# POTENTIAL OF CYCLODEXTRINS TO INCREASE THE PETROLEUM HYDROCARBONS BIODEGRADATION

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**Abstract**

Pollution phenomena cause important changes in the phytosphere and zoosphere, as well as in microorganisms, leading to the disappearance of a large number of species, causing a decrease in soil fertility, its most important property, which allows the support of plant and animal life. In this paper will be presented a review concerning the potential of cyclodextrins to increase the petroleum hydrocarbons biodegradation. Cyclodextrins are natural compounds, non-toxic for microorganisms existing in the soil with great use in medical applications. Their involvement in microbial degradation, such as the purification of pesticide or phenol waste water, was also investigated. Cyclodextrins absorb very little or not at all on solid soil particles. In unsaturated soils, they increase the desorption of contaminants from the solid particles. The low bioavailability of polluting hydrocarbons is a limiting factor of biodegradation by existing microorganisms in the soil. Cyclodextrins have the role of promoting the desorption of non-polar compounds from the surface of solid particles and their mobilization in the aqueous phase where hydrocarbon-degrading microorganisms carry out their activity. Cyclodextrins have the role of activating a series of bacteria, such as: *Bacillus macerans*, *B. subtilis*, *B. coagulans, Flavobacterium* spp., but also soil fungi such as *Trichoderma* spp.

**Key words**: biodegradation, cyclodextrins, petroleum hydrocarbons

Introduction. With the establishment of the first human collectivities, with the start of socio-economic development, with the exacerbation of the industrial and post-industrial revolution, etc., man was not satisfied with nature as such, with the intelligence and creative spirit that define him, but began to adapt it and transforms according to his needs.

Unfortunately, harmful side effects of human activity have also appeared, becoming increasingly frequent and aggressive, some with completely unpredictable and even irreversible impacts on the quality of the environment, plant and animal life, and human existence itself.

All of this led to the emergence, development and worsening at a particularly accelerated rate of a completely new, very complex and increasingly large and dangerous phenomenon that encompassed, on a global scale, all countries and continents, identified under the name of pollution of the environment (Reid B.J. *et al*, 1998; Fenyvesi E. *et al*, 2002, 2005; Bardi L. *et al*, 2007; Hajdu C. *et al*, 2011).

By pollution is meant any unwanted change in the physical, chemical and biological characteristics of water, air and soil that can adversely affect the health, survival or activities of humans or other living organisms, a term for which a clearer understanding is necessary to take into account, at the same time, by the term contamination, which refers to the presence, regardless of quantity, of dangerous and unwanted elements or substances in water, air or soil, due to human activities that are categorically not harmful (Leitgib L. *et al*, 2008; Riding M.J. *et al*, 2013).

The immediate and very obvious harmful effects of soil pollution with crude oil, sometimes very serious, quickly alerted public opinion, requiring the decontamination of soils polluted with oil products, especially in areas with large oil exploitations.

In the meantime, this concern has expanded over an increasingly wider area, becoming an increasingly diversified sphere of activity, with the use of more and more categories of methods and procedures for depollution, known more and more frequently also under the name of decontamination, of polluted soils with oil, both in the fields of scientific research and in the field of practical applications to prevent and combat soil pollution with oil and/or oil products. Depending on the type of pollution, the source and the intensity of the pollution, the ecological restoration

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measures of the polluted soils are established (Trellu C. *et al*, 2016).

Physical, chemical and biological methods can be used for the decontamination of soils polluted with petroleum hydrocarbons. The physico-chemical processes that take place between the pollutant particles and the soil particles are important and essential in soil remediation (Molnár M. *et al*, 2005).

The main physical-chemical processes that occur in the case of oil-polluted soils are the biodegradation or microbial degradation of hydrocarbons, the evaporation of hydrocarbons, the sorption or retention of soil, the solubility of hydrocarbons and the volatilization of hydrocarbons.

