PRELIMINARY DATA REGARDING BIOREMEDIATION OF SOILS POLLUTED WITH PETROLEUM HYDROCARBONS BY USING CYCLODEXTRINS

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Abstract

The main characteristics of the pollution caused by refineries and petrochemical plants is that the source of pollution is active, most of the time, for a short period of time, but has an important intensity, the pollutiong agent being usualy made up of limited pretoleum fractions. Also, in most of tha cases of accidental spills of petroleum products, the affected soil surface is much smaller than the contaminated surface of the first aquifer encountered by the pollution front. The crude oil resulting from the extraction, having a complex composition, acts on the soil depending on the amount, composition and properties of the organic and inorganic components contained. β-Cyclodextrin is a chemical substance with the molecular formula $C_{42}H_{70}O_{35}$*. Cyclodextrins were the first compounds studied in terms of complexation behaviour and catalytic properties, the objective being to mimic enzymes. The paper presents data concerning the soil physical, chemical and biological characteristics and chemical characteristics of crude oil that will be used in the Greenhouse experiment. Also, in the paper is achieved the state of the art for β-cyclodextrins used in bioremediation of soils polluted with petroleum hydrocarbons.*

***Key words**: bioremediation, petroleum hydrocarbons, polluted soils, cyclodextrins*

INTRODUCTION

Product and utility pipes that connect installations and appliances can be located above ground, in channels or buried. Breaking some seals or even the pipe leads to the penetration of part of the product into the soil. In the case of buried pipes, detecting these leaks is more difficult. Also, the aggression on the "Petrotrans" and "Conpet" pipelines, warehouses and loading - unloading areas of crude or finished petroleum products, resulting in hundreds of thousands of "small pollutions", the theft of communication cables of the same companies, which persist control of pumping installations, pollution with used oils discharged into sewers, riverbeds or directly on the ground are so many new phenomena that are added to the traditional sources of pollution that Romania faces. Due to the spills that occur following the attack of these pipes by thieves, the agricultural lands crossed by the perforated pipes are polluted, the water table is affected, there is a danger of explosions, fires or poisoning (Ivănuş et al., 2004).

Spent catalysts from technological processes can be sources of soil pollution if they are not properly stored. Although they are stripped when removed from the reactors, they still contain traces of petroleum products, and when they are stored directly on the ground, they can be washed away by meteoric waters and thus impure the soil and the subsoil. Compounds of heavy metals in catalysts can become pollutants with a cumulative effect over time. Meteoric waters can wash the oil products accidentally appeared on roads, platforms, loading-unloading ramps or in other places, and a part of these waters contaminated with oil products contaminates the soil and the subsoil (Neag, 1997, Sadler and Connel, 2003).

MATERIALS AND METHODS

The methodology is adapted to the type of experimentation in the laboratory, with representative experimental variants to highlight the proposed objectives. Determinations and sample collections will be made at different time periods, which reflect the dynamics of the disappearance of the pollutant from the soil, respectively at 1, 15, 30, 45, 60 and 90 days after the soil pollution and the application of the experimental technological links.

The experimental variants were made of controlled polluted soil with a concentration of 5% crude oil. Different doses of cyclodextrins were applied, respectively 0.2%, 0.6% and 1.2% of β-cyclodextrin and hydroxy-propyl-β-cyclodextrin.

The experience will include 7 experimental variants with 5% polluted soil, treated with 0.2%, 0.6% and 1.2% β-cyclodextrin according to the following experimental scheme:

- ✓ V1, soil polluted with 5% oil;
- ✓ V2, soil polluted with 5% oil + 0.2% β-cyclodextrin;
- ✓ V3, soil polluted with 5% oil + 0.6% β-cyclodextrin;
- ✓ V4, soil polluted with 5% oil + 1.2% β-cyclodextrin;
- ✓ V5, soil polluted with 5% oil + 0.2% hydroxy-propyl-β-cyclodextrin;
- ✓ V6, soil polluted with 5% oil + 0.6% hydroxy-propyl-β-cyclodextrin;
- ✓ V7, soil polluted with 5% oil + 1.2% hydroxy-propyl-β-cyclodextrin.

RESULTS AND DISCUSSIONS

The soil to be used in the laboratory experiment is a cambic chernozem from Teleorman county from the arable layer of 0-20 cm.

