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Enhancement of Microbial Petroleum Degradation by Oil Spill Bioremediation Products

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It is found that the use of peat-based ameliorants, which also contain nitrogen and phosphorus fertilizers, would promote microbial growth in petroleum-contaminated soil, enhancing thereby the extent and rate of petroleum hydrocarbon biodegradation by 1.5-2 times. The extent and rate of petroleum-contaminated soil remediation was investigated using peat-based ameliorants containing various amounts of carbamide and ammonia nitrate fertilizers. It is found that the use of peat-based ameliorant containing carbamide fertilizer provides for a significant enhancement of microbial activity.

Keywords: petroleum hydrocarbons (PHCs), soil contamination, peat, ameliorant, biodegradation, bioremediation.

Introduction

Crude oil production, processing and transportation create a grave environmental hazard worldwide [1]. We are concerned, in particular, with the extensive soil contamination in the oil-producing regions of Northern Siberia (Russia). Thus various adsorbents used by professionals to cleanup oil spills help mitigate serious threats to shorelines banks and other sensitive habitats. However, the cleanup and remediating of petroleum-contaminated soil still poses a grave problem. Much attention has been

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paid lately to the development of biological agriculture methods, which are based on biodegradation of petroleum hydrocarbons (PHCs) in soil. Peat is known to be a high-efficiency adsorbent of PHCs; moreover, it contains various species of naturally occurring community that are capable of PHC degradation [2]. The sorption capacity of peat with respect to PHCs is determined by its decomposition degree. The sorption capacity was determined for high-moor and valley peats; the values obtained are 8-10 g and 6-8 g of crude per g of oven-dry peat, respectively [3]. The aim of our study was to obtain an environmentally appropriate peat-based ameliorant enriched with active PHC-oxidizing microorganisms, which would have high sorption capacity.

Experimental methods and investigated materials

The peat samples were collected from various sites of the Tomsk region (Western Siberia). Microbial enumeration involved culturing bacteria naturally occurring in the peat samples, using a liquid growth agar medium containing the contaminant (PHCs); the mineral composition of the medium was similar to that of the liquid Muntz medium. Using a gravimetric method, we determined the in-situ response to PHCs of the naturally occurring microbial community, which is an important indicator of bioremediation potential. The extraction of PHCs from the soil samples was performed in a Soxhlet extractor, using chloroform+methanol mixture in the 93:7 ratio [4]. After stripping the solvent in a rotor evaporator, the mass of recovered PHCs was determined by weighing on analytical laboratory scales. The carbon and hydrogen contents were measured using pyrolysis in an oxygen flow and a subsequent gravimetric analysis. The sulphur content was determined by the Schöniger flask method and the nitrogen content, by oxidative destruction method using nickel oxide as a catalyst [5].

The simulation of PHC biodegradation processes was carried on for low-humus podzolic soil samples contaminated with commercial oils from an oil field of Western Siberia. Peat ameliorants (PA) were prepared on the base of peat sampled from the lowland peat soil horizon Tyomnoe (Tomsk region, Western Siberia). The peat samples studied had decomposition degree of 25–30% and ash content of 15% (by mass). In order to achieve an enhanced bioremediation of soil, carbamide or ammonium nitrate mineral fertilizers were added the peat ameliorants (PA). Thus PA1 and PA2 contained carbamide and PA3 and PA4, ammonium nitrate.

To stimulate intrinsic bioremediation, i.e. the in-situ response to PHCs of the naturally occurring microbial community, and thus provide for PHC-contaminated soil remediation, PA1 and PA3 should be applied in amounts required for C:N:P to be in the 20:1:0.15 ratio [6, 7]. To provide for soil melioration and improvement through crop rotation techniques, PA2 and PA4 should be applied in amounts required for C:N:P to be in the 863:1:1 ratio, which is equivalent to 60 kg of $N+P_20_5$ per hectare. The peat-based ameliorants considered herein were applied for PHC-contaminated soil remediation in amounts of 50 tons per hectare.

Dry soil used for laboratory testing was collected from a depth of 0-20 cm. The soil was sifted through a 3-mm mesh sieve and moistened to the specific retention of moisture. Using soil, petroleum and peat ameliorant, saturated paste was prepared by stirring until homogeneous. Then the mixture was placed into laboratory vessels and moistened now and then to maintain optimal moisture content. Soil samples were taken for analysis three days, one month or six months after the preparation.

The group composition of PHC-contaminated soil samples was examined by the method of liquid-adsorption chromatography on a column packed with silica gel [8]. The carboxyl group content was determined by potentiometric titration of the analyte, using alcohol alkali solution in accordance with the protocol of the State Standard GOST 11362-96 (Russia).

The enumeration of microorganisms and urobacteria involved using meat-peptone agar and Fyodorov's medium [9], respectively. The microbial enumeration was performed using a medium having the following composition (g/dm^3): NH_4NO_3 – 1.45; KNO_3 – 1.0; $MgSO_4 \times 7H_2O$ – 0.1; K_2HPO_4 – 2.4; KH_2PO_4 – 0.6; NaCl – 1.0; $(NH_4)_2MoO_4$ – 0.004; $ZnSO_4 \times 7H_2O$ – 0.009; $FeSO_4 \times 7H_2O$ – 0.014; $CoCl_2 \times 5H_2O$ – 0.008; agar-agar – 20.0; crude oil – 10.0. The number of fungi was determined on potato-glucose agar medium (pH 4.5 – 5.0) [9]. The activity of catalase and dehydrogenase was assessed by gas-measuring method involving hydrogen peroxide decomposition and by photo-calorimetric method, respectively [10].

