BIOREMEDIATION OF OIL POLLUTED SOILS BY INDIGENOUS BACTERIA AT SUPLACU DE BARCAU, ROMANIA

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Abstract. *Bioremediation is the enhancement of live soil organisms as fungi, bacteria and plant to break down hydrocarbon and organic contaminants. For achieving the bioremediation method one took soil samples with different concentrations of petroleum products, from different parts of the petroliferous exploitation Suplacu de Barcau. Out of these collected soil samples, the microorganisms were isolated and analyzed all their characters and for identification were used the Galleries API 20E, 20NE and 20Strep. The obtained pure cultures were tested on agar media enriched with different concentrations of crude scaffolding oil, as the sole source of carbon. The bacteria that were validated in this study constituted then the bacterial consortium used for bioremediation. This consortium was applied on polluted soil with initial different TPH concentration. Regarding the initial concentration of mineral oils and extractable petroleum products, this varies in a very large range but during the whole bioremediation process, one noticed a decrease of the petroleum products concentration.*

KEYWORDS: BIOREMEDIATION, OIL POLLUTED SOILS, BACTERIA

1. Introduction

Petroleum hydrocarbons (PHC) released into the environment can pose risk to ecosystems and human health. The hydrocarbons (PHC and PAH - polycyclic aromatic hydrocarbons) are a class of toxic pollutants that have accumulated in the environment due to both natural and anthropogenic activities (Kästner, 2000). They are mainly produced from incomplete combustion of organic materials, fossil fuels, petroleum product spillage and various industrial activities, and partly also from natural processes such as forest fires and volcanic eruptions. Some compounds in petroleum products are known to be mutagenic and carcinogenic. Petroleum and its product enter the soil via crude oil pipe leakages, oil tank ruptures and indiscriminate disposal of refinery products leading to changes in soil properties (Trofimov and Rozanova, 2003). Crude oil is a highly complex mixture of hydrocarbons amounting to hundreds of individual compounds with different chemical structure and molecular weight plus a series of lower molecular weight compounds other than hydrocarbons (phenols, thiols, naphthenic acids, for example heterocyclic compounds with N (pyridines, pyrrole, indole etc.), compounds with S (alkyl thiols, thiophene, etc.). Soils polluted with petroleum hydrocarbon (PHC) are low in fertility and hence, do not support adequate crop growth and development (Abii and Nwosu, 2009).

Bioremediation is a widely accepted method of remediation because it is an environmentally friendly method that requires less cost and techniques. To a large extent, bioremediation equally accomplishes complete clean-up of the polluted soil. When compared with other conventional remediation techniques, results have shown that bioremediation reduces PHC concentrations in polluted soils with minimum site disturbance and at lower costs (Buzea and DeStefanis, 2001). Bioremediation is the enhancement of live soil organisms as fungi, bacteria and plant to break down hydrocarbon and organic contaminants. Many microorganisms have the ability to use gaseous hydrocarbons, liquid and solid aliphatic series, asphalt and aromatic as the sole source of carbon and energy and they decompose these in water, CO2 and lower molecular weight compounds. Bacteria and fungi are the main microorganisms responsible for biodegradation of petroleum hydrocarbons (Abbassi and Shquirat, 2008). Generally, the biological methods for the remediation of petroleum hydrocarbons are based on the cooperation of more bacterium species because a pure culture of bacteria has not the metabolic ability to easy degrade certain compounds or it does not have the necessary biomass needed to degrade fast enough the toxic compounds.

The objective of our study was to isolate and identify species of indigenous bacteria from the polluted soils of the petroliferous exploitation site Suplacu de Barcau, Romania, to test their biodegradable capacity *in vitro* and *in vivo*.