Of all these, research has proven that bioremediation, especially in the case of oil pollution, is a superior, efficient and much cheaper method compared to physical or chemical methods. Indeed, in recent years, the bioremediation of soils polluted with petroleum hydrocarbons represents a real challenge for modern scientific research (Rahman K.S. *et al*, 2003, Molnár M. *et al*, 2007).

## MATERIAL AND METHOD

Cyclodextrins can be considered as a truncated cone in which the secondary hydroxyl groups are arranged on the large base, and the primary hydroxyl groups on the small base of the truncated cone.

The dimensions of the three shapes presented above, respectively the diameters of the inner cavity, are: 5.7; 7.8 and 9.5 Å for α-, β- and γ-cyclodextrin, respectively. With the increase in the diameter of the cyclodextrin cavity, it can host a larger number of water molecules, therefore in aqueous solution the bound water molecules will differ less and less energetically from those in the solvent mass (Szejtli J., 1982).

α, β and γ-cyclodextrins are cyclic oligosaccharides formed by 6, 7 or 8 α-1,4-glycosidic units (Szaniszlo N. *et al*, 2005). Because they have hydrophobic cavities and hydrophilic shells, they are soluble in water. In this way, the solubility of hydrocarbons increases. The 3 natural cyclodetrins α, β and γ are completely and easily biodegradabl.

The effect of β-cyclodextrins on the solubility of hydrocarbons in the aqueous phase was investigated by Huipeng G. *et al* (2015). The relationship between the solubility of hydrocarbons and the concentration of β-cyclodextrins was determined, and the distribution coefficient of hydrocarbons between β-cyclodextrins and water (Kcw) was calculated.

## RESULTS AND DISCUSSIONS

Pollution phenomena cause important changes in the phytosphere and zoosphere, as well as in microorganisms, leading to the disappearance of a large number of species, causing a decrease in soil fertility, its most important property, which allows the support of plant and animal life and, implicitly, of humans (Viglianti C. *et al*, 2006).

Although it possesses the capacity of self-regeneration, the specific formation conditions mean that, once destroyed, the soil cannot be restored as it was, because the conditions and the millennial history of its formation cannot be reproduced. At most one body with similar functions can be created. Until the last decades, the soil was regarded, mainly due to its fertility, i.e. the ability to sustain plant life, only as the main means of production in agriculture, lately it has been recognized that the existence and development of human society will also be conditioned in future of the abundance and quality of higher terrestrial plants, which must provide people with food and raw materials for clothing, shelter, medicine and other requirements. Among the potentially polluting elements of the soil, there are petroleum hydrocarbons, considered among the strongest soil pollutants (Molnár M. *et al*, 2005).

Crude oil radically changes the properties of the soil, both physical and chemical as well as biological. This forms an impermeable film on the surface of the soil, which prevents the circulation of water in the soil and the exchange of gases between the soil and the atmosphere, producing asphyxiation of the roots and favoring the manifestation of reduction processes. As the soil becomes more anaerobic, the number and metabolic activity of bacteria decreases. Crude oil being rich in organic carbon (98% hydrocarbons), increases the C/N ratio in the soil, negatively influencing microbiological activity and plant nutrition with nitrogen.

Considering that pollution with crude oil, products and petroleum residues affects the capacity of soils to support life, the improvement of the remediation methodology, adapted to the conditions in Romania, is imperative.

Microbial degradation or biodegradation is the main process by which microorganisms degrade crude oil. The biodegradation of hydrocarbons can take place under aerobic conditions or, more difficult, under anaerobic conditions. In the presence of oxygen, most organic compounds are rapidly mineralized by microorganisms. During the biodegradation process, the carbon is completely oxidized to carbon dioxide which provides energy, and a part is used in the formation of new microorganisms. Cycloalkanes represent a minor component in oil and are relatively resistant to the attack of microorganisms. Through the process of biodegradation, petroleum hydrocarbons can be partially or totally transformed by a series of microorganisms. Most of the time, polynuclear aromatic hydrocarbons are not totally degraded by processes mediated by microorganisms.