Chernozomies (Russian: black earths) are defined by a dark soft A horizon (Am) with chromas less than 2 in wet material, an intermediate horizon (AC, Bv or Bt) with values and chromas below 3, 5 to the material in a wet state, at least in the upper part (10–15 cm) and at least on the faces of the structural aggregates, followed by a horizon of accumulation of secondary calcium carbonates, present in the first 125 cm or in the first 200 cm, if the soil texture is coarse.

Chernozioms include the following subtypes: typical (Am-AC-Cca), psamic (chernoziom with a coarse texture in the first 50 cm), pelic (chernoziom with a fine texture at least in the first 50 cm), vertic (chernoziom with a vertical horizon located between the base of the horizon A and 100 cm), maronic (chernoziom with soft forest A horizon), gleic (chernoziom with gleic properties between 50 and 100 cm), calcareous (chernoziom with calcium carbonates from the first 50 cm), castanic (calcareous chernozioms having chrome colors equal to 2 in wet material), cambic (Am-Bv-Cca), argic (Am-Bt-Cca), Greek (Am-Ame-Bt-Cca), saline (chernoziom with a hyposalic horizon in the first 100 cm or salic horizon between 50 and 100 cm), sodic (chernozem with hyposodic horizon in the first 100 cm or natric horizon between 50 and 100 cm) (Ivănuş et al., 2004).

Cambic chernozems are leached chernozems, which are notable for the presence of the Bv horizon with wave and 3.5-wet and 5.5-dry chromes and the Am horizon with 2 chromes.

Cambic chernozems have a medium or medium-fine texture and are rarely sandy or clayey. The structure is glomerular, giving the soil a good permeability for water and air, but also average values of the hydrophysical indices (water capacity in the field and useful water capacity). The humus (3-5% in the soil) is of good quality "calcic mull", the degree of saturation in bases exceeds 85%, the soil reaction is weakly acidic or neutral, the pH values being between 6 and 8 (Tzimas, 2005).

Cambial chernozems have good fertility and are cultivated with cereals (wheat, corn), technical plants (sunflower, sugar beet), vegetables, vines and trees. The application of irrigation to supplement the water deficit in dry periods, the administration of organic and mineral fertilizers contributes to obtaining higher yields (Blaga et al., 2005).

The soil was analysed from a physical, chemical and biological point of view. It was subjected to some analyses and determinations:

- ✓ total petroleum hydrocarbons - gravimetric method according to SR 13511/2007;
- ✓ pH (H2O) – soil:water ratio 1:2.5 potentiometric;
- ✓ Organic carbon (humus) - Walkley-Black method, Gogoașă modification;
- ✓ Total nitrogen – Kjeldahl method;
- ✓ C/N ratio, by calculation;
- ✓ Mobile phosphorus (PAL) – Egnèr-Riehm-Domingo method;
- ✓ Mobile potassium (KAL) – Egnèr-Riehm-Domingo method;
- ✓ Quantitative and qualitative determinations of heterotrophic bacteria (NTB - total number of bacteria) and quantitative determinations of microfungi (NTF - total number of microfungi) - method of dispersing decimal dilutions of soil on solidified culture media: Topping and Czapeck.

Thus, the physical characteristics of the soil used in the experiment are presented in table 1.

Table 1 Physical characteristics of the soil used in the experiment (n = 3)

Soil type	Particle size fractions (mm) (% from the mass of the mineral part of the soil)				Carbonates %	Textural class symbol
	Corse sand	Fine sand	Dust	Clay		
	2.0-0.2	0.2-0.02	0.02-0.002	<0.02		
Cambic chernozems	2.1	25.0	26.6	46.3	0.0	AL

The soil has a clay-clay texture with a content of 2.1% coarse sand, 25.0% fine sand, 26.6% dust and 46.3% clay. The soil is devoid of carbonates.

The chemical characteristics of the soil used in the experiment are presented in table 2.

Table 2 Chemical characteristics of the soil used in the experiment (n = 3)

Characteristics	U.M.	Medium value
pH_{H2O}	-	8.09
Total nitrogen content	%	0.279
Organic carbon content	%	2.99
Humus content	%	5.16
C/N ratio	-	12.5
Mobile phosphorous content	$mg \cdot kg^{-1}$	50
Mobile potassium content	$mg \cdot kg^{-1}$	215

The soil material analyzed shows a slightly alkaline reaction (pH = 8.09); a small content of humus (2.99%); placed in the high class in terms of total nitrogen content (0.279%); with a high content of soluble phosphorus (P_{AL} = 50 $mg \cdot kg^{-1}$) and high content of soluble potassium (K_{AL} = 215 $mg \cdot kg^{-1}$).

