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A REVIEW CONCERNING THE EFFECTS OF CYCLODEXTRINS ON HYDROCARBONS BIODEGRADATION IN SOIL

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Abstract

Organic chemicals as hydrocarbons provided by crude oil represent serious environmental and health risk. For the remediation of contaminated soil different physical, chemical and biological technologies can be applied. The most promising remediation technologies are based on biodegradation. Bioremediation is an inexpensive, safe and environmental friendly technology. The end product of bioremediation is the harmless, decontaminated soil. Bioremediation of hydrocarbon polluted soils can be improved by the increase of hydrocarbon availability. In present review, it is discussed various effects of cyclodextrins on hydrocarbons biodegradation, bioremediation strategies, mechanisms involved in hydrocarbons biodegradation, factors and some technologies in bioremediation approaches.

Key words: *biodegradation, hydrocarbons, cyclodextrins, soil.*

INTRODUCTION

The release of hydrocarbons into the environment, accidental or due to human activities, is a main cause of soil pollution. Many bioremediation technologies have been developed to remove these contaminants, as some biological treatments are cheaper than chemical and physical treatments and sometimes result in complete mineralization (Holliger et al., 1997). One of the most important requirements is certainly the presence of microorganisms with the appropriate metabolic skills, but even the chemical physical characteristics of the oil and the interactions between the oil phase and the aqueous phase (containing the microorganisms) are very important for the success of bioremediation which relies on augmenting the natural biodegradation rate of oil (Ron and Rosenberg, 2014). Several petroleum aliphatic and polycyclic aromatic hydrocarbons can act as source of carbon and energy for the growth of soil microorganisms (Galli, 1998). One main factor that influences the extent of their biodegradation is their bioavailability and this is a priority research objective in the bioremediation field (Brusseau, 1998). Their hydrophobicity and low water solubility mean that hydrocarbons pass very slowly from a non-aqueous to the aqueous phase liquid in which they are metabolised by microorganisms (De Jonge et al., 1997). Moreover, in the soil they are adsorbed to clay or humus fractions (Tabak, 1997).

MATERIALS AND METHODS

Cyclodextrins are natural compounds, non-toxic to soil microorganisms and released enzymes (Szejtli, 1988), with great use in medical applications (Szejtli, 1994). Their involvement in microbial degradation, such as the purification of residual pesticide waters (Olah et al., 1988) or phenols (Banky et al., 1985), has also been investigated. Cyclodextrins absorb little or no soil solids (Brusseau et al., 1994). In unsaturated soils, they increase the desorption of contaminants from solid particles (Olah et al., 1988). The low bioavailability of pollutants is a limiting factor for biodegradation by microorganisms existing in the soil. Cyclodextrins act as a promoter for desorbing non-polar compounds from the surface of solid particles and mobilizing them in the aqueous phase where hydrocarbon-degrading microorganisms (Steffan et al., 2001) are active. Cyclodextrins act as a series of bacteria such as *Bacillus macerans, B. subtilis, B. coagulans, Flavobacterium*, and soil fungi such as *Trichoderma* sp. (Oros et al., 2001).

Cyclodextrins are non-reducing cyclic oligosaccharides (Figure 1). Of this class, the most important and accessible is β-cyclodextrin. Cyclodextrins can be obtained by a relatively simple technology, following the fermentative prehydrolysis of starch. Synthesis and characterization of new polymeric materials by chemical transformation of cyclodextrin, with the production of polyelectrolyte products being justified for: i) diversification of the range of biodegradable natural polyelectrolytes with biomedical uses; ii) the superior capitalization of polysaccharides as renewable resources; iii) completing the database on chemical modification of polysaccharides and solution behavior of rigid skeletal polyelectrolytes, given that there is little literature data on obtaining soluble polysaccharide derivatives with phosphoric groups (Szejtli, 1982).

