POTENTIAL USE OF CYCLODEXTRINS IN SOIL BIOREMEDIATION

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ABSTRACT

Bioavailability and toxic effect of contaminants are the main limitations during soil bioremediation. Cyclodextrins may influence bioavailability of the contaminants during biodegradation and also toxicity of the pollutant on soil microbes and plants since their ability to form inclusion complex with organic compounds. The effect of cyclodextrins on bioremediation and on toxic effect of hydrocarbons was investigated by testing the activity of hydrocarbon degrading microflora and of plant growth. The effect of cyclodextrins could be demonstrated in both cases: biodegradation of hydrocarbons could be enhanced and toxic effect of hydrocarbons on plants and soil microbes could be decreased by adding cyclodextrins.

1. INTRODUCTION

Different *in situ* and *ex situ* physical, chemical and biological technologies can be used for remediation of soils contaminated by hydrocarbons. The role of biotechnologies in soil decontamination is to upgrade biodegradation by ensuring nutrients and oxygene for the indigenous or the inoculant's microflora and by reducing limiting factors [1,2].
In case of biodegradation of hydrocarbons **bioavailability** is one of the main limitations. Hydrocarbons are strongly bound to the surface or built into the organic structure of the soil particles. The strongly absorbed hydrophobic contaminant is hardly available for the microflora which lives in the hydrophilic biofilm. Some of the biotechnologies apply surfactants to improve contact between contaminant and microflora, or such microbes, which can produce surfactants by themselves. Contaminants, due to their toxic effect, can inhibit microbial activity and plant growth [3]. Even biodegrading microbes could be inhibited by too high concentrations of toxic organic contaminants, eg. oily wastes, polyaromatic hydrocarbons (PAH) or polychlorinated biphenyls [4].
The effect of cyclodextrin (CD) on soil bioremediation and soil toxicity was investigated.

2. MATERIALS AND METHODS

Soil was contaminated with Diesel oil, mineral oil, polycyclic aromatic hydrocarbons, or transformator oil containing polychlorinated biphenyls. The soils were originated from contaminated sites or were polluted artificially.

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The intensity of biodegradation; the count of the living- and oil degrading microbes, the amount and the composition of the residual hydrocarbon contamination were investigated on the effect of cyclodextrin addition. RAMEB (DS: 1.8, Wacker Chemie) a randomly methylated β-cyclodextrin was applied in 0.1-5 % concentration range. The investigations were carried out in 100-500 g laboratory scale experiments, with the maximal duration of 3 months.
The content of extractables was measured by gravimetry after CCl_4 extraction, their composition was determined by gaschromatography and by liquid chromatography.
The toxic effect of soil contaminated with hydrocarbons was tested by measuring the growth and dehydrogenase enzyme activity of soil microbes and by seed germination and root growth inhibition of higher plants, like *Sinapis alba* (white mostard).

3. RESULTS AND DISCUSSION

The effect of cyclodextrin addition into the soil contaminated with hydrocarbons was investigated by studying the efficiency of biodegradation and the changes in toxicity.

3.1. Effect of cyclodextrin on oil biodegradation.

Loess soil contaminated artificially was used to investigate the effect of cyclodextrin on biodegradation of Diesel-oil and mineral oil. The results of long bioremediation experiments with and without cyclodextrin were compared.

TABLE 1. Changes in soil characteristics during bioremediation of hydrocarbons with and without cyclodextrins