Following microbiological analyses, the soil used in the experiment has a total number of heterotrophic bacteria (NTB) of 2.05 x 10^6 cfu/g soil. The soil presented three bacterial species and *Actinomycetes*, *Arthrobacter globiformis*, *Bacillus cereus*, *Pseudomonas* sp., genera of great value in realizing the biogeochemical cycles of matter in the soil. The value of the total number of fungi (NTF) was 30.86 x 10^3 cfu/g soil.

All these values are within the limits corresponding to a fertile soil.

On average, crude oil contains 85% C, 12-14% H, the rest is N, O, S, V, Co, Cu, P, K, I, Fe, etc. The oil is oily, florescent, dark brown or black with greenish or greenish yellow shades. There is also light-yellow oil, translucent, "white" oil found in Baku and Pennsylvania. Crude oil with water forms emulsions, it is insoluble in water and lighter than water, its density varying between 700 and 930 kg/m^3.

The density of the crude oil used in the experiment is:

$$\rho = 836{,}9 \text{ kg/m}^3$$

The total hydrocarbons extracted from the crude oil by the "cold" method with a non-polar solvent are then separated on a chromatographic column and determined by gravimetric analysis.

In order to achieve the separation of hydrocarbons into oil fractions from the obtained extract, a first phase of precipitation of asphaltenes is required following the interaction with a specific solvent. After precipitation, the solid phase is separated from the liquid phase by filtration; wash the filter very well and follow the drying operation until the constant mass of the fraction called "asphaltenes".

The method of separating total hydrocarbons is carried out by column chromatography, which consists in the adsorption of hydrocarbons on an activated stationary phase followed by their selective desorption, on petroleum fractions (aliphatic, aromatic and resins), by elution with solvents of different polarities and/or solvent mixtures. After this stage, each fraction is determined by gravimetry.

The content of each fraction (aliphatic, aromatic hydrocarbons, resins and asphaltenes) is expressed in mass percentages.

The data obtained from the analysis of the crude oil used in the laboratory experiment on petroleum fractions are presented in the following table.

Table 3 The content in petroleum hydrocarbons of crude oil

The content in petroleum hydrocarbons	U.M.	Medium value
Aliphatic hydrocarbons	%	31
Aromatic hydrocarbons	%	32
Resins	%	25
Asphaltenes	%	12

As can be observed, the concentration of aliphatic hydrocarbons is approximately equal to that of aromatic hydrocarbons, 31% and 32% respectively, followed by resins 25% and asphaltenes 12%.

β-Cyclodextrin is a chemical substance, the molecular formula is $C_{42}H_{70}O_{35}$. The white crystal, in water, is relatively easy to crystallize.

The solubility in water is relatively low at 1.85% at room temperature and increases with increasing temperature. It is not hygroscopic, but easily forms a stable hydrate. The degree of hydration between 50–70% relative humidity is equivalent to 10–11 water molecules per β-Cyclodextrin molecule (13.7–14.8% water content), and the humidity isotherms are two phases.

Insoluble in organic solvents in general, but may be slightly soluble in pyridine, dimethylformamide, dimethylsulfoxide and ethylene glycol.

Widely used in the separation of organic compounds and for organic synthesis, but also for medical excipients, food additives, there are natural cyclodextrins and modified cyclodextrins, and some do not have biocompatible molecules with drugs prepared in the inclusion complex. It not only increased the biocompatibility of drugs but also played a role in slow release.

Because the outer edge of cyclodextrin is hydrophilic and the cavity is hydrophobic, it can provide a hydrophobic binding site as an enzyme, as a host guest, in which organic molecules, inorganic ions and gas molecules are the guests. Its hydrophobic - hydrophilic and external - internal characteristics make it possible to form molecular integration and assembly systems with many organic and inorganic molecules, according to the van der Waals force, the hydrophobic interaction force, the matching effect between host and host molecules, and becomes chemical research.

Cyclodextrins were the first compounds studied in terms of complexation behaviour and catalytic properties, the objective being to mimic enzymes. The first publication appeared in 1891, when Villiers reported the isolation of two different types of crystalline compounds from the degradation of starch treated with Bacillus amylobacter bacteria. Villiers characterized these compounds by their physical properties and named them "cellulosins". A few years later Schardinger published more physical data relative to these compounds. He continued with the isolation of the bacterium responsible for the formation of cyclodextrins (Bacillus macerans) which is today the most used source in the world for the production of cyclodextrins. Because of his pioneering work, cyclodextrins are often referred to as Schardinger dextrins. Other names, such as those from the old literature, are cycloglucans, cyclomatlooligosaccharides and cycloamyloses (Szejtli, 1988).