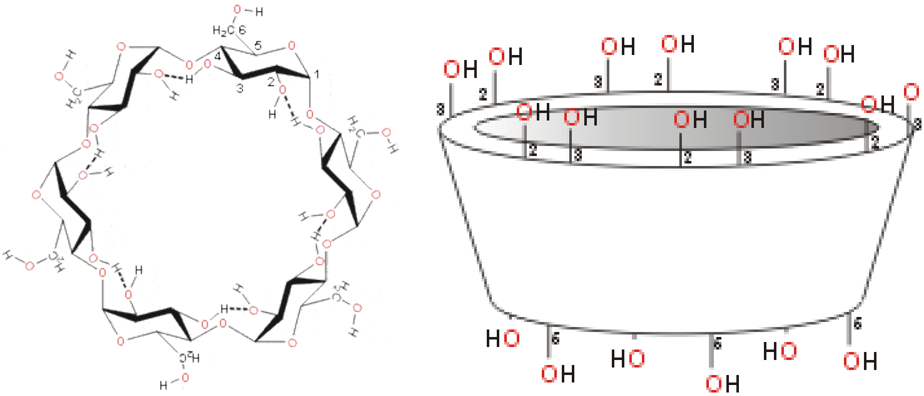

Figure 1. Cyclodextrin structure (Szejtli, 1982)

RESULTS AND DISCUSSIONS

The randomized methylated β-cyclodextrin (RAMEB) is industrially produced and has a high solubility capacity. According to the studies performed by RAMEB, it is capable of enhancing the biodegradability of many organic compounds, such as polynuclear aromatic hydrocarbons (Fenyvesi et al., 1996; Fava et al., 2002). This compound can reduce the toxic effects of contaminants on bacterial microflora, plants and animals (Gruiz et al., 1996). The effects of RAMEB different amounts on the bioremediation of soils polluted with petroleum hydrocarbons have been achieved through a laboratory study. Three types of sandy, clay and loam soil were used which were treated with RAMEB at concentrations of 0-1%. A significant increase in hydrocarbon biodegradability and microbial activity due to the presence of RAMEB (Molnár et al., 2007) was observed in the 4 week study. The RAMEB concentration of soil samples contaminated with petroleum hydrocarbons was determined by the HPLC method. The efficiency of RAMEB extraction from soil samples depended on soil properties. The extraction method had a good recovery rate for sandy and loam soil, and with less efficiency for soil with a higher clay content (Fenyvesi et al., 2002). Steffan et al. (2002) conducted a study on the effects of β-cyclodextrin on dodecane biodegradation. The results obtained showed that β-cyclodextrin significantly influenced the kinetic biodegradation process. A study by Molnár et al. (2005) showed that RAMEB can be applied as an additive in bioremediation of soils polluted with crude oil, and the results were similar to those obtained in the field. These bioremediation technologies based on cyclodextrins have a high potential for enhancing bioremediation compared to convectional biolological technologies in which time is a limiting factor. RAMEB is also a non-toxic and easily degradable product in the soil, does not pose a risk to soil life. The addition of

RAMEB, nutrients and a CO_2 stream has led to very good results in in-situ technologies and seems to be the most effective remediation method (Leitgib et al., 2008).

Bacterial degradation of toluene with an organic solvent and p-methyl benzoic acid, a water-soluble aromatic compound is enhanced by the addition of β-cyclodextrin (Schwartz and Bar, 1995). Also, the phenanthrene biodegradation of a polynuclear aromatic hydrocarbon in the presence of hydroxypropyl-β-cyclodextrin (HPBCD) has been removed (Wang et al., 1998).

Bardi et al. (2000) have conducted a study in which they made research in hydrocarbons degradation by the microbial population using β-cyclodextrin to increase their bioavailability. This work presents ways in which β-cyclodextrin increases the biodegradation of aliphatic hydrocarbons and polynuclear aromatics in high concentrations. Petroleum hydrocarbons having the following meanings were studied: 2 medium-chain (C12-dodecane) and one high chain (C24-tetracosan) and 2 polynuclear aromatic hydrocarbons with 2 benzene rings (naphthalene) and 3 benzene rings (anthracene). A study by Cuypers et al. (2002) demonstrated that hydroxypropyl β-cyclodextrin solutions can accelerate the degradation of polynuclear aromatic hydrocarbons.