2. Material and methods

There were randomly collected 6 soil samples at depth of 0-15 and 30-60 cm as follows: 4 points from the petroliferous site, 2 points from sludge sediment and 1 point form unpolluted soil (as control). Subsamples of 1g were suspended in 99 ml of 0.1% saline solution, agitated on a water-bath shaker (100 rpm at 28^{o}C for 30 min). A serial decimal dilution was performed to 99 ml of 0,1 % saline solution. Aliquots of 0.1 ml from each dilution were transferred on different types of agar medium: Topping, Nutrient Agar (Merk), Thornton, Pseudomonas agar F Base and Pseudomonas agar P base. The morphological characterization of each isolate was first performed by noticing cultural, morphological and tinctorial characters (color, size, colony characteristics - form, margins and elevation and Gram staining reaction). The microscopically examination revealed bacilli, coccobacillus and cocci Gram stained positive and negative. For identification there were used biochemical tests with different API Galleries (20E, 20NE and 20Strep). The bacteria were tested in vitro for the biodegradable capacity on mineral medium Difco Bushnel-Hass enriched with different quantities of oil from Suplacu de Barcau (2ml/l – mineral medium I and 5 ml/l – mineral medium II). The bacteria that were validated in this study formed the bacterial consortium used for bioremediation. This consortium was applied on polluted soil from Suplacu de Barcau, with initial different TPH (total petroleum hydrocarbon) concentration in two experiments. In the first experiment were collected 7 samples of polluted soil form Suplacu de Barcau and for the monitoring of the bioremediation were used flasks in 3 replicates. In every flask was introduced 100g polluted soil, inoculated it with 20 ml bacterial consortium and agitated 24 hours for homogenization. The TPH content was monitored during the bioremediation process at 8, 18 and 24 weeks. In the second experiment of 175 days were collected 5 types of polluted soils from Suplacu de Barcau area. 10 kilo of each soil, in 3 replicates were placed in transparent boxes and inoculated with 2000 ml of bacterial consortium.

Before applying the consortium, in both experiments, at all the soil samples were determined the physicochemical properties and the TPH (1-st experiment), mineral oils (2 experiment) and EPH (extractable petroleum products – 2-nd experiment) by GC-FID and FTIR methods of the soil samples collected for bioremediation.

3. Results

We`ve isolated 15 bacterial strains. The bacteria isolated from the polluted soil of Suplacu de Barcau which showed good and very good results, in the terms of their ability in the biodegradation of hydrocarbons consisted of species of the following genera: *Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Brevibacterium, Clostridium, Corynebacterium, Flavobacterium, Mycobacterium, Micrococcus, Pseudomonas and Streptomyces.* The microbial consortium was created of these bacteria. We can see in table 1 and figure 1 the in vitro testing of the bacteria ability to degrade the crude oil from Suplacu de Barcau.

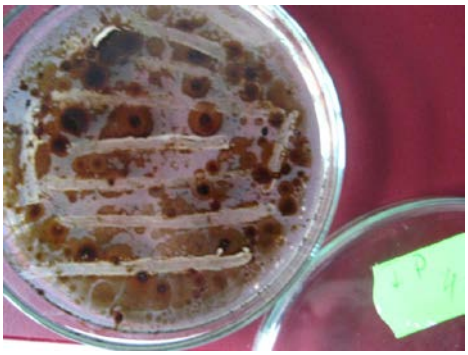

Figure 1. In vitro testing bacteria ability of oil degradation

Table 1. *The evolution of bacterial strains on mineral media enriched with crude oil*

Bacterial strains	Mineral medium I		Mineral medium II	
	without oil	with oil	without oil	with oil
1	+	+	+	+
2	+	+	-	-
3	+	+	+	+
4	+	+	-	+
5	+	0	-	0
6	+	+	+	-
7	-	+	-	+
8	+	+	+	+
9	+	+	0	+
10	+	0	0	0
11	+	+	-	+
12	+	+	-	+
13	+	+	-	+
14	+	+	0	0
15	-	+	-	+

This consortium was applied in flasks, on polluted soil with initial different TPH concentrations. The initial concentration of crude oil varies in a very large range, from 8850 to 73700 (in sludge sediment) exceeding the intervention threshold (2000 mg/kg) for soils with less sensible usages as we can observe in table 2.

