Asphaltenes Biodegradation in Biosystems Adapted on Selective Media

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The aims of this paper was to test the bioremediation capacity for three neutralization media over waters polluted with petroleum residues, a special attention being paid to asphaltenes biodegradation. To prepare neutralizing media there were used as raw materials three biological media: hump, biological sludge and chemosorb. The biodegradation degree is influenced by several factors, their monitoring in the analyzed systems offering data about microbiological behavior of microorganisms in these media and revealing the system with the best performance for bioremediation of waters polluted with oil residues.

Keywords: asphaltenes, biological sludge, oil residues

Biodegradation is natural phenomena with high impact in environment chemical equilibrium recovery, representing the metabolic capacity of microorganisms to convert or to mineralize organic pollutants in substances with lower harmful effects by reducing the hazard. Industrial wastes, regardless of the circumstances that produced them, have a negative impact on the environment and on the human health also. The oil industry, as well the drilling as the refining process, is part of the considerable share in environmental pollution, since all stages of technological processes related components industry run in a closed system and continuously and products, waste and residues in this industry can generate remanence in the environment, with negative consequences.

Petroleum residues resulting from specific activities of oil industry are: oil refining residues, sludges from desalination, sludges from tanks, oily residues, oily sludges from maintenance operations of the plant and equipment, etc. The storage of oil residues from oil refining may be done knowing at micro-scale the effects on the environment over time. The applied laboratory tests come to complete the data concerning storage of these residues, preventing pollution risk.

Valorization of oil residues is a necessity due to the pollution they bring to the environment. There is therefore the opportunity to be harnessed in imposed conditions to protect the environment by using them as fuel substitutes in other industries. If is not possible the valorization of oil residues in order to obtain an interesting product for petrochemistry, finding biodegradation solutions is desirable to reduce the impact on the environment.

Nowadays, extracted crude oils are becoming increasingly rich in character asphaltic compounds, which is why thereof residues from processing have higher content in asphaltenes. These create problems both in the stage of oil recovery from reservoirs and in valorization and storage stages. Asphaltenes are compounds with asphaltic character, with high molecular weight, which contain beside carbon and hydrogen, and heteroatoms as nitrogen, oxygen and sulfur, and metals. From structural point of view asphaltene molecules are made up of naphthenic and aromatic rings, condensed with heterocycles and having paraffinic chains attached. This complex structure makes asphaltenes to be hardly biodegraded [1].

Between the methods used to remediate the waters pollutes with petroleum products there are oil – water separator as mechanical method, using different surfactants as chemical method or using different neutralization media for biodegradation of pollutants as biological method [2-5]. This latest method seems to become widely used, because it turns out to be the least harmful to the environment, not expensive, and with good performance in polluted waters remediation, even if the process takes longer. Moreover, the aerobic activity of natural microorganisms population is the primary mechanism for organic pollutants elimination, as many studies show [2, 4-7].

The literature presents several studies for using microorganism to biodegrade or to mineralize the asphaltenes. Pineda – Flores et co. drafted a microbial consortium able to mineralize asphaltenes form crude oil, at room temperature, but on weak saline and almost neutral polluted waters [8]. In addition, during the mineralization process the micellar structure of asphaltenes is destroyed and thereby decreases the amount of CO_2 released without metabolic activity of microorganisms. Tavassoli et co. have isolated five microorganisms with high growth rate on asphaltenes [9]. Among the studied microorganisms, Bacillus lentus TMU5-2 showed the greatest capacity of asphaltene degradation – 46%, almost near by the activity of a mixture of five similar bacteria – 48%. The predominant genera of isolates microorganisms which use asphaltenes as a sole source of carbon and energy are Pseudomonas, Acinetobacter, Alcaligenes, Flavo-bacterium, Citrobacter amalonaticus, Enterobacter cloacae, Staphylococcus hominis, Bacillus cereus and Lysinibacillus fusiformis [10 - 12]. These showed a good activity in asphaltenes biodegradation, even though it was weaker than the degradation rate of other lighter components of crude oil.

