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CYCLODEXTRINS IN BIOREMEDIATION OF CONTAMINATED SOIL

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Application of cyclodextrins (CDs) in environmental management has been recently reviewed (1,2). Due to the multiple benefits offered by these complex forming carbohydrates such as improving the solubility, decreasing the volatility, and changing the partition of the contaminants between the soil phases as well as enhancing the bioavailability (toxicity) of the contaminants these additives have been thoroughly studied in soil remediation technologies, that is in technologies cleaning the contaminated soil.
Bioremediation is an innovative technology which uses microorganisms to degrade organic contaminants in soil either excavated or in situ (3). The microorganisms break down contaminants by using them as a nutrient or cometabolizing them with a nutrient. Aerobic processes require an oxygen source, and the end products typically are carbon dioxide and water. Anaerobic processes are conducted in the absence of oxygen, and the end products can include methane, hydrogen gas, sulfide, elemental sulfur, and dinitrogen gas. Both under aerobic and anaerobic conditions the bioavailability can be the rate limiting factor. In such cases, solubilization with CD complexation can help.

Microbial degradation under aerobic conditions

The CDs can improve biodegradation by enhancing the bioavailability of organic pollutants. For instance, phenanthrene biodegradation by aerobic bacteria was accelerated by HPBCD (4); fluorene biodegradation in a slurry soil that was augmented by fungi (such as *Absidia cylindrospora)* was enhanced by maltosyl β-cyclodextrin (5); and the microbial decomposition of PAHs and phenols and biphenyl was intensified by the addition of HPBCD (and nutrients) (6,7). Amendment with HPBCD at as low concentration as 14 mg kg^{-1} soil enhanced the biodegradation of hexadecane and phenanthrene (8).

Even the unsubstituted BCD, which usually forms complexes of low-solubility, may have a beneficial effect on biodegradation, as the availability of the pollutant molecules that are molecularly encapsulated is higher when compared with those adsorbed to the soil. The rate of degradation of model aliphatic and aromatic compounds was enhanced in the presence of BCD (9), and was increased with increasing BCD concentration (10). BCD was beneficial for biomass production, as it improved the bioavailability of hydrocarbons and ensured an additional carbon source for the microbes. In addition to the type and concentration of the CD, the soil properties were also found to have an influence on biodegradation: the rate followed the order: clay < loamy soil < sand (11). Alpha-CD was inefficient but gamma-CD (GCD) improved slightly the biodegradation of hexadecane (12) as well as of polychlorinated biphenyls (13).

Figure 1. Ex situ pilot scale experiment at a former military site

In an *in situ* field experiment, a hydrocarbon-polluted soil (total petroleum hydrocarbon (TPH) content 310–660 ppm) was treated with 1 g BCD/m^2, N and P nutrients, and augmented with an adapted culture of indigenous microflora (14). Three months later, the hydrocarbons were practically biodegraded (TPH reduced to 5–23 ppm).

We used randomly methylated β-cyclodextrin (RAMEB) to enhance the biodegradation of diesel and transformer oil, as well as of mazout (black oil, the residue of petroleum

distillation) in spiked soils and also in soils with aged contamination (15,16). RAMEB was selected because it has a higher solubilizing potential than HPBCD (17,18) and can form inclusion complexes of higher stability with the typical hydrocarbons (19). RAMEB has a beneficial effect on the soil pore structure, thus improving the habitat of the microbes (20). Although less readily biodegradable by the soil microflora than HPBCD but its 1-1.5 year half life time makes it a suitable additive to enhance bioremediation (21,22).

Adding RAMEB to the soil led to a pronounced increase in the bioavailability of the contaminants at the beginning of the treatment. In its presence, biodegradation started earlier in soils contaminated with either diesel or transformer oil, resulting in more efficient removal of contaminants depending on the RAMEB concentration (15). In the laboratory experiments, 0.7%, 0.5% and 0.5% RAMEB concentrations were found to give optimum results in clay, sandy and loamy soils contaminated with transformer oil, respectively. Even mazout, which is difficult to biodegrade, became partly bioavailable in the presence of RAMEB: the number of specific degrading bacteria was increased by one to two orders of magnitude, and 0.7% RAMEB yielded a 40% decrease in mazout concentration compared with <10% achieved without using RAMEB.

The optimum RAMEB concentration was 1–3% for PCB-contaminated soil, resulting in an enhanced specific PCB-degrading biomass and improved biodegradation, first of all in a slurry-phase treatment (23).

The efficiency of RAMEB was demonstrated both in *ex situ* and *in situ* field experiments in soils contaminated by transformer oil (24). The treatments were monitored by the Soil Testing Triad, including physico-chemical, biological and ecotoxicological methods (25). Applying 0.8% RAMEB in the *ex situ* experiment resulted in a shortened adaptation phase at the beginning of the treatment. The initially observed remarkable differences in the decrease of hydrocarbon content compared with the control soil (without RAMEB treatment) disappeared after a six-month treatment.

In the *in situ* field experiment at a transformer station in Hungary, a combined technology (ventilation, nitrogen and phosphorus) was applied. RAMEB addition, continuous removal of groundwater, continuous moisture supply (by slow infiltration of the treated water) and *ex situ* treatment on activated carbon were also used. The soil was flushed with a RAMEB solution from time to time through the injection well, and, after a few days' pause, the groundwater was continuously pumped out from the extractor wells on the other side of the transformer (23,26). This continuous groundwater removal reduced the risk of spreading the contaminants that were solubilized by RAMEB. The few days' delay after flushing was enough for adsorption of RAMEB on the soil, resulting in a long-term effect on the biodegradation. Technology monitoring was based on the analysis of soil-gas and

groundwater. After the addition of RAMEB, the CO_2 content of the soil gas increased suddenly, indicating improved microbial activity. At the end of the treatment, the hydrocarbon content of the soil decreased from approximately 25 000 mg/kg to <300 mg/kg. The reduced toxicity was shown by the plant (*Sinapis alba*), animal (*Folsomia candida*) and bacterial (*Vibrio fisheri*) test organisms.