Table 2. *The evolution of TPH (mg/kg) in polluted oil soil samples under the influence of bioremediation (experiment 1)*

Sample	TPH (mg/kg) after:			
	0 week	8 weeks	18 weeks	24 weeks
S1	46600	44300	27000	22000
S2	37600	35000	19000	14000
S3	21500	21000	5700	5700
S4	8850	5500	1400	1200
S5	21500	19400	13700	9750
S6	9670	7800	1500	1400
S7	73700	69000	66000	62000

Along the bioremediation process in all the variants the TPH decreased with the increasing of the contact time between the soil and the microorganisms.

For studying the dynamics of the TPH we`ve calculate the percentage of TPH remained in samples after every stage of bioremediation, considering 100% the initial concentration of TPH in every sample. As we can see in table 3 in soils with TPH between 0-10000 mg/kg (S4 and S6) the concentration decreased with 85%. In samples with TPH between 10000 – 50000 mg/kg (S1, S2, S3, and S5) the oil content decreased variable between 26 and 47%. The smallest decrease (15%) was found in sludge sediment.

Table 3. TPH percentage remained in polluted soil samples during the bioremediation

Sample	TPH percentage after:			
	0 week	8 weeks	18 weeks	24 weeks
S1	100	95.1	57.9	47.2
S2	100	93.1	50.5	37.2
S3	100	97.7	26.5	26.5
S4	100	62.1	15.8	13.6
S5	100	90.2	63.7	45.3
S6	100	80.7	15.5	14.5
S7	100	93.6	89.6	84.1

The dynamics of this process is presented in figure 2. We can observe that after a period of adaptation between the components of the consortium and with the new source of nutrition (weeks 0-8) when the decrease of TPH was slower, follows a period of intense decrease (weeks 8-18). In the last period of bioremediation, weeks 19-24, the decrease was slower again, maybe because of the low volume of soil.

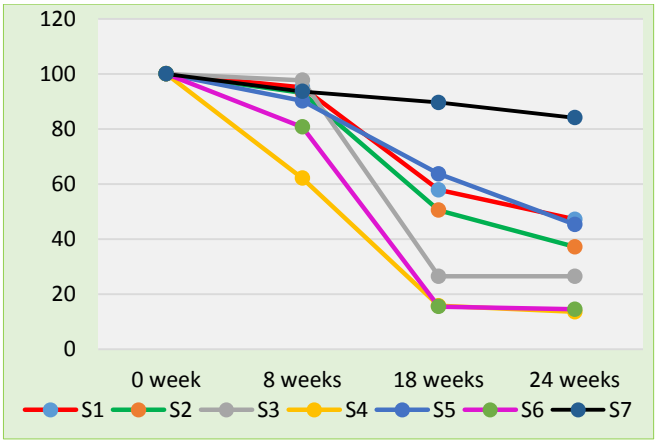

Figure 2. *The variation of TPH from polluted soil samples during the bioremediation period (experiment 1)*

Table 4. *The evolution of mineral oils content (mg/kg) in polluted oil soil samples under the influence of bioremediation (experiment 2)*

Sample	Mineral oils (mg/kg) after:			
	0 day	63 days	125 days	175 days
S1	47667	42333	35000	31000
S2	28667	26333	23667	19667
S3	16000	13667	13500	10667
S4	3733	3267	2933	2833
S5	7333	6933	6733	6267

Data regarding the second experiment of bioremediation are presented in table 4 (mineral oils) and 5 (EPH) and figure 3 (mineral oils) and 4 (EPH).

In table 4 we can observe that the initial mineral oils content varies between 3733 and 47667 mg/kg and decreases constantly in all the soil samples.