Bioremediation can be: in-situ and ex-situ. Bioremediation is based on the ability of microorganisms to use petroleum hydrocarbons as a source of carbon and energy. It is considered to be the most effective because, in addition to the lower cost, it does not have irreversible effects on the pedogenetic characteristics of the affected soil (Voiculescu A.R. *et al*, 2003).

Cyclodextrins are natural compounds, non-toxic for microorganisms existing in the soil and released enzymes (Szejtli J., 1988, 1996; Reid B.J. *et al*, 2000), with great use in medical applications (Szejtli J., 1994). Their involvement in microbial degradation was also investigated, such as the purification of pesticide waste water (Olah J. *et al*, 1988) or phenols (Banky B.K. *et al*, 1985).

Cyclodextrins absorb very little or not at all on solid soil particles (Brusseau M.L. *et al*, 1994). In unsaturated soils, they increase the desorption of contaminants from solid particles (Olah J. *et al*, 1988). The low bioavailability of polluting hydrocarbons is a limiting factor of biodegradation by existing microorganisms in the soil. Cyclodextrins have the role of promoting the desorption of non-polar compounds from the surface of solid particles and their mobilization in the aqueous phase where hydrocarbon-degrading microorganisms carry out their activity. Cyclodextrins have the role of activating a series of bacteria, such as: *Bacillus macerans, B. subtilis, B. coagulans, Flavobacterium*, but also soil fungi such as *Trichoderma* sp. (Steffan S. *et al*, 2001, 2002; Boving T. *et al*, 2003).

Randomly methylated β-cyclodextrin (RAMEB) is industrially produced and has a high solubilization capacity. According to the studies carried out by RAMEB, it is able to intensify the biodegradability of many organic compounds, such as polynuclear aromatic hydrocarbons (Fenyvesi E. *et al*, 1996; Fava F. *et al*, 1998). This compound can reduce the toxic effects of contaminants on bacterial microflora, plants and animals (Gruiz K. *et al*, 1996).

Only a few authors have investigated the influence of cyclodextrins on biodegradation mechanisms and kinetics. Therefore, it is quite difficult to discuss the obtained results and highlight clear trends. The availability of petroleum hydrocarbons for microbial degradation can be greatly affected by their preferential interaction with non-aqueous phases and soil organic matter content. It has been shown that the biodegradation effect of the surfactant depends on both the solubilizing power of the cyclodextrins and the bioavailability of petroleum hydrocarbons to form an inclusion complex. Based on the Monod equation, was proposed to describe the substrate and biomass evolution during the biological treatment of petroleum hydrocarbons in the presence of cyclodextrins.

Electrokinetic migration (EK) of β-cyclodextrin (β-CD), which also applies to total petroleum hydrocarbons (THP), is a friendly, economically and environmentally beneficial remediation process for crude oil contaminated soils. Remediation studies of crude oil contaminated soils generally use certain hydrocarbons. This study investigates the removal of hydrocarbons from oil by electromigrating microbial cells in an electrokinetic field. Both hydrocarbon content and soil respiration decreased during the electrokinetic remediation process. Bacterial strains from the soil include *Bacillus* sp., *Sporosarcina* sp., *Beta proteobacterium*, *Streptomyces* sp., *Pontibacter* sp., *Azorhizobium* sp., *Taxeobacter* sp., and *Williamsia* sp. Electromigration of microbial cells reduced the biodiversity of the soil microbial community. At 200 $Vm^{-1}$ for 10 days, 36% of total petroleum hydrocarbons were removed with a small population of microbial cells, thus demonstrating that electrokinetic migration remediation is effective for crude oil contaminated soils (Chunli W. *et al*, 2011).