	Cell number [cell/g soil]		Oil degrading cells [cell/g soil]		Extract [mg/kg]	
Sample	start	end	start	end	start	end
Diesel oil+inoculant	$2.7 \cdot 10^7$	$3.1 \cdot 10^7$	10^3–10^4	$>1.1 \cdot 10^5$	46220	7100
Diesel oil+CD+inoculant	$4.3 \cdot 10^7$	$10.0 \cdot 10^7$	10^3–10^4	$>1.1 \cdot 10^5$	46290	5900
Mineral oil+inoculant	$2.6 \cdot 10^7$	$1.4 \cdot 10^7$	10^3–10^4	$9.3 \cdot 10^4$	46280	19300
Mineral oil+CD+inoculant	$1.9 \cdot 10^7$	$2.9 \cdot 10^7$	10^3–10^4	$>1.1 \cdot 10^5$	47170	17400
Cyclodextrin	$6.9 \cdot 10^6$	$1.4 \cdot 10^7$	10^3–10^4	—	4000	400
Cyclodextrin+inoculant	$4.1 \cdot 10^6$	$6.5 \cdot 10^7$	10^3–10^4	$9.3 \cdot 10^4$	4000	500

The decrease of oil content is considerable during the 2.5 months period. CD resulted in further decrease in residual oil amount and increase in cell number compared with CD untreated. Cyclodextrin itself was degraded in the soil after 2.5 months.
The results of the gaschromatographic measurements gives more detailed information about the composition of the extract and its oil content. Hydrocarbon under C17 were completely degraded. Table 2. showes the amount of hydrocarbons under carbon number C25. In case of Diesel oil the chromatograms show considerable biodegradation, but no significant difference between CD treated and untreated samples could be observed. In case of mineral oil, the treatment with cyclodextrin resulted in a highly increased biodegradation of hydrocarbons under C25. In spite of the increased biodegradation only slight decrease in the amount of the extractables was measured. It suggests that two contrary processes take place: an increase in the amount of extractables and a decrease in the amount of biodegradable hydrocarbons. The explanation for both changes may be a general increase in degradational activity of the soil microflora. This may due to CD, which effects not only the contaminant but also the organic compounds of the soil matrix.

TABLE 2. Gas chromatographic characterisation of the residual oil

Sample	Cromatographed amount [mg]	CH under C_{25} [mg]	CH under C_{25} [%]	Amount of the extract [mg/kg]
Diesel-oil+inoculant	20	2.4	12.0	7100
Diesel-oil+CD+inoculant	23	2.9	12.6	5900
mineral oil+inoculant	113	2.8	2.5	19300
mineral oil+CD+inoculant	469	1.1	0.2	17400

The chromatograms of residual mineral oils show the increased biodegradation by the decreased amount of residual oil, compared to an inner standard (IS).

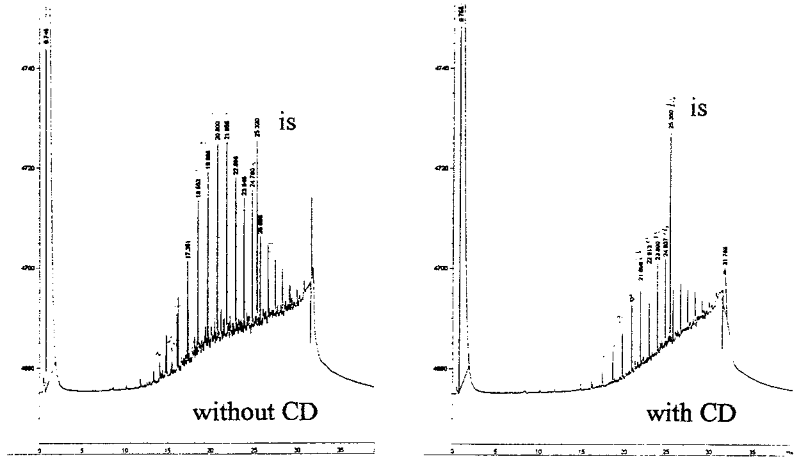

Fig. 1 Gas chromatograms of residual mineral oil, after CD enhanced biodegradation

3.2. Effect of cyclodextrin on biodegradation of PAHs in contaminated soil.

In case of soils derived from sites contaminated with PAHs the above mentioned phenomenon could be also indicated: changes in the amount of extractables does not correlate with the hydrocarbon content of the extract. The relationship depends on the soil type. The amount of the extracts could be either less or more after CD treatment, but the PAH content was always considerable lower. Also the cell number of oil degrading microbes in the soil increased to a higher value on the effect of CD.