The evolution of the bioremediation process, presented in figure 3, show us a continuous decrease of the mineral oils content. This decrease is more pronounced in the samples with higher initial concentration of oil. After 63 days from inoculation the content of mineral oils decreases with 2-12%. In the next stage the decreasing is slow too, in 4 variants with the exception of variant S1 where the decrease is of 32%. In the last stage of bioremediation, after 50 days the decrease is more pronounced, between 29 and 46%. So, after 175 days of bioremediation the content of mineral oils decreased with 42-66% and the highest decrease was in the sample with maximum level of contamination – S1.

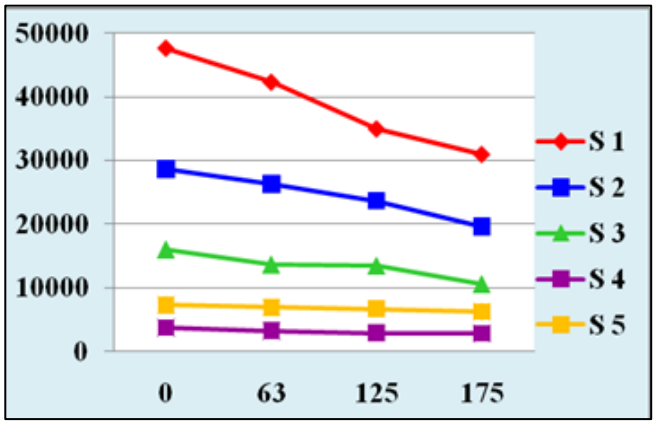

Figure 3. *The variation of mineral oils (mg/kg) content during the bioremediation process (experiment 2)*

The levels of the EPH as we can see in table 5 and figure 4 are lower than those of the mineral oils in all the samples. The evolution of the EPH is characterized by constant and slow decrease in the first 125 days as we could see at the evolution of the content of mineral oils but after 175 days the decrease is significant.

Table 5. *The evolution of EPH (mg/kg) in polluted oil soil samples under the influence of bioremediation (experiment 2)*

Sample	EPH (mg/kg) after:			
	0 day	63 days	125 days	175 days
S1	32000	31233	29667	11333
S2	16967	15300	12933	6167
S3	10800	9733	6467	3333
S4	3500	3367	2233	1143
S5	4867	4300	4267	2833

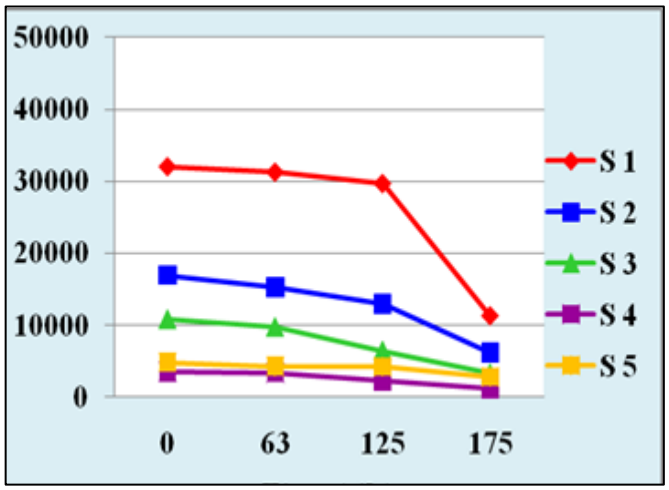

Figure 4. *The variation of EPH (mg/kg) during the bioremediation process (experiment 2)*

4. Conclusion

Generally, the biological methods for the remediation of petroleum hydrocarbons are based on the cooperation of more bacterium species which use the crude oil hydrocarbons as carbon source.

In the first experiment because of the low quantity of soil the bioremediation capacity of the consortium leveled in the last stage.

In the second experiment the bacterial consortium isolated from the polluted soil determines a decrease of the content of mineral oils with 15-30% and of the EPH with 30-60%. To decrease the values of TPH under the intervention threshold (2000 mg/kg) we can either continue the process of bioremediation or we can increase the added quantity of consortium/ soil unit.

6. Literature

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