analyses (Gruiz et al., 2001).

After application of the technology *material balance* was calculated on the basis of the results of soil, soil-gas and

Table 4 – Chemical-, biological and ecotoxicological results of the soil central core on the 85th week

Soil depth [m]	EOM content [mg kg ⁻¹]	EPH content [mg kg ⁻¹]	Aerob heterotrophic cell concentration CFU [10 ⁵ g ⁻¹]	Oil-degrading cell concentration [10 ³ g ⁻¹]	<i>Vibrio fischeri</i> luminescence inhibition ED ₂₀ [g soil]	Nitrification activity inhibition [%]	<i>Tetrahymena pyriformis</i> reproduction inhibition [%]	<i>Folsomia candida</i> mortality LD ₂₀ [g soil]	<i>Sinapis alba</i> root elongation inhibition ED ₂₀ [g soil]	<i>Sinapis alba</i> shoot elongation inhibition ED ₂₀ [g soil]
0	2265 (2039–2492)	977 (879–1075)	239 (206–272)	24 (5–112)	66.9 Non-toxic	91	0 Non-toxic	20 Non-toxic	>5 Non-toxic	>5 Non-toxic
0.5	7395 (6656–8135)	4744 (4270–5218)	141 (113–169)	46 (10–215)	11.4 Toxic	98	27 Toxic	13.5 Slightly toxic	>5 Non-toxic	>5 Non-toxic
1	3945 (3551–4340)	3226 (2903–3549)	39 (25–53)	15 (3–70)	7.0 Toxic	n.m.	22 Toxic	>20 Non-toxic	>5 Non-toxic	0.9 Very toxic
1.5	5560 (5004–6116)	5226 (4703–5749)	63 (45–81)	1100 (235–5148)	2.0 Very toxic	n.m.	26 Toxic	4.1 Toxic	<0.9 Very toxic	0.9 Very toxic
2	3575 (3218–3933)	2938 (2644–3232)	7 (4–10)	11 (2–5)	9.0 Toxic	n.m.	22 Toxic	10.1 Toxic	<0.9 Very toxic	<0.9 Very toxic

EOM content: Extractable Organic Material content, EPH content: Extractable Petroleum Hydrocarbon content, CFU: Colony Forming Unit, ED: Effect dose of the soil, LD: Lethal dose of the soil, n.m.: not measured.

Data in parentheses represent the lower and the higher values in 95% confidence interval.

groundwater monitoring. The material balance includes the eliminated or modified amount of pollutant. The efficiency of the technology compartments can be evaluated by this calculation. The material balance of the carbon proved the efficiency of the bioremediation technology, especially the effects of the RAMEB and the bioventilation. RAMEB as a solubility-enhancing agent made the hydrocarbon pollutant in motion and enhanced its solubility in the ground water. The bioventilation completed with nutrients and RAMEB stimulated the indigenous microbial consortia removing a large amount of the mixture of the diesel end engine oils.

Cost assessment and the comparative evaluation of the costs of the potential technology-alternatives is one of the basic arguments in the decision making process. The cost assessment always depends on the future land use. Comparison to the physical and chemical technologies the costs are more favourable, but to the natural attenuation it is more expensive due to the application of nutrients, agents and the *ex situ* ground water treatment. The application of these technological steps can be necessary in those cases when the saved time has advantages and compensates the higher costs.

Characterisation of the Strengths, Weaknesses, Opportunities and Threats makes the technologies comparable to the other commercial available remediation technologies.

Strengths: The technology is an *in situ* technology; it is not necessary soil removal, excavation and transport. Moreover, it uses the indigenous communities for decontamination of the pollutant. For saving remediation time a natural molecule: RAMEB is applied. As bioavailability enhancing agent it improves the biodegradation activity of the microbes towards the poorly degradable contaminants. As solubility enhancing agent it helps desorption of the contaminants from the soil particles.

Weaknesses: the remediation time of this technology due to the microbiological function takes long in comparison to the sole physical-chemical techniques. Emission from the technology should be monitored. An integrated technology monitoring including methods with different sensitivity to the contaminants is required.

Opportunities: the technology is suitable to combine with other techniques in order to decontaminate the other types of pollutants existing. With application of the additives the treatment time can be reduced. The decreased treatment time results in sooner utilisation of the site and benefits from future land use.

Threats: The technology presented in this paper is limited to hydrocarbon pollutants. The price of RAMEB is relatively high yet. *In situ* soil remediation increases environmental risk but monitoring and applying depression in the ground water, the risk due to emission can be reduced.

5. Conclusion

Before application of a remediation technique it is necessary the site-specific assessment, investigation of the contaminated environment elements in lab-scale experiments, and planning the optimal technology. A technology can be excellent on one site, but absolutely not effective on another one, this is the reason why a site-specific evaluation is so important.

We have to emphasize the importance of the appropriate technology monitoring. Mobile soil phases (soil gas and ground water) were monitored during remediation. For the technology monitoring an integrated methodology was applied, which was able to give relevant information about the effect of technology on the soil processes as well as the behaviour and the effects of the pollutant. At the end of the soil remediation it was absolutely necessary to analyse also the solid phase of soil in order to make sure that the acceptable target value has been achieved.

The feasibility of the innovative combined cyclodextrin-enhanced bioremediation was proved.

The 448-day active function of the complex technology caused continuous changes both in the saturated and the unsaturated soil. The soil flushing with RAMEB resulted in a large amount of the contaminants solubilizing from the soil solid parts to the ground water, which was pumped out and treated on the surface. RAMEB also stayed for a long time in the three-phase soil zone and enhanced the bioavailability of the contaminants to the biofilm, where the hydrocarbon-degrading microbes work. Due to the bioventilation the CO₂ content of the soil gas significantly increased. The amendment with RAMEB and nutrients brought an additional rise in the CO₂ production, which indicated the optimal conditions for the biodegradation. Data were usable to calculate material balance and evaluate the efficiency of the technology.

The risk of the technology is lower than an *ex situ* technology because of the application of the technology. The quality of the pollutant could allow the application of *ex situ* treatment because it has not contained volatile organic compounds, which can contaminate the air because of the direct contact with the atmosphere. However the *ex situ* application should be completed with a leachate treating system, while *in situ* the elimination of the water soluble compounds can be controlled by creating water depression. The pumping wells continuously kept the level of the ground water low in order to restrict the contamination from the *in situ* treated soil. The risk of the technology was low and there was no measurable emission at the demonstration site.

Consequently, our results serve as evidence that RAMEB-enhanced combined bioremediation technology can be considered as a potential technological version for remediation of sites polluted with poorly degradable hydrocarbons, e.g. mixture of diesel and engine oils.

Acknowledgements

The research was supported by the National Research and Development Programs (NKFP-3/002/2001) and the Hungarian Ministry of Education (BIO-00066/2000). Thanks are due to dr. Péter Szabó for realization of the complex remediation technology.

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