Bioremediation Use in Order to Decontaminate Polluted Soils Infested with Liquid Hydrocarbons

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Soils and subsoils decontamination technologies using microbiological processes are based on the ability of microorganisms to assimilate the existing compounds from the petroleum pollutant. Biologically applied decontamination processes may be executed ex situ and in situ. To increase the efficiency and speed of decontamination we can combine it with physico-chemical methods which does not involve extra costs. This paper presents the results of the experimental research on bioremediation technologies, where were used microbiological preparations and adjuvants existing on the market.

Keywords: bioremediation, contaminated site, surfactants, bioreactors, microbiological preparations.

Regardless the application manner, in situ or ex situ, the bioremediation technologies require the following steps [3-5]:

- establishing the pollution level of the area subject to remediation;
- pedo-ameliorative works (excavation, crushing);
- laboratory measurements to determine the type and quantity of the vegetable, adsorbent of the microbiological substances, of the nutrients;
- vegetal adsorbent treatment;
- microbiological analysis;
- treatment with microbial bio-preparations activated with nutrient;
- mellowing, mixing, homogenizing works.

The success of a microbiological remediation technology is obtained if appropriate microorganisms are selected for the type of pollutant to be removed and it is used the appropriate way to "multiply" them during remediation [5].

Bioremediation process consists of transforming the pollutant molecules into compounds simpler and simpler (metabolites) until it reaches the final phase of water and carbon dioxide. For certain classes of hydrocarbons and / or other organic and inorganic compounds, the microbiological decontamination technologies have as specific aspect the microorganisms selectivity towards these pollutants [1, 6].

Suppliers of microbiological preparations, for bioremediation of soils contaminated with petroleum products, produce products with high specificity for the type of hydrocarbons involved in pollution. Microbial preparations already marketed in wide world for bioremediation of polluted environments are based on microbial consortia where exist the predominant representatives, in particular, of the Bacillus, Pseudomonas and Xanthomonas genus [2, 5].

Microorganisms capable of utilizing petroleum hydrocarbons as the sole source of carbon and energy have been termed Ahearn in 1973, hydrocarbon-oxidizing microorganisms or "hydrocarbonoclastic" [6].

For laboratory and field tests conducted within this paper were used microbiological preparations purchased from the existing offer on the Romanian market (Biosol company).

They are present as a mixture of bacterial growth, obtained by selection for the degradation of a wide range of petroleum fractions, starting with benzines and ending with the residues from the vacuum distillation.

The product is presented as a gray- beige powder with a relative density of 0.6 to 0.8 and a *p*H in the range of 6.0 to 8.5. It contains an average of about $2 \cdot 10^9$ microorganisms /g of product.

The recommended dosage is of max. 500 g product / m^3 polluted soil and depends on the type of pollutant and of the polluted soil. There have been no studies on this sense.

After sowing, bacterial growth requires nutrients. These are fertilizers with nitrogen and phosphorus.

Bacteria working parameters are specified by the manufacturer as follows:

-temperature 12 to 30°C;
-*p*H 6.0 to 8.5;
-soil moisture 50-60%.

The consortium of microorganisms producer states that a technology in order to use them to decontaminate soil polluted by hydrocarbons foresees a preculture of microorganisms in water achievement, with less than 24 h before seeding, at a temperature of 15°C. During the achievement of the pre-culture should be done the aeration in a closed system by a pump.

Microbiological products for bioremediation of soils contaminated with petroleum products are not toxic and fulfill the conditions that attest the non-pathogenicity of the contained microorganisms.

It was considered that, to reduce the time to develop the experiment, should be used surfactants as by their accumulation at the water - polluting petroleum interface, they favour the extraction of hydrocarbon molecules from the soil structure and water and it is performed their emulsification in water [1, 7].

Surfactants are part of surface active agents class and they have a detergent action.

Experimental part

During the studies, in order to test the soil decontamination process using microorganisms it was analyzed the model described in paper [8, 9], which presents, in general, researches on decontamination by bio-remediation of polluted soils by organic substances. The performed experiments aimed at finding solutions to