The mass balance proved that most of the contaminants (>98%) were removed as a consequence of the enhanced microbial activity, and less than 2% were removed by groundwater treatment. The quantification of the initial and residual risk, i.e. the predicted environmental concentration (PEC) of the site compared with the predicted no-effect concentration (PNEC) at the beginning and at the end of remediation, showed that the technology effectively reduced the risk to the soil. The treatment time was considerably reduced. The results of the demonstration at the transformer station, as well as previous experiences, prove that the two-and-a-half-year period required for bioremediation without RAMEB can be reduced to one to one and a half years, including one or two summer-periods, to reach the target concentration. This time saving compensates for the higher costs incurred by using RAMEB. The evaluation and verification of the technology, based on mass balance, risk assessment and cost-efficiency assessment, as well as SWOT analysis[*1], demonstrated the advantages of the technology (27).

A similar combined technology was used at a former tank-station on an agricultural site in Hungary contaminated with aged diesel and engine oil from leaking underground tanks (28,29). In this case, however, the injection and extraction were performed alternately in the same well (i.e. using a push–pull technique). As a matter of fact, a combination of push–pull and drive-through techniques was applied: half of the additives (RAMEB and nutrients) were applied through the combined injection–extraction well, and the other half through the five additional injection wells arranged around the combined well in a circle. The elements of the combined technology include: (1) intensification of spontaneous biodegradation by *in situ* bio-ventilation in the unsaturated soil zone by the addition of nutrients and RAMEB as additives to enhance the bioavailability; (2) *ex situ* physico-chemical treatment of the pumped groundwater; and (3) impulsive flushing of the unsaturated zone by a RAMEB solution. After the addition of RAMEB, the hydrocarbon concentration in the extracted groundwater and the specific oil-degrading bacteria increased ten- to 40-fold and two- to ten-fold, respectively. This combined technology effectively reduced the risk posed by the site: the contaminant concentrations decreased significantly from 30 000 mg/kg to 3500 mg/kg and from >1000 mg/L to <200 mg/L in soil and water, respectively, at the end of the treatment. The mass balance based on the

1* Strategic planning method used to evaluate the **S**trengths, **W**eaknesses, **O**pportunities, and **T**hreats.

monitoring of groundwater and soil gas and on the contaminant concentrations in the soil before and after the treatment, showed that approx. 99% of the contaminant was removed by biodegradation, and only approx. 1% by groundwater treatment. The specific feature of this combined technology is that it applies the same additive (RAMEB) for the enhancement of both soil flushing and bioremediation. The depressed level of groundwater and its *ex situ* treatment ensure that the contaminants with enhanced solubility cannot spread into the surrounding environment.

Microbial degradation under anaerobic Fe(III)-reducing conditions

Numerous examples show the efficacy of cyclodextrins in enhancing the aerobic biodegradation of various contaminants. The anaerobic processes also require the improved transport and availability of the pollutants.

The Fe(III) ion is the most abundant electron acceptor in soils; it plays an important role in natural attenuation of PAH contamination. Applying HPBCD under Fe(III)-reducing conditions to improve the bioavailability of PAHs, the initial phenanthrene mineralization rate was increased with increasing HPBCD concentration (30). At a high (5 g/L) HPBCD concentration, however, the biodegradation ceased after 25 days. A possible explanation is that too much CD protects the contaminants included in their cavity from the microbes, in a similar way to the surfactants at concentrations above the critical micelle concentration (CMC).

Co-metabolites and cyclodextrins

Metabolites are intermediates and products of metabolism. A primary metabolite is directly involved in normal growth, development, and reproduction. The secondary metabolites are not involved directly in these processes.

Cometabolism involves the injection of a dilute solution of nutrients into the contaminated groundwater or soil. The microbes that metabolize these nutrients produce enzymes that react with the organic contaminant and degrade it to harmless minerals.

Amending the soil with biphenyl can enhance polychlorinated biophenyls (PCB) degradation. A slight further enhancement can be achieved by jointly applying biphenyl and cyclodextrins, e.g. HPBCD and GCD (12), and RAMEB (22). Other studies showed that there is competition between biphenyl and the PCBs for the cyclodextrin cavity, resulting in decreased depletion of PCBs compared with HPBCD alone, especially in the low-chlorinated region (31).

Applying co-metabolites of plant origin (naringin, cumarin, limonene, isoprene and carvone) together with HPBCD, considerable synergism was observed: in soil with a low organic carbon content (55–85%) higher PCB depletion was observed in the presence of carvone and HPBCD together, as compared with carvone alone (31). HPBCD improved the availability (solubility) of these secondary plant metabolites by the formation of complexes. It was also observed that HPBCD treatment changed the phospholipid fatty acid (PLFA) composition: several lipids indicative of Gram-negative bacteria were found to be enriched, and lipids suggesting the presence of Gram-positive bacteria were found to be depleted.

Summary

The application of CDs in soil bioremediation proved to have beneficial effects resulting in enhanced efficiency and decreased time requirement. Both unsubstituted CDs and their highly soluble hydroxypropyl, methyl and maltosyl derivatives were successfully applied as additives. A great advantage of CDs as bioavailability-enhancing additives compared to surfactants and cosolvents is that they are biodegradable and do not cause any harm to the soil microflora. As soon as price level reaches the feasibility CDs will really find practical utility in protection of environment.

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