Each experiment was repeated thrice. Variants of experiments are as follows (here the symbols S and O denote Soil and Oil, respectively):

1. S+PA1;
2. S+O+PA1 (C:N:P in the 20:1:0.15 ratio);
3. S + O+ PA2 (C:N:P in the 863:1:1 ratio);
4. S + O+ PA3 (C:N:P in the 20:1:0.15 ratio);
5. S + O+ PA4 (C:N:P in the 863:1:1 ratio).

Results and Discussion

It is found that in order to achieve enhanced bioremediation, both types of peat ameliorants should be applied in sufficient amounts required for C:N:P to be in the 863:1:1 ratio. As is seen from Fig. 1, the PHC degradation achieved within a six-month period is 42.5 and 54.3% (variants of experiments 3 and 5) and 21.6 and 32.4% (variants of experiments 2 and 4). In the latter case a significantly lower extent of PHC degradation is evidently achieved within the same period, which might be due to the toxic effect of the mineral fertilizer on the soil biota. Thus, the application of peat ameliorants containing a

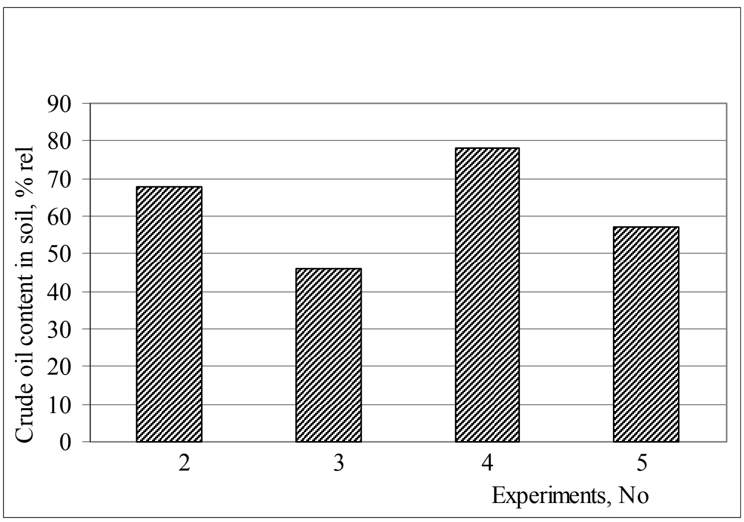

Fig. 1. Variation in the degraded crude oil content of the soil samples within a six-month period (variants of experiments 2 through 5)

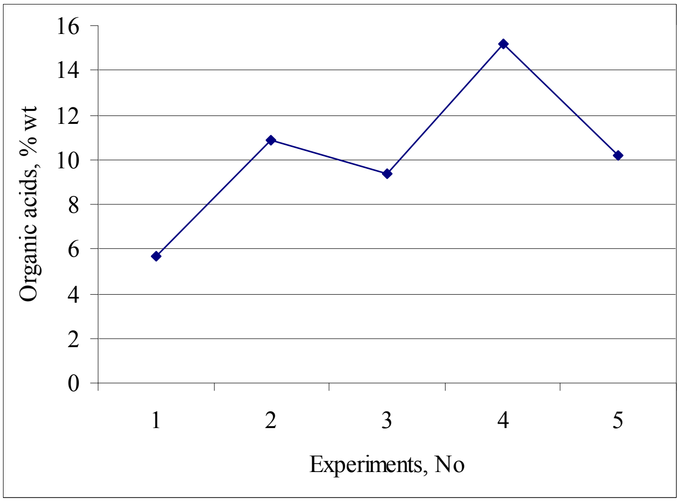

Fig. 2. Variation in the organic acid content of the PHC-contaminated soil samples within a six-month period (variants of experiments 1 though 5)

carbamide fertilizer are expected to bring about a higher extent of PHC degradation (32.4 and 54.3%) relative to those containing an ammonium nitrate fertilizer (21.6 and 42.5%), no matter what the C:N:P ratio.

The PHC degradation processes occurring in the crude-contaminated soil samples involve organic acid formation (Fig. 2).

As is seen from Figure 2 (variants of tests 2 and 3), the application of peat ameliorants containing a carbamide fertilizer have caused a significant change in the organic acid content of the PHC-contaminated soil samples within a six-month period, which is suggestive of intensive PHC oxidation processes. Evidently, the highest acid content is determined for the PHC-contaminated soil samples, which had been treated with peat ameliorants containing an ammonium nitrate fertilizer six months before (variants of experiments 4 and 5).

An analysis of the structural group composition suggests that in all the variants of experiments the PHC content has decreased considerably relative to the original PHC-contaminated soil samples (Fig. 3).

Due to the application of peat ameliorant containing a carbamide fertilize, the highest extent of PHC degradation was of experiments 3 and 5. An increase in the resin content of the test samples is probably due to the accumulation of residual resins and resins formed as PHC biodegradation products (Fig. 3).

Microbiological and fermentation analyses were made of PHC biodegradation products. The enumeration of PHC-oxidizing microorganisms was performed for oil-contaminated soil samples containing a carbamide or an ammonium nitrate fertilizer.

The data obtained also suggests that a carbamide fertilizer is preferred over an ammonium nitrate one (see Table 1 and Fig. 4).

It is found that the application of a carbamide fertilizer brings about a two-fold increase in the number of PHC-oxidizing bacteria after three days of observation. This appears to be a convincing