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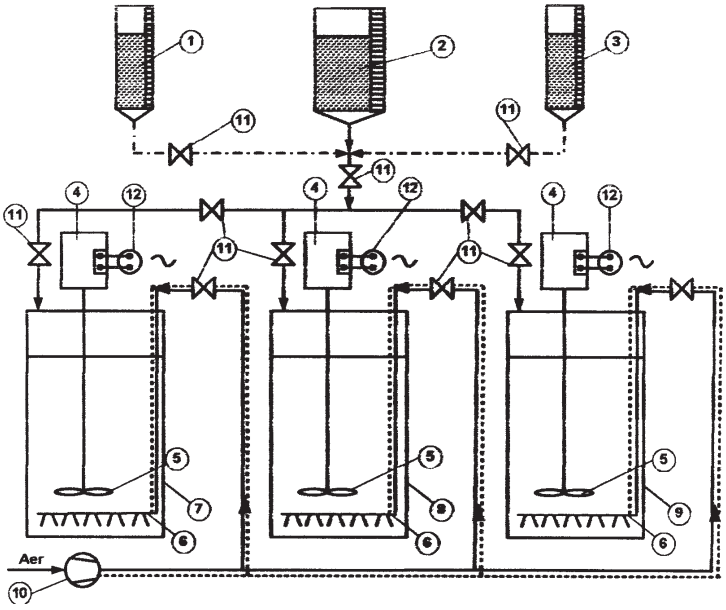

Fig. 1. Appliance for soil decontaminating by biodegradation of petroleum products contained by it
Marks of figure 1 have the following meanings:
1 - containing nutrient vessel, 2-vessel containing microorganisms (Consortium), 3 - vessel with surfactant, 4 - electric motor 5 - stirrer, 6 - air distributor 7 - bioreactor containing polluted soil by liquid petroleum products, 8 - bioreactor containing polluted soil by liquid petroleum products, 9 - bioreactor containing polluted soil by liquid . petroleum products 10 - air compressor, 11 - valves, 12 - power source.

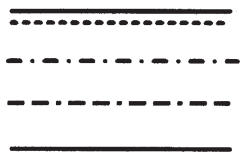

Fig. 2. Material flows signification. The notes are: a – air flow, b – nutrient flow, c – surfactant flow, d – mixed flow

the particular case of decontamination of soils in Romania, polluted by liquid hydrocarbons with the same origin. The experimental fitting was designed and conducted in the laboratories of the Oil and Gas Engineering Faculty, within the Petroleum - Gas University of Ploiesti. The protocol for experiments performing is original.

The realised experiments had the premise highlighting the effect of microorganisms in Consortium (E1) preparation and also the effect of using two types of surfactants: Super 100 and LCD. Table 1 presents the characteristics of the two surfactants.

To achieve decontamination experiments we chose bioreactors version. This consists of using a bioremediation system which allows an advanced control of the working parameters and provides an increased speed of the reactions.

Bioremediation has been achieved in three ways:

- Experiment noted E1, where has been used only the microbiological preparation of Consortium type;
- E2 Experiment - it was used Super 100 surfactant before the introduction of microbiological preparation of Consortium type;
- E3 Experiment where was used LCD surfactant before adding microbiological preparation of Consortium type.

Figure 1 shows the experimental appliance designed to decontaminate soils using microbiological and surfactant preparations.

The materials flow significations are presented in figure 2.

E1 experiment was carried out in the bioreactor which was noted with 7 in figure 1 using only the treatment with the Consortium preparation.

In bioreactor noted with 8, in figure 1, is performed E2 experiment in which the treatment with Super 100 surfactant is used before the microbiological preparation.

In bioreactor denoted with 9, in figure 1 is performed E3 experiment, in which it is used the LCD surfactant for the treatment before using a microbiological preparation.

Each bioreactor is formed of a cylindrical vessel in which we introduce the sample of contaminated substrate. The vessels are supplied continuously with water, where is inserted a microbiological preparation of the container - 2, the surfactant of the bowl - 3 and the nutrient solution from the vessel -1 through some valves. Each reactor was equipped with a mechanical stirrer, propeller type - 5, driven by an electric motor -4, fed from a power supply -12.

The intensification of metabolic activity of microorganisms in biological preparations is achieved by an intensive aeration with an air distributor 6, placed at the bottom of the reactors. Air is introduced by means of a compressor 10.

In the three versions of the experiment we have used a substrate from an uncontaminated area located in the vicinity of a contaminated area with location in sub-Carpathian hills in Romania.

For each experiment, E1, E2, E3 was taken 1 kg of substrate, consisting of a non-homogenous conglomerate of free of pollution aggregates, which was then contaminated with oil in a controlled way.

The experiments lasted 20 days. Daily, a visual inspection was performed.

