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The role of biopreparation in bioremediation of soil polluted by hydrocarbons

The efficiency of various bioremediation methods including natural attenuation, bioaugmentation with addition of oil-degrading microbial consortium and biosurfactant for the purification of crude oil contaminated soil was studied. It was shown that joint introduction of microorganisms and biosurfactant promoted greatest removal of pollutant from the studied soil samples.

Исследована эффективность различных методов биоремедиации включая природное восстановление, биоаугментация с внесением ассоциации нефтеокисляющих микроорганизмов и биоПАВ для очистки почвы, загрязненной нефтью. Показано, что совместное внесение микроорганизмов и биоПАВ способствует достижению наивысшего уровня деградации загрязнения в исследуемых образцах почвы.

The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving methods for the removal and degrading of many environmental pollutants including the products of petroleum industry. In addition, bioremediation technology is believed to be non-invasive and relatively cost-effective. There are three main technologies for oil spill bioremediation: natural attenuation, bioaugmentation, in which know oil-degrading bacteria are added to supplement the existing microbial population and biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting co-substrates.

The aim of this work was to evaluate the possibility of enhancing the natural bioremediation process by introducing biosurfactant into soil samples and/or inoculating them with biopreparations containing strains of microorganisms capable of detoxifying and degrading crude oil contamination in soil samples and comparing the variations and efficiency of the applied agents of different compositions.

The bioremediation process was carried out by ex situ method for seven weeks using soil contaminated by Russian crude oil (10g/1kg of soil, density=0,875mg/cm^3). During the experiment the following parameters were controlled: humidity WHC=70%, pH of soil, degrading activity of microorganisms by INT test (Shawn 2006), amount of bacteria in soil, the content of oil product (extraction with DCM) and the quantitative composition of product of biodegradation before and after the experiment (GC-FID, ^{1}HNMR).

The first – V1 is a control, where the processes of self-purification will be enriched by mechanical mixing in order to aerate the sample. In V2 sample processes of self-purification were enriched by the introduction of water. The next sample (V3) was enriched by the introduction of biogenic substances – nitrogen and phosphorus in appropriate ratio C:N:P, moreover the processes of self-purification was enriched also by introduction of water (70%WHC) and mechanical mixing in order to aerate the sample (as in V2). Variants of the other samples were enriched by the introduction of bacterial biopreparation (V4, V5, V6, V7) and biosurfactant solution (V4). Remaining conditions were the same as in sample 3 (V3). Microbial biopreparation consist strains of bacteria *Ochrobactrum anthropi* E1 *Strenotrophomonas maltophilia* E2 and yeast strain *Candida lipolytica* PT. Rhamnolipid biosurfactants were received from Dr Elena Karpenko (Ukrainian

Academy of Sciences), which was obtained from the strain of *Pseudomonas* sp. PS-17 (Karpenko 1996).

At the beginning of the experiment humidity of soil and the content of nitrogen and orthophosphate was determined. It was necessary to keep constant humidity of soil (WHC=70%) and to set up the ratio C:N:P equal 100:10:1 using $(NH_4)_2SO_4$ i $NaNO_3$ as a nitrogen source and KH_2PO_4 as a phosphorus source.

Analysis of the soil microbial counts showed that bioaugmentation (V4-V6) and biostimulation (V3) had a significant increase of this index in samples V3-V6 when compared to the control sample. It should be noted that the counts of bacteria in the control samples (V1, V2) were also high, which indicated the presence of indigenous bacteria capable of degrading contaminants such as petroleum. Sample V7 contained microorganisms sealed in capsules, hence the amount of bacteria was similar to the control sample (V1). In the course of bioremediation count of bacteria in the soil also gradually increased in a sample with alginate capsules (V7) probably due to their destruction and releasing of bacteria into surrounding soil. In the last week, due to the depletion of organic carbon sources the number of bacteria decreased.

The analysis showed the loss of oil in all the vases and controls, however, the effectiveness of bioremediation was highest in soils treated with both biostimulation and bioaugmentation (Fig. 1). The rate of decomposition of oil was also high in nutrient-enriched soil, which means that the indigenous microorganisms had a high degradation capacity. The most effective proved to be enrichment with a surfactant biopreparation (V4), due to its ability of desorption of oil from the soil matrix and increase its bioavailability.

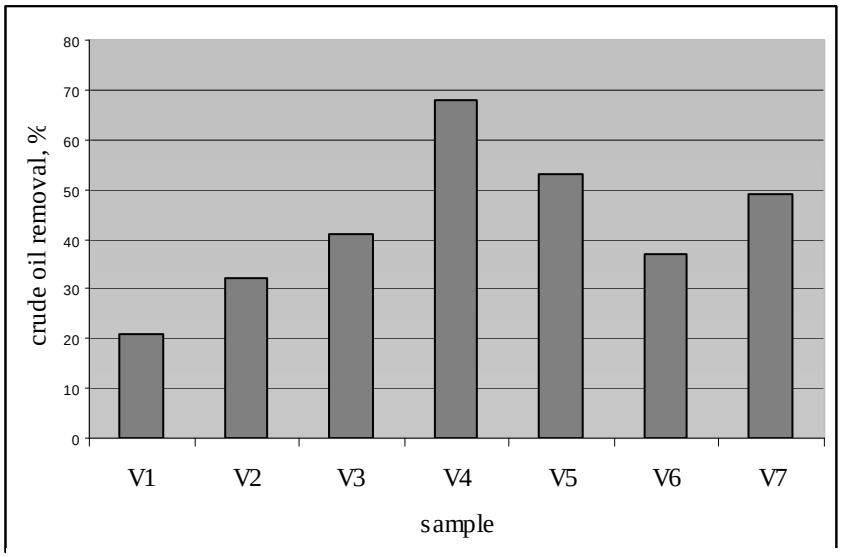

Fig. 1. Crude oil removal after 7 weeks of bioremediation

The samples of both the output of crude oil and products obtained in the biodegradation process were analyzed by ^{1}HNMR (Śliwka 2012). Qualitative analysis of residues of petroleum biodegradation showed that the biodegradation products have more aromatic character than the substrates (% HA was 1,3-fold higher than the products of biodegradation in crude oil), which shows the removal of aliphatic compounds, which are readily biodegradable.

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