Cyclodextrins do not have terminal reducing groups. In general, they show the characteristic reactions of non-reducing sugars; they therefore give the color reaction with anthrone, which can be used for their quantitative determination (Szejtli, 1988).

Partial acid hydrolysis of cyclodextrins leads to glucose and a series of acyclic malto-saccharides. This series is completed by oligosaccharides that contain as many glucose units as the original cyclodextrins.

The stability of the intact ring to acid hydrolysis is two to five times higher, depending on temperature and acidity, than that of acyclic dextrins. The opening of the cycle, by splitting the first glycosidic bond, is a slower process than the hydrolysis of the malto-oligosaccharides thus formed.

During the hydrolysis of oligo- and polysaccharides, the glucosides bonds of the terminal glucose units are split faster than the bonds between the non-terminal members. Since cyclodextrins do not contain terminal glucose units, it follows that the activation parameters of the 1,4-a-glucosidic bonds forming the ring must be different from those of the analogous bonds in an acyclic dextrin (Szejtli, 1988; Venema, 1996).

This association (host / guest) is maintained by hydrophobic intermolecular interactions similar to those encountered in many biological systems (enzyme / substrate, antigen / antibody). Therefore, it rarely happens that water-soluble molecules associate with cyclodextrin. The main requirement for this pairing is that the guest has an appropriate size and shape to fit into the empty space of the host. There are different compound inclusion classes:

- ✓ Polymolecular inclusion compounds - the host consists of a network of several free bound molecules that form a channel (urea, cholic acids) or a cage (hydroquinone), trapping a guest molecule.
- ✓ Macromolecular inclusion compounds and "molecular sites" - the host presents a three-dimensional crystalline side capable of retaining molecules. These host compounds, widely used in industry, consist of zeolites, dextrans, polyacrylamide, agarose, silica gels.
- ✓ A particular case of inclusion is the blue iodine solution product - the blue color in the iodine / starch reaction results from the attachment of a polymerized chain of iodine through the starch helix.
- ✓ Monomolecular inclusion compounds or molecular encapsulation - The host is a molecule characterized by the presence of a cavity in which the guest molecule is included.
- ✓ The advantages of inclusion complexes
- ✓ There are numerous potential applications of cyclodextrins, and in

particular betacyclodextrins, in the pharmaceutical field:

- ✓ Improving the solubility of compounds with low water solubility, facilitating greater bioavailability of drugs (when solubility or dissolution rate are the limiting factors of bioavailability).
- ✓ Increasing the shelf life of expensive flavors and perfumes by encapsulating volatile compounds.
- ✓ Control of the release of drugs, perfumes or flavors.
- ✓ Improving the organoleptic properties of medicines and obtaining tastier preparations.
- ✓ Avoiding incompatibility between active substances in the same formula.
- ✓ Stabilization of reactive ingredients against oxidation, hydrolysis.
- ✓ Stabilization against temperature degradation, hydrolysis, oxidation, photolysis.
- ✓ Improving fluidity and compressive behavior of active compounds.
- ✓ Transforms liquid assets into solid complexes that are easy to handle.
- ✓ Betacyclodextrin can also be used as a manufacturing aid for the extraction of unwanted compounds (eg cholesterol in butter).

CONCLUSIONS

The soil has a clay-clay texture with a content of 2.1% coarse sand, 25.0% fine sand, 26.6% dust and 46.3% clay. The soil material analysed shows a slightly alkaline reaction; a small content of humus; placed in the high class in terms of total nitrogen content; with a high content of soluble phosphorus and high content of soluble potassium. Following microbiological analyses, the soil used in the experiment has a total number of heterotrophic bacteria (NTB) of 2.05 x 10^6 cfu/g soil. The soil presented three bacterial species and *Actinomycetes*, *Arthrobacter globiformis*, *Bacillus cereus*, *Pseudomonas* sp., genera of great value in realizing the biogeochemical cycles of matter in the soil. The value of the total number of fungi (NTF) was 30.86 x 10^3 cfu/g soil. All these values are within the limits corresponding to a fertile soil.

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