In this paper we studied the possibility to remediate waters infested with heavy petroleum products by using various neutralization media prepared from hemp, biological sludge from a bioremediation plant and chemosorb. The microbiological media were contacted in optimal working conditions (temperature, oxygen saturation, nutrients etc.) on the same exposure time on several toxic pollutants. The obtained results were summarized for finding the media with the best performances in petroleum residues biodegradation, in order to valorize the biodegradation potential in presence of these contaminates. We choose to pay more attention to biodegradation of asphaltenes because these compounds have a complex structure, hardly biodegradable, blocking the crude oil extraction and transport pipes, involving sever problems at transport and storage. Furthermore, the complex structure of asphaltenes

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Stage	System 1	System 2	System 3
	Oil residue, %		
Day 1	2.5	2.5	2.5
Day 5	5	5	5
Day 10	7.5	7.5	7.5
Day 15	10	10	10

Table 1
SYSTEMATIZATION OF OIL RESIDUE DOSAGE STAGES

molecules makes them more resistant to biodegradation and produces accumulations of asphaltenes in places. On the other hand, a good activity in asphaltenes biodegradation means that other lighter components of crude oil are biodegraded at high rate. The other compounds present in petroleum residues were not analyzed in this study.

The degree of biodegradation is influenced by several factors. In analyzed systems were monitored the nutrients proportion in these three media, the amount of dissolved oxygen, *p*H and the evolution on asphaltenes content.

Experiemntal part

Materials and methods

In order to prepare the artificial infested water, in this study was used a petroleum residue from one refinery from Romania, from atmospheric distillation of a paraffin-naphthenic crude oil, with a moderate content in asphaltic compounds. This residue was characterized from physical – chemical point of view (density, pour point, flash point, suphur content and asphaltenes content).

There were used three biological media. The first one was prepared in our laboratory, starting from hemp stems, with 10-12% fibers content, provided by local growers. The second biological media was biological sludge used in bioremediation plants, and the last one was Chemosorb provided by Supelco. The water used to prepare the artificial polluted water was also analyzed.

In the first culture media, named System 1, has been tested a mixture formed by 100g dry hemp as biomass and 500 mL waste water, rich in nutrient. The waste water was filtrated before blending. In the second culture media, called System 2, it was tested 100 mL mixture of biological sludge, adapted biomass from petroleum industry, having sludge index I_n = 150 mL/L and 500 mL waste water, filtered before. The test media was enriched in nutrients. In the last culture media, named System 3, was made by Chemosorb, 50 g/L in 500 mL waste water, filtered before, and enriched in nutrients.

For the development of bacterial media in all three systems were added nutrients, essential for bacterial growth: nitrogen, phosphorus, potassium, iron, calcium in doses of 10 mg/L, and each element being dosed once daily throughout the experiment. As a source of nutrients have been used aqueous solutions of Na_3PO_4, $NaNO_2$ and KI in appropriate concentration to provide the desired amount of nutrients. During the nutrients dosing in the systems was monitored *p*H variation. In the same time was studied by microscope the bacterial development in presence of contaminates.

The tests to adapt biological media to favorable conditions to biomass developing and microbiological evolution in analyzed media, in the presence of oxygen, dosed continuously with a compressor system, lasted 20 days. After adapting time there were made microscopic tests to determine the adaptability of microorganisms to the prepared media.

After microorganisms adapting time to the nutritive media created in our laboratory were dosed, in each system, 2.5; 5.0, 7.5 and 10.0 mL of oil residue (table 1). Dosing was made during 20 days, dosed 2.5 mL once every 5 days, in the mentioned amount, in the same time and with respecting the temperature and oxygen conditions. During experiments were made measurements regarding *p*H value and nutrients level, microscopic and asphaltenes biodegradation evolution tests for all three studied media. Microscopic examinations were made in order to evaluate the activity of formed microorganisms in analyzed systems in contaminants presence, by using a MOTIC B1-223ASC Trinocular Super Contrast Compound microscope and the images are recorded with MOTICAM 352 camera.

