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PhD Thesis

INTENSIFICATED BIOREMEDIATION OF CONTAMINATED SOIL WITH CYCLODEXTRIN

FROM THE LABORATORY TO THE FIELD

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1. INTRODUCTION, MAIN OBJECTIVES OF THE WORK

Inherited contaminated sites still present high concentration of pollutants like petroleum hydrocarbons, pesticides and polycyclic aromatic hydrocarbons. These chemicals represent serious environmental and health risk. About 18 000 registered contaminated sites are waiting for remediation in Hungary on the basis of the survey of the National Environmental Remediation Programme. The registered pollutions endanger 86 % of the soil and groundwater.

For the remediation of contaminated soil many accessible physical, chemical and biological technologies can be applied. The technologies based on natural biological processes are often received with mistrust and lack of understanding, although these are most promising. A series of running European projects has focused on the development of innovative soil remediation technologies and deals with collection and dissemination of these novel technologies.

An important rate-limiting factor in the bioremediation of historically contaminated soils is often the low bioavailability of the hydrocarbon-type contaminants. Cyclodextrins (CDs) can be used to improve the bioavailability of soil contaminants. The applicability of CDs for soil treatments was mainly tested in soil extraction and soil washing methods. Application of CDs for intensification of soil bioremediation was only studied in some laboratory experiments.

With this international R&D trends in view, **the primary aim of my research was to establish the scientific background for a cyclodextrin-based soil bioremediation technology** (***CDT***) using randomly methylated ß-cyclodextrin (RAMEB). The main objective of my PhD work is to develop *CDT* to high level, to prove that CDT is a possible alternative biotechnology for the reduction of the environmental risk of hydrocarbons-contaminated sites in comparison with other not-intensified biotechnologies.

The **second objective** of my research and development work **was to compile and test of a high quality generally applicable, integrated methodology** for site assessment, planning, development and monitoring of remediation.

2. METHODS APPLIED IN TECHNOLOGY-DEVELOPMENT

Complex approach and integrated methodology are required to develop a new soil remediation technology.

My research was the integral part of the R&D work of the Environmental Microbiology and Biotechnology Group of the Department of Agricultural Chemical Technology, which means to get a better inside view of the soil „black box system”, and aims to work out new environment-friendly bioremediation technologies. My task was to plan, to perform and to evaluate the laboratory experiments from the small to the large scale; the field application was the result of the collective work of our Research Group.

The applied innovative tools for supporting the technology-development were assembled from two main parts:

I. *Scale-up* conception for establishment of the technology

II. *Integrated* chemical-biological-ecotoxicological *methodology* for monitoring and evaluation of the experiments.

2.1. Establishment of *CDT* with scale–up experiments

Biodegradation of different organic contaminants in spiked soils was investigated in large number of small-scale laboratory *biodegradation experiments* (**1st phase** of the scale-up).

I have tested the applicability of the developed *soil respiration system* to follow biodegradation processes and to investigate the influence of some technological parameters on bioremediation in *technological microcosms experiments.* The feasibility of bioventing and slurry phase biotreatment for an aged, highly creosote-contaminated soil was modelled and compared.

The influence of RAMEB-concentration (0–0.7 %) on biodegradation of organic contaminants in soils with different sorption capacity, and in actual site mazout contaminated soil was also studied and evaluated (**2nd phase** of the scale-up).

The main goal of the **3rd phase** (the *pilot-scale laboratory experiments)* was to assess the importance of nutrient-supply during bioremediation and to prove the bioavailability-enhancing effect of RAMEB in a larger scale, in transformer oil contaminated soils. The suitability of biological and ecotoxicological methods for evaluation of microbiological processes in contaminated soils was also tested.