Surfactants name	Super 100	LCD
Composition	**Sodium tripolyphosphate** **Alkyl benzene sulfonic acid** **Sodium hydroxide** **Sodium xylene sulfonate** **Butylglycolate**	**Tricloramină** **Alkyl benzene** **Sodium sulphonate** **Methylparaben** **Laureate sodium sulfate** **Sodium xylene sulfonate** **Urea** **Styrene / acrylate**
Physico-chemical properties	**Transparent green liquid** **pH = 9.5 to 10** **Biodegradability 90%**	**Opaque orange liquid** **pH = 7 to 8** **Biodegradability 95%**

Table 1
SURFACTANTS CHARACTERISTICS

Experiment code	Type treatment applied	The concentration of oil product, mg/kg				
		Day 0	Day 5	Day 10	Day 15	Day 20
E1	Consortium microorganisms	52 630	46 800	42 400	33 200	22 500
E2	Consortium microorganisms + surfactant Super 100	52 630	41 300	34 100	26 500	15 200
E3	Consortium microorganisms + surfactant LCD	52 630	39 500	28 500	16 500	7800

Table 2
VARIATION IN TIME OF THE CONCENTRATION OF OIL PRODUCT REMAINING IN THE SOIL

Experiment code	Type treatment applied	Total plate count, UFC/ ml				
		Day 0	Day 5	Day 10	Day 15	Day 20
E1	Consortium microorganisms	300	600	1200	2000	3000
E2	Consortium microorganisms + surfactant Super 100	300	900	2000	3500	4500
E3	Consortium microorganisms + surfactant LCD	300	900	3000	5000	6000

Table 3
VARIATION OF TOTAL NUMBER OF GERMS

Experiment code	Type treatment applied	Variation in the level of pollution while, %				
		Day 0	Day 5	Day 10	Day 15	Day 20
E1	Consortium microorganisms	0	11.1	19.4	36.9	51.2
E2	Consortium microorganisms + surfactant Super 100	0	21.5	35.2	49.6	71.1
E3	Consortium microorganisms + surfactant LCD	0	24.9	45.8	68.6	85.2

Table 4
LEVEL OF REMEDIATION VARIATION

Initial concentration of oil was of 52 630 mg oil/ kg dry soil. Water was added upon each sample until a consistency was obtained in order to permit mechanical stirring and air bubbling.

The samples were maintained in laboratory conditions at a temperature of 20°C and were daily subjected to mechanical stirring and air bubbling. Operations were started simultaneously, but mechanical agitation lasted 1 hour and bubbling 1.5 hours.

At each interval of five days, from the three bioreactors, samples were taken which have been analyzed for oil content and for the number of active biological germs. Microbiological analyzes were carried out by a qualified and authorized company.

In the E2 experiment, carried out in the bioreactor denoted with 8, in figure 1 was used Super 100 surfactant, and in E3 experiment, carried out in the bioreactor denoted with 9, in figure 1, the LCD surfactant was used.

The surfactants were initially introduced in the two bioreactors at a concentration of 5%, together with the dilution water.

Results and discussions

The results of the analyzes regarding the variation of the amount of remaining oil product in the soil, during the experiment performance, are shown in table 2.

Analyzing these results we find the followings:

-continued decline in time of the oil products content during the experiment;

- comparing the results obtained in E2 and E3 variants of the experiment we note that the use of surfactants increase bioremediation activities of microorganisms, so the amount of oil product decreases during the experiment, the LCD surfactant has a higher efficiency.

Analysis of the total number of germs expressed in colony forming units / ml has resulted in obtaining the results shown in table 3.

The survey data in table 3 leads to the following observations on the variation of the number of germs:

-the total number of germs has increased over the course of the experiment, leading to an improvement in decontamination level;

- in addition, the LCD surfactant using favors the increase of germs number compared to Super 100 surfactant, which indicates a better cleaning-up.

The results obtained after determining the degree of cleaning-up during the experiment are presented in table 4.

The results in table 4, which refer to the variation manner of the degree of cleaning-up lead to the following observations:

-the degree of decontamination of soil samples contaminated with liquid hydrocarbons in a controlled manner, increases during the experiment development;

- when LCD surfactant was used, it was achieved a better decontamination degree than in the case with Super 100 surfactant, fact explained by the superior effect of dispersion provided by LCD surfactant composition, towards the pollutant liquid hydrocarbons.

Conclusions

Decontamination of soils by means of bioremediation using microbiological preparations and surfactants is effective.

Effectiveness of using selected microorganisms, existing on the specific market, with bioremediation technologies, is possible under effective conditions.

The use of surfactants in bioremediation processes, has the effect of increasing the number of germs and increasing the decontamination of soils level, polluted with liquid hydrocarbons.

Comparing the two used surfactants LCD surfactant was found to have superior efficacy than Super 100 surfactant.

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