Szaniszl *et al* (2005) determined using headspace gas chromatography (SHSGC) the association constants for the inclusion of different hydrocarbons (linear alkanes, cycloalkanes, aromatics) in various cyclodextrins (CD) in aqueous solution. Thus, the relationship between structure and stability was examined.

Bacterial degradation of toluene, an organic solvent, and p-methyl benzoic acid, a water-soluble aromatic compound, is increased by the addition of β-cyclodextrin. The increase of biodegradation of phenanthrene, a polynuclear aromatic hydrocarbon, in the presence of hydroxypropyl-β-cyclodextrin (HPBCD) was also demonstrated.

Bardi *et al* (2000) conducted a study in which they followed the degradation of hydrocarbons by the microbial population by using β-cyclodextrin to increase their bioavailability. Four representative petroleum hydrocarbons were studied: 2 aliphatic hydrocarbons, one with a medium chain (C12-dodecane) and one with a long chain (C24-tetracosane) and 2 polynuclear aromatic hydrocarbons with 2 benzene rings (naphthalene) and with 3 benzene rings (anthracene). The largest decrease in hydrocarbons was recorded in the case of naphthalene. 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 carried out by Hickman et al., 2008. According to the results obtained, the method can also be applied at the soil level.

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

In order to evaluate the effect over time of the availability of the polynuclear aromatic hydrocarbon pyrene, accumulation in the body of the earthworm (*Eisenia fetida*) and chemical extraction by unexploited techniques in the soil, a soil was artificially contaminated with various concentrations of pyrene and were measured at different time periods. The results showed that the amount of pyrene accumulated by earthworms did not change much over time at high concentrations, but changed significantly at lower concentrations. In addition, the chemical availability of pyrene decreased significantly over time. The relationship between soil bioaccumulation of hydroxypropyl-β-cyclodextrin (HPCD) and organic solvent extraction was investigated to find a suitable and rapid method to increase the bioavailability of pyrene. The results showed that at different concentrations of pyrene, the average values absorbed by rams and extracted with HPCD were 10–40% and 10–65%, respectively. The results obtained and their correlation for pyrene removal suggest that HPCD extraction was a better method to increase pyrene bioavailability in soil compared to organic solvent extraction (Khan M.I. *et al*, 2011).

The research carried out by Sivaraman C. *et al* (2010), focuses on studying the degradation of hydrocarbons by *Pseudomonas* species isolated from waters contaminated with petroleum hydrocarbons in the presence of cyclodextrins. Among the three cyclodextrins tested at different concentrations, the 2.5 mM concentration of b-cyclodextrin showed the highest biodegradation when n-hexadecane was used as the test hydrocarbon. The percentage of residual hexadecane remaining in the medium in which 2.5 mM β-cyclodextrin solution was added at 120 hours was 15% compared to the biotic medium which was 43%. In the following experiment, the degradation of the mixture of hydrocarbons (tetradecane, hexadecane and octadecane) by Vidl (*Pseudomonas*-like species) at a concentration of 2.5 mM β-cyclodextrin was studied. The residual percentage 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% were recorded, respectively. The studies showed that in the case of a mixture of hydrocarbons (tetradecane, hexadecane and octadecane) in the presence of β-cyclodextrin, the highest hydrocarbon degradability was registered for tetradecane, then hexadecane, and octadecane, respectively.

Ramadass K. *et al* (2015) conducted a study on soils contaminated with petroleum hydrocarbons collected from an arid region in Australia and a significant biodegradation of hydrocarbons in three of the five soils by optimizing the nutritional status and physical characteristics. The data obtained supported the reduction of THP concentration along with the increase of bacterial diversity in these soils. Analysis of microbial diversity in soils demonstrated the existence of hydrocarbon-degrading bacterial communities in these soils. However, the bioremediation was not efficient in these soils, even after adding the surfactant (surfactant - Triton), due to the high concentrations of hydrocarbons (123,757 mg $kg^{-1}$). Furthermore, it was necessary to apply the biopile technology to these soils. It was found that microbial diversity depends on the degree of pollution and the solubility (bioavailability) of petroleum hydrocarbons in soils, which can be accelerated by HPCD extraction. This study provides an overview of the major parameters to be considered when evaluating the applicability of remedial technology using biopiles.