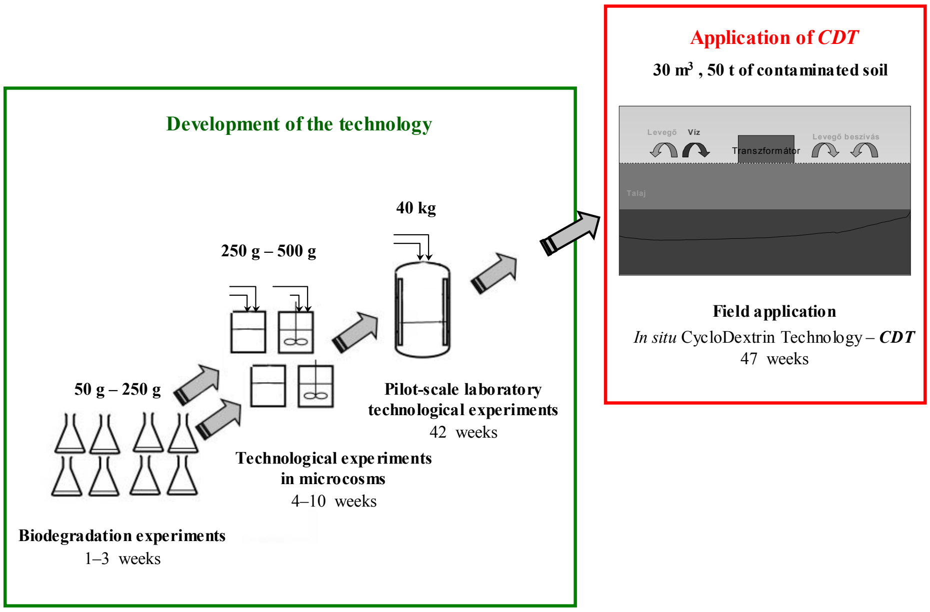

Figure 1. Experimental scale-up

2.2. Integrated methodology to monitor and evaluate the experiments

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 site. I have modified and developed biological and ecotoxicological methods for soil and integrated in the complex methodology for monitoring and evaluation of the remediation.

2.2.1. Chemical methods for measuring the contaminants

The soil organic extract was quantified gravimetrically after ultrasonic extraction. Extractable Petroleum Hydrocarbon (EPH) content of the soils was measured by Fourier Transform Infrared Spectroscopy (FT-IR).

2.2.2. Biological methods for characterization of microbes and microbial activity in soil

Number of aerobic heterotrophic bacterial cells, cell concentration of the pollutant-degrading microbes and soil respiration were determined for characterisation of the biological activity of the soil.

2.2.3. Ecotoxicity for testing the effect of the contaminated soil

For direct contact ecotoxicity testing of the contaminated soil bacterial, plant and animal testorganisms were used:

- *Vibrio fischeri* inhibition of bioluminescence
- *Azotobacter agile* inhibition of dehydrogenase enzyme activity
- *Pseudomonas fluor.* inh. of dehydrogenase enzyme activity
- *Sinapis alba* inhibition of root- and shoot-elongation
- *Folsomia candida* mortality.

2.2.4. Statistical evaluation of microcosms experiments

The endpoints (ED_{20}/LD_{20}, ED_{50}/LD_{50}), used for the bacterial, plant and animal tests were determined from dose-response curve (inhibition percent values of different dilutions) after sigmoidal fitting of data by *Microcal Software^{TM} ORIGIN® 6.0* software.

I have used *Pearson* correlation analyses using *StatSoft Statistica ® 6.1* program for the investigation of the correlation between results of chemical and biological, ecotoxicological methods.

For evaluation of the effect of RAMEB and soil characteristics in technological microcosms, ANOVA analysis of variance, using *StatSoft Statistica ® 6.1* program was carried out by classification by means of three factors (RAMEB, time, soil).

3. SUMMARY OF THE NEW SCIENTIFIC RESULTS

The scientific background and basis of the CycloDextrin Technology has been created by the stepwise growing scale technological experiments.
I have demonstrated and proved the bioavailability-enhancing effect of RAMEB in laboratory microcosms and in laboratory pilot experiments. Increased biodegradation rate, higher microbial activity and decreased toxicity were observed in a consequence of the enhanced bioavailability.

The new scientific results presented in my PhD dissertation can be summarised in the following thesis:

3.1. **Effect of RAMEB was more significant in solid phase treatments**, than in slurry phase soils. In case of solid phase treatment the contaminant-removal was 1.5–2.5 times higher on the effect of RAMEB-addition, than without cyclodextrin.

Higher efficiency of RAMEB in slurry phase treatments was only observed in case of lower contamination level (10 000 mg/kg). In this case increase of contaminant-degradation was ~20–50 % on the effect of RAMEB compared with untreated soil.

3.2. I have confirmed that the **RAMEB-treatment was more efficient in transformer oil contaminated soil**, than in case of more easily biodegradable diesel oil contamination.
Increase in diesel oil removal on the effect of CD-addition was ~30–60 %. Whereas in case of transformer oil contaminated soils the rate of oil-degradation was ~1.5–3.5-folds in RAMEB-treated soils, compared with untreated soils.
The results demonstrated that the bioavailability is not a rate-limiting factor in case of bioremediation of diesel oil contaminated soil.