Before each addition of petroleum residue, from each system was taken samples from the organic phase and chemical analyzes were carried out, in order to determine asphaltenes content after each stage of biodegradation. The asphaltenes content was determined by extraction, using Soxhlet extraction apparatus in a large excess of n-heptane (Sigma – Aldrich, 99.0%). After the precipitation, the suspension was filtered using Whatman No.42 filter paper and the asphaltenes phase was dried at 70°C.

Results and discussions

For the analysis carried out to the oil residue and for the water used to prepare artificial infested water, the experimental results are depicted in tables 2 and 3.

The measured parameters for used water are at the limit of values recommended by standard.

The hemp fibers have length cells between 3 to 55 mm (the average being 20 mm), with thickness between 5 to 50 μm (the average being 20μm) and density in the range

Properties	Value	Method
Density, (at 20°C, kg/m³)	863.5	EN ISO 3675
Refractive index, n_D^{20}	1.4980	ASTM D-1218
Freezing point, (°C)	-1	ASTM D-2500
Pour point, (°C)	2	ASTM D-2500
Flash point,(°C)	71	ASTM D-93
Sulphur content, (wt%)	0.70	ASTM D-2622
Asphaltenes content, (wt%)	5.57	ASTM D3279 - 12

Table 2
THE CHARACTERISTICS OF OIL RESIDUE

Properties	Value	Method
pH	8.5	STAS 612-325-75
Chemical oxygen demand CCO-Cr, (mg/l)	150	SR ISO 6060-1996
Biochemical oxygen demand CBO_5, (mg/l)	25	STAS 6560-82
Particles in water, (mg/l)	35	STAS 6953-81
Ammonia nitrogen, (mg/l)	10	STAS 8683-70

Table 3
THE PROPERTIES OF WASTE WATER

Day	pH value		
	System 1	System 2	System 3
Initial	6.5	6	7
After 5 days	7	6.5	7
After 10 days	7	7.5	7
After 15 days	7.5	8	7
After 20 days	7.5	7.5	7

Table 4
*p*H VARIATION OVER NUTRIENTS DOSAGE

(a) (b) (c)

Fig. 1. The culture media before adapting stage
(a)hemp; (b) biological sludge; (c) chemosorb

1.43 – 1.48 g/cm^3. The chemical composition of hemp fibers shows: 77% cellulose, 9.31% pectic substances; 8.08% water; 0.56% waxes and fats; 1.57% minerals; 3.48% soluble [13-15]. All culture media are presented in figure 1.

The microscopic tests were carried out after adapting time in order to determine the adaptability of microorganisms to prepared media. Tests showed the sludge agglomerates appearance, the development of Zoogleale mass (formations with 0.07 mm length and 0.02 mm width) and the presence of microorganisms – indicators for nutrient medium stabilization - Paramecium sp.

At the beginning of the experiment, for the system 1 there are not present microorganism agglomerations in the analyzed samples, as can be seen in figure 2.

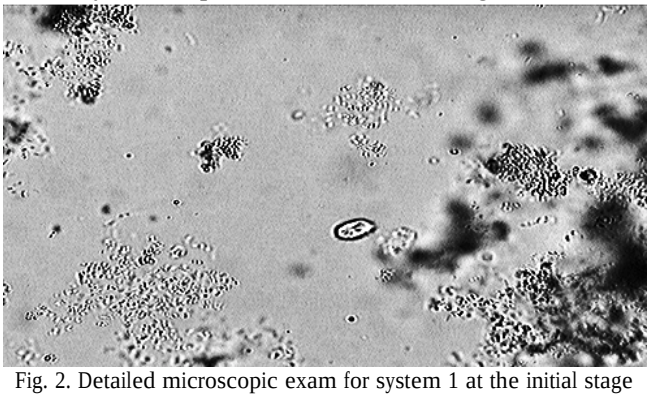

Fig. 2. Detailed microscopic exam for system 1 at the initial stage

After adding nutritive factors, the bacterial growth was accelerated (fig. 3). By adding nutrient culture media, pH value was raising slightly, most pronounced increase being observed for system 2 (table 4). Initially, this system is slightly acid, and after 20 days the *p*H value passes into