Hajdu C. *et al* (2011) conducted a study in which RAMEB (randomly methylated b-cyclodextrin) was applied, which has a high solubilization capacity for many typical soil contaminants. RAMEB influenced the evolution and behavior of the contaminant in the soil and the soil solution, and the results depend on the concentration of RAMEB in the bioassay, the contact time, the type of organism tested and the characteristics of the contaminant complex - RAMEB.

## CONCLUSIONS

Bioremediation technologies can be in-situ and ex-situ. Bioremediation is based on the ability of microorganisms to use petroleum hydrocarbons as a source of carbon and energy. It is considered to be the most effective because, in addition to the lower cost, it does not have irreversible effects on the pedogenetic characteristics of the affected soil. Depending on the degree of pollution, the appropriate soil bioremediation technology is chosen: biostimulation; inoculation with selected microorganisms; bioventilation; stimulation with surfactants; phytoremediation; the use of agricultural land; natural attenuation; biobubble; co-metabolism, bioreactors; loosening the ground; biopiles; composting (static/mechanical agitation).

The biodegradation of hydrocarbons can take place under aerobic conditions or, more difficult, under anaerobic conditions. In the presence of oxygen, most organic compounds are rapidly mineralized by microorganisms. During the biodegradation process, the carbon is completely oxidized to carbon dioxide which provides energy, and a part is used in the formation of new microorganisms. Through the process of biodegradation, oil hydrocarbons can be partially or totally transformed by a series of microorganisms. Most of the time, oil hydrocarbons are not completely degraded by processes mediated by microorganisms.

Research results indicate that cyclodextrins significantly increase the biodegradation of hydrocarbons. There is a possibility that non-inclusive interactions play a role in increasing bioavailability. Studies using mixtures of hydrocarbons in the presence of cyclodextrins indicate that the reduction of hydrocarbon concentration, both in the presence and absence of cyclodextrins, is influenced by chain length.

Cyclodextrins are also non-toxic and easily degradable products in the soil, they do not pose any risks to life in the soil.

The application of cyclodextrins has the role of improving the biological method of remediation of soils polluted with oil 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.

## ACKNOWLEGMENTS

This work was supported by grant of the Romanian Ministry of Research, Innovation and Digitization, project number 44 PFE /2021, Program 1 – Development of national research development system, Subprogramme 1.2 – Institutional performance – RDI Excellence Financing Projects.

## REFERENCES

**Banky B.K., Recseg K., Novak D. 1985** – *Application of water soluble betacyclodextrin in microbiological decomposition of phenol*, Magy Kem Lapja, 40, p. 189.

**Bardi L., Mattei A., Steffan S., Marzona M. 2000** – *Hydrocarbon degradation by a soil microbial population with b-cyclodextrin as surfactant to enhance bioavailability*, Enzyme and Microbial Technology 27:709–713.

**Bardi L., Martini C., Opsi E.F., Bertolone E., Belviso E., Masoero G, Marzona M., Ajmone Marsan F. 2007** - *Cyclodextrin-enhanced in situ bioremediation of polyaromatic hydrocarbons-contaminated soils and plant uptake,* J Incl Phenom Macrocycl Chem, 57:439–444, DOI 10.1007/s10847-006-9231-x.

**Boving T., McCray J.E., Blanford W. 2003** – *Comparison of Two Cyclodextrin Remediation Approaches: Line-drive versus Push–pull*, Abstracts Programs of the Geological Society of America, 35(6), p. 371.