3.3. In case of transformer oil contamination the **0.1 % of RAMEB** was found to be **effective** for intensification of bioremediation. Removal of transformer oil was 1.5–2.5-folds higher in the presence of 0.1 % RAMEB concentration that achieved without RAMEB. So take into account the cost of remediation, it could be sufficient 0.10–0.15 % of RAMEB-application for soil.

3.4. In solid-phase experiments carried out on sandy, clay and humic-loamy soils, different soil characteristics appeared different extent of transformer oil biodegradation in the sandy, humic-loamy and clay soil. An enhanced degradation was observed in all soil in the presence of RAMEB. The highest oil degradation rate and extent was found in the humic-loamy soil (after 4 weeks treatment). Humic-loamy soil is the „best-quality" habitat for microorganisms due to its favourable physico-chemical properties.

The **statistical evaluation** (ANOVA) of the results **proved**, that the **contaminant-removal and the biodegradation-rate depend significantly on RAMEB concentration.** The rate of hydrocarbon-removal was significantly different in the sandy, loamy and clay soils. Dependence on RAMEB-concentration was found linear.

3.5. A high quality methodology including different combinations of selected suitable physical-chemical, biological and ecotoxicological methods **was created and applied** to follow, monitor and evaluate of remediation experiments.

The results of the experiments carried out on coal tar contaminated soil illustrated that the soil toxicity is influenced by the availability of contaminants, and underline the need to take ecotoxicological effects into account in order to monitor the technology, particularly at the end of remediation.

I have made a **proposal for ecotoxicity test-batteries** applicable for investigation and determination of toxic effect of complex, organic contaminants: *Vibrio fischeri* bioluminescence inhibition test, *Sinapis alba* shoot-elongation test and *Folsomia candida* mortality test.

3.6. I have confirmed the **applicability of the self-designed soil respirometer system**, **for the description of biodegradation processes** in contaminated soil, **for determination and characterisation of technological parameters** influencing soil bioremediation.
The influence of type, concentration and age of contaminants and effects of aeration-intensity on biodegradation was found to be well documented by the CO_2 production measured by in the soil respirometer.

3.7. I have performed **methodological developments** in ecotoxicological testing of soil.

I have modified the Hungarian Standard (HS 21976-17) and developed **a direct contact biotest** to examine the harmful effect of the contaminated soil **using plant testorganism**. I have investigated directly the whole soil, instead of the investigation of the soil-water extracts. So these direct contact tests are more sensitive and characterize the soil as a matrix and habitat, and show the interactions between the soil and testorganism.
I have **demonstrated and proved**, that the inhibition of the **shoot-elongation** particularly in case of *Sinapis alba* testorganism is **more sensitive parameter** (as an end-point) in case of organic contaminants than the inhibition of root-elongation.

4. APPLICATIONS

4.1. The *field demonstration and verification of the CDT* was done at the highly contaminated site of the Nepliget transformer station (~25 000 mg transformer oil/kg soil). The bioavailability-enhancing effect of RAMEB was utilized in the unsaturated soil, solubilizing ability of RAMEB was used to influence the soil-groundwater interaction during the *in situ* bioremediation of *CDT*.

The results of the integrated methodology and the complex technology-verification (material-balance, the characterization of the environmental risk, the cost-efficiency and the SWOT analysis) showed the benefits of the applied technology. The transformer oil removal in the soil was approximately 99 % during the 47 weeks of the *in situ* bioremediation. The concentration of transformer oil in the soil measured at the end of the field experiment (210–260 mg/kg) was under the Hungarian limit value. The ecotoxicity tests with the bacterial, plant and animal testorganisms performed at the start and at the end of the remediation, showed the decrease of the toxicity, with an acceptable end-value.

Decrease of duration of the technology can be one of the most important benefits by the evaluation and assessment of the technology.

The developed ***CDT is applicable for risk reduction*** of contaminated sites polluted with poorly available, and degradable organic contaminants, so it can be one of the tools of the risk-reduction tasks.

4.2. The innovative *CDT* got into **the *database of EURODEMO***. EURODEMO was funded by the European Union’s Research Directorate in 2005. This new platform was established with the ambition to boost technology demonstration in the field of soil and groundwater remediation. Top priorities of the project are to make demonstration of promising, innovative soil and groundwater technologies easier to implement.

5. PUBLICATIONS related to PHD topic

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