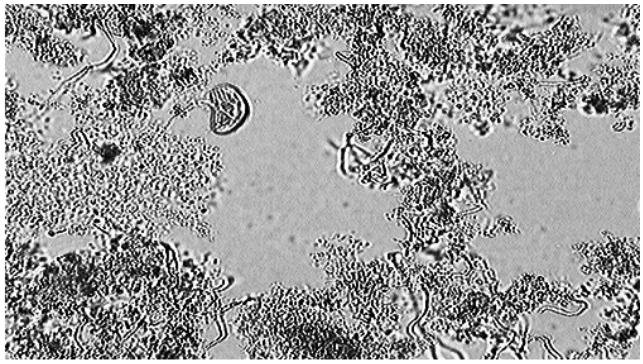

Fig. 3. Detail regarding the adaptability and zoogleale mass forming in system 1, at the end of experiment

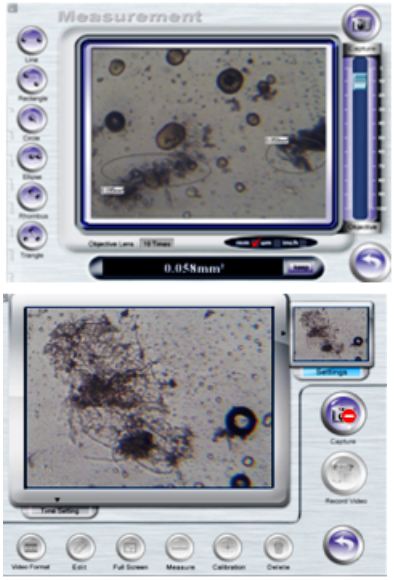

Fig. 4. Microscopic view of microbiological formations during the dosing period of contaminant (system 1 at the beginning and end of the experiment)

alkaline medium, that fosters the growth of microorganisms.

The experiments have shown a gradual increase of petroleum residue biodegradation degree. Compared to the first images viewed in adapted media without oil residue, now the images are clear, bacterial activity is intense, they formed a large number of formations, with larger size of conglomerates, having 0.83 mm length and 0.60 mm width (fig. 4). Asphaltenes concentrations after three sets of analyzes were recorded in table 5.

On the first stage, the decrease of asphaltenes content in system 1 has a significant speed, and then the

Analyzed system	Asphaltenes concentration, %		
	Stage 1 (after 5 ml biodegraded residue)	Stage 2 (after 10 ml biodegraded residue)	Stage 3 (after 15 ml biodegraded residue)
System 1	0.92	0.24	0. 09
System 2	1.18	0.33	0.12
System 3	1.62	0.38	0.28

Table 5
THE ASPHALTENES CONCENTRATIONS AFTER GRADUAL BIODEGRADATION IN ANALYZED SYSTEMS

biodegradation rate decreases gradually. The high biodegradation speed of asphaltenes at the beginning of the experiment it may be due to degradation of lateral aliphatic chains, more susceptible than the ring structures. Moreover, lateral chains are more exposed to bacterial attack.

After a given period of contacting contaminated water with clean up environment the system set adaptation of microorganisms, bacterial activity is more intense, which generates a large number of microbiological formations. Therefore the asphaltenes concentration after 15 day after beginning of the experiment has low values, even the aromatic condensed structures from asphaltenes are harder to be degraded. The agglomerations of bacteria with the formation of Zoogleea ramigera have varying sizes, most frequently having 0.83 mm length and 0.60 mm of width.