**Brusseau M.L., Wang X., Hu Q. 1994** – *Enhanced transport of low-polarity organic compounds through soil by cyclodextrin*, Environmental Science and Technology 28(5):952-956.

**Chunli W., Maoan D., Duu-Jong L., Xue Y., Wencheng M., Zheng Z. 2011** - *Electrokinetic remediation and microbial community shift of β-cyclodextrin-dissolved petroleum hydrocarbon-contaminated soil*, Appl Microbiol Biotechnol, 89:2019–2025, DOI 10.1007/s00253-010-2952-1

**Fava F., Di Gioia D., Marchetti L. 1998** – *Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil*, Biotechnology and Bioengineering 58:345-355.

**Fenyvesi É., Szeman J., Szejtli J. 1996** – *Extraction of PAHs and Pesticides from Contaminated Soils with Aqueous CD solutions*, Journal of Inclusion Phenomena and Molecular Recognation Chemistry 25:229-232.

**Fenyvesi É., Csabai K., Molnár M., Gruiz K., Muranyi, A., Szejtli J. 2002** - *Quantitative and Qualitative Analysis of RAMEB in Soil*, Journal of Inclusion Phenomena and Macrocyclic Chemistry 44:413–416.

**Fenyvesi É., Gruiz K., Verstichel S., De Wilde B., Leitgib L., Csabai K., Szaniszlo N. 2005** – *Biodegradation of cyclodextrins in soil*, Chemosphere, 60:1001–1008.

**Gruiz K., Fenyvesi É., Kriston E., Molnár M., Horvath B. 1996** – *Potential Use of Cyclodextrins in Soil Bioremediation*, J. Incl. Phenom. Mol. Recogn. Chem., 25:233-236.

**Hajdu C., Gruiz K., Fenyvesi E., Nagy S.M. 2011** - *Application of cyclodextrins in environmental bioassays for soil*, J Incl Phenom Macrocycl Chem, 70:307–313, DOI 10.1007/s10847-010-9855-8.

**Huipeng G., Xiaorong G., Yaming, C., Li X., Lingyun**

**J. 2015** - *Influence of Hydroxypropyl-β-cyclodextrin on the Extraction and Biodegradation of p,p'-DDT, o,p'-DDT, p,p'-DDD, and p,p'-DDE in Soils*, Water Air Soil Pollut (2015) 226: 208, DOI 10.1007/s11270-015-2472-9.

**Khan M.I., Cheema S.A., Shen C., Zhang C., Tang X., Malik Z., Chen X, Chen Y. 2011** - *Assessment of Pyrene Bioavailability in Soil by Mild Hydroxypropyl-b-Cyclodextrin Extraction,* Arch Environ Contam Toxicol, 60:107–115, DOI 10.1007/s00244-010-9517-2.

**Leitgib L., Gruiz K., Fenyvesi É., Balogh G., Murányi A. 2008** – *Development of an innovative soil remediation: "Cyclodextrin-enhanced combined technology"*, Science of the Total Environment 392:12-21.

**Molnár M., Leitgib L., Gruiz K., Fenyvesi É, Szaniszló N., Szejtli J., Fava F. 2005** – *Enhanced biodegradation of transformer oil in soils with cyclodextrin – from the laboratory to the field*, Biodegradation 16:59–168.

**Molnár M., Gruiz K., Halász M. 2007** – *Integrated methodology to evaluate bioremediation potential of creosote-contaminated soils*, Chemical Engineering 51(1), p. 23–32.

**Olah J., Cserhati T., Szejtli J. 1988** – *Beta-cyclodextrin enhanced biological detoxification of industrial wastewaters*, Water Research 22, p. 1345-1352.

**Rahman K.S., Thahira-Rahman J., Kourkoutas Y., Petsas I., Marchant R., Banat I.M. 2003** – *Enhanced bioremediation of n-alkanes in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients*, Bioresource Technology 90:159–168.