The effect of hydroxypropyl β-cyclodextrin (HPBCD) on the biodegradation of polynuclear aromatic hydrocarbons (ΣPAH) in complex matrices was performed by Hickman et al., 2008. According to the results, the method can be applied to soil level.

The extraction capacity of hydroxypropyl-α-cyclodextrin provides information on the bioavailability of aliphatic hydrocarbons in the soil. A study by Stroud et al. (2009) investigated the potential for the extraction of hydroxypropyl-α-cyclodextrin in biodegradation of soil hexadecane. The soil was artificially polluted with 10-100 mg kg^{-1} hexadecane. Following the obtained results, hydroxypropyl-α-cyclodextrin can be used in biodegradation of aliphatic hydrocarbons from polluted soils.

Fenyvesi et al. (2005) conducted a study in which they followed cyclodextrins biodegradation in soil. It have been studied 8 different cyclodextrins on four soil types in a laboratory experiment. According to the results obtained, the cyclodextrins studied are all biodegradable by the microorganisms in the following order α-cyclodextrin ≈ β-cyclodextrin γ-cyclodextrin › acetyl β-cyclodextrin › cellulose › hydroxypropyl β-cyclodextrin (HPBCD) › peracetyl α-cyclodextrin › peracetyl-β-cyclodextrin » methylated β-cyclodextrin (RAMEB). It has been concluded that RAMEB is the least biodegradable. These conclusions confirm that RAMEB is a good solution for enhancing the bioremediation process, reducing it by 40% relative to its initial concentration over two years in a hydrocarbon polluted soil in an experimental field.

To assess the effect over time of the availability of pyrene (polynuclear aromatic hydrocarbon), earthworm accumulation (*Eisenia fetida*) and chemical extraction by unexploited techniques in the soil was artificially contaminated a soil with different pyrene concentrations and were measured at different times. The results showed that the amount of earthquake-rich pyrene did not change over time at high concentrations, but significantly changed at lower concentrations. In addition, the chemical availability of the pyrene has significantly decreased over time. The relationship between the bioaccumulation in the soil of hydroxypropyl-β-cyclodextrin (HPCD) and organic solvent extraction has been investigated to find a suitable and rapid method to increase the pyrene bioavailability. Results showed that at different pyrene concentrations the mean values absorbed by earthworm and HPCD extracts were 10-40% and 10-65%, respectively. The results obtained and their correlation for pyrene removal suggest that HPCD extraction was a better method of increasing bioavailability of pyrene in soil compared to organic solvent extraction (Khan et al., 2011).

According to studies conducted by Viglianti et al. (2006) addition of cyclodextrin (CD) in aqueous wash solutions has been shown to increase the efficiency of hydrocarbons removal from soil, being at the same time a non-toxic agent. In this study, the efficacy of cyclodextrins to remove polynuclear aromatic hydrocarbons (PAHs) occurring in industrial areas was investigated. The β-cyclodextrin (BCD), hydroxypropyl β-cyclodextrin (HPCD) and methyl β-cyclodextrin (MCD) solutions were used for soil washing by column tests to evaluate certain influential parameters that can significantly increase the hydrocarbon removal efficiency. The process parameters chosen were the CD concentration, the ratio between the volume of the washing solution and the soil mass and the temperature of the washing solution. These parameters proved to be significant and the effect was almost linear on the removal of PAHs from the contaminated soil, except for the temperature at which an insignificant influence on PAH extraction was observed for the temperature range between 5 and 35°C. The removal capacity of HPCD and MCD was higher than BCD.