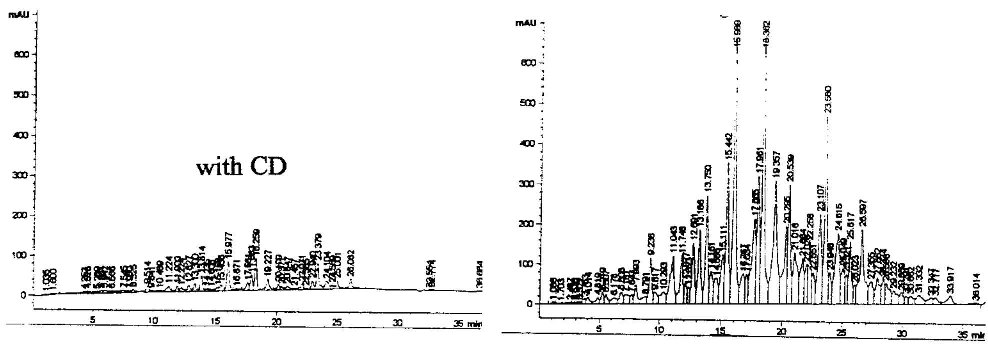

Fig. 2 HPLC of the residual polyaromatic hydrocarbons after biodegradation of soils treated and untreated with cyclodextrin

Two liquid chromatograms show the difference between the PAH content of the extracts of the untreated and the CD treated soils after 3 months biodegradation.
We suppose, that in the presence of cyclodextrin not only the contaminant, but also some components of the hardly available, structural organic matter are affected by the soil microflora, and degraded to smaller, more soluble and extractable molecules.

3.3. Effect of CD on soil toxicity: plant root growth in mineral oil and PAH contaminated soils.

In a soil contaminated with high, 30 000 ppm Diesel oil, root growth has been inhibited in a large scale: root size decreased from 50 mm to 5 mm. The addition of 0,1-5 % CD increased the root length from 5 mm to 20 mm, proportionally with CD concentration. Growth and enzyme activity of soil microbes showed the same tendency.
In case of lower contamination, 6500 ppm of transformator oil, root growth inhibition could be compensed by 0,5 % of CD. It suggests that a stochiometric relationship exists. It means that application of CD is realistic and effective in case of not too concentrated, but highly toxic contaminants.

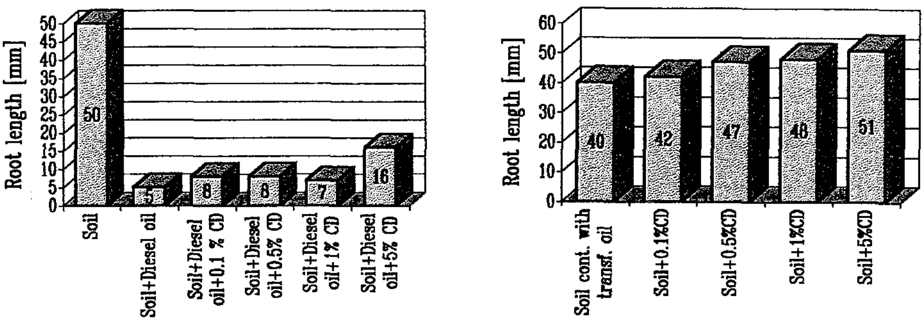

Fig. 3 Changes in the white mostard root growth inhibition in soils contaminated with Diesel oil and transformator oil by addition of cyclodextrin

4. CONCLUSION

The application of CD in soil bioremediation resulted in an increase in the efficiency of biodegradation of hydrocarbons. Addition of cyclodextrin decreased soil toxicity, which positively influenced both degrading microflora and plants growing in contaminated soils. Both phenomena may be due to the encapsulating effect of the cyclodextrin.

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