**Ramadass K., Smith E., Palanisami T., Mathieson G., Srivastava P., Megharaj M., Naidu R. 2015** - *Evaluation of constraints in bioremediation of weathered hydrocarbon-contaminated arid soils through microcosm biopile study*, Int. J. Environ. Sci. Technol. 12:3597–3612, DOI 10.1007/s13762-015-0793-2.

**Reid B.J., Semple K.T., Jones K.C. 1998** – *Prediction of bioavailability of persistant organic pollutants by a novel extraction technique*, In: Contaminated Soil '98, vol. 2, Thomas Telford, London, p. 889-990.

**Reid B.J., Stokes J.D., Jones K.C., Semple K.T. 2000** – *Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability*, Environmental Science and Technology 35:3174-3179.

**Riding M.J., Doick K.J., Martin F.L., Jones K.C., Semple K.T. 2013** - *Chemical measures of bioavailability/bioaccessibility of PAHs in soil: Fundamentals to application*, Journal of Hazardous Materials 261:687– 700.

**Sivaraman, C., Ganguly, A., Mutnuri, S. 2010** - *Biodegradation of hydrocarbons in the presence of cyclodextrins*, World J Microbiol Biotechnol, 26:227–232, DOI 10.1007/s11274-009-0164-6.

**Steffan S., Bardi L., Marzona M. 2001** – *Biodegradation of hydrocarbons in polluted soils using β-cyclodextrin as a coadiuvant*, Biology Journal of Armenia, Special issue: Cyclodextrins, p. 218–25.

**Steffan S., Tantucci P., Bardi L., Marzona M. 2002** – *Effects of cyclodextrins on dodecane biodegradation*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 44:407-411.

**Stroud, J. L., Tzima, M., Paton, G. I., Semple, K. T. 2009** - Influence of hydroxypropyl-β-cyclodextrin on the biodegradation of 14C-phenanthrene and 14Chexadecane in soil. Environmental Pollution, 157.2678–2683.

**Szaniszlo N., Fenyvesi, E., Balla, J. 2005** - *Structure-stability Study of Cyclodextrin Complexes with Selected Volatile Hydrocarbon Contaminants of Soils*, Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 53:241–248, DOI 10.1007/s10847-005-0245-6.

**Szejtli J. 1982** – *Cyclodextrins and their inclusion complexes*, In: Proc First Int Symp on Cyclodextrins, Akademiai Kiado, Budapest, D. Reidel Publishing, Dordrecht, p. 95–109.

**Szejtli J. 1988** – *Cyclodextrin thechnology*, Kluwer Academic Publishers, Dordrecht, The Netherland, p. 1–393.

**Szejtli J. 1994** – *Medicinal applications of cyclodextrins*, Med Res Rev, 14(3):353– 86.

**Szejtli J. 1996** – *Inclusion of Guest Molecules, Selectivity and Molecular Recognition by Cyclodextrins*, In Szejtli J & Osa T (Eds) Comprehensive Supramolecular Chemistry, Vol 3, p. 189-203, Pergamon - Oxford.

**Trellu C., Mousset E., Pechaud Y., Huguenot D., van Hullebusch E.D., Esposito G., Oturan M.A. 2016** – *Removal of hydrophobic organic pollutants from soil washing/flushing solutions: A critical review*, Journal of Hazardous Materials 306:149–174.

**Viglianti C., Hanna K., De Brauer C., Germain P. 2006** - *Use of Cyclodextrins as An Environmentally Friendly Extracting Agent in Organic Aged-contaminated Soil Remediation*, Journal of Inclusion Phenomena and Macrocyclic Chemistry, 56:275–280, DOI 10.1007/s10847-006-9094-1.

**Voiculescu A.R., Dumitru M., Toti M. 2003** – *Decontaminarea Solurilor Poluate cu Compuşi Organici*, Ed. SITECH, Craiova, ISBN 973-657-939-5.