β-cyclodextrin (β-CD) significantly accelerates the hydrocarbon biodegradation, but its efficacy during the final degradation steps is not yet clear. Moreover, it is not yet known whether the absorption of hydrocarbons by plants is influenced by the presence of cyclodextrins. A field study was conducted by creating two lots (A and B). Crude oil was spread across the surface, and bacterial inoculum and β-CD on B lot. The soybean was sown on both lots. Soil samples were harvested from 10 in 10 cm at a depth of 0 to 60 cm before soil contamination with crude oil, immediately after sowing and after harvesting the plant. Chemical and microbial analyzes were performed throughout the process to characterize soil and determine residual PAH. Soybean seeds were analyzed for PAH content. It was observed that β-CD induced a significant increase in the rate of PAH degradation. Bacterial inoculum did not improve degradation; biodegradation was stronger in superficial layers, and leakage of PAHs was observed that was reduced by this CD. PAH analysis in soybeans showed that there was an absorption of hydrocarbons and that it was more significant in B lot. This suggests that β-CD has accelerated the bioremediation process but can be improved by phytoremediation which could lead to also to obtain an additional profit through the production of biofuels (Bardi et al., 2007).

The research achived by Sivaraman et al. (2010) focuses on studying the degradation of hydrocarbons by *Pseudomonas* isolated from crude oil contaminated soil in the presence of cyclodextrins. Of the three cyclodextrins tested at different concentrations, the 2.5 mM β-cyclodextrin concentration showed the highest biodegradation when n-hexadecane was used as the hydrocarbon test. The percentage of residual hexadecene remaining in the medium in which 2.5 mM β-cyclodextrin solution was added at 120 hours was 15% compared to the biotic environment that was 43%. In the following experiment, degradation of the hydrocarbon mixture (tetradecane, hexadecane and octadecane) by Vid1 (*Pseudomonas* species) was studied at a concentration of 2.5 mM β-cyclodextrin. The residual percent of tetradecane, hexadecane and octadecane at 120 hours was 32, 43 and 61% compared to the biotic environment where biodegradations of 50, 58 and 67%, respectively, were recorded. Studies have shown that in the case of a mixture of hydrocarbons (tetradecane, hexadecane and octadecane) in the presence of β-cyclodextrin, the highest hydrocarbons degradability was registered in tetradecan, then hexadecane and octadecane, respectively.

Ramadass et al. (2015) conducted a study on soils contaminated with petroleum hydrocarbons sampled from an arid Australian region and a significant hydrocarbons biodegradation in three of the five soils by optimizing nutritional status and physical characteristics. The data obtained supported the reduction of THP concentration with increasing bacterial diversity in these soils. Analysis of microbial diversity in soils has demonstrated the existence of bacterial communities of hydrocarbon degradation in these soils. However, bioremediation was not effective in these soils, even after the addition of surfactant (surfactant - Triton) due to high hydrocarbon concentrations (123.757 mg kg^{-1}). Further application of biopile technology to these soils was required. Microbial diversity has been found to depend on the degree of pollution and the solubility (bioavailability) of petroleum hydrocarbons in soils that can be accelerated by HPCD extraction. This study provides an overview of the major parameters to be taken into account when assessing the applicability of remediation technology by using biopiles.

CONCLUSIONS

Research results indicate that cyclodextrins increase hydrocarbons biodegradation significantly. Non-inclusion interactions may play a role in increasing bioavailability. Studies in which hydrocarbon mixtures were used in the presence of cyclodextrins indicate that the reduction in hydrocarbon concentration, both in the presence and absence of cyclodextrins is influenced by chain length. Cyclodextrins are also nontoxic and readily degradable in soil and do not pose any risk to soil life.

Cyclodextrins application has the role of improving the remediation biological method of soils polluted with petroleum hydrocarbons by increasing the efficiency of the biodegradation process. They have the ability to favor the development of existing bacteria in the polluted soil and to increase the rate of biodegradability of petroleum hydrocarbons.

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