This process of adaptation and, by default, the development of the bacterial population in the presence of asphaltenes remains the most powerful, the explanation being offered by the structure of the culture medium.

The presence of cellulose fibers favors Zoogleal mass population growth, which promote, by chemical reactions, the asphaltenes biodegradation. Zoogleea ramigera is the structural unit of the activated sludge, able to biodegrade the chemical contaminates. Seen under the microscope, it appears as a gelatinous mass, secreted by bacteria that have adapted to environmental conditions and in the presence of asphaltenes. Probably, the overcrowding bacteria include inert inorganic and organic substances and other microorganisms that develop through, e.g. ciliated Paramecium caudatum. This microorganism favors asphaltenes biodegradation. On the other hand, because alternating of aeration periods with rest periods during the experiment, anaerobic microorganisms can occur. In the same time, during biodegradation, the micellar structure of asphaltenes is unfolded and heteroatoms as oxygen, nitrogen and suphur are more accessible. These heteroatoms are, probably, an abundant potential source of electron donors for anaerobic metabolism, promoting the mechanism of anaerobic organisms' development having oil residue as the sole source of carbon and energy

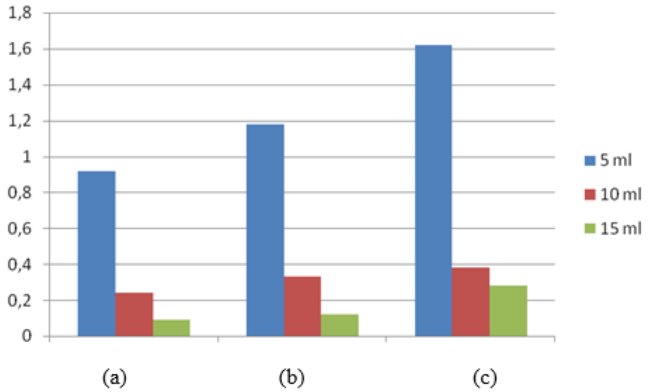

Fig. 6. The evolution of asphaltenes concentrations in the three systems during experiments (a)hemp; (b) biological sludge; (c) chemosorb

and helping thereby increasing the biodegraded aspaltenes amount [16].

If initially the proposed new system has a higher inertia as compared to conventional systems, by increasing the period of contacting contaminated water with clean up environment, the system set adaptation of microorganisms and the concentration of asphaltenes decrease gradually under 0.1%.

Conclusions

The biodegradation of petroleum residues can be accelerated through the use of microorganisms already existing in natural medium and which can be adapted to analyzed contaminant (asphaltenes form petroleum residues) by using growing nutritive agents.

The efficiency of biological method used for de-contamination is influenced by the procedures for combining multiple elements: the characteristics of the environment undergone to remediation, knowledge about existing contaminants and their biodegradability, the choice of the oxidant, nutrients and microorganisms used.

Following carried out tests it can be seen that residual oil, namely asphaltenes, are biodegraded by the system 1, made from natural biodegradation media, consisting of hemp, when nutritive factors promoting bacterial growth are present.

On first stage, the decrease in the percentage of asphaltenes content in this system has a significant speed, and then the biodegradation rate decreases gradually. As time passed and the system set adaptation of microorganisms, bacterial activity is intense, which generates a large number of microbiological formations. The size of the agglomeration of bacteria with the formation of Zoogleea ramigera has varying sizes, most frequently having 0.83 mm length and 0.60 mm of width.

This process of adaptation and thus the development of the bacterial population in the presence of asphaltenes remains the most powerful, the explanation being offered by the structure of the culture medium.

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Initially, the asphaltenes degradation yield in the system with biological sludge and with chemosorb is lower compared to the biosystem with hemp. After the adaptation period of a population of microorganisms in biological sludge to new environmental conditions, the bio-

degradation in the system with biological sludge is reasonably high. Thus, the yield of asphaltenes biodegradation is close to the yield obtained for the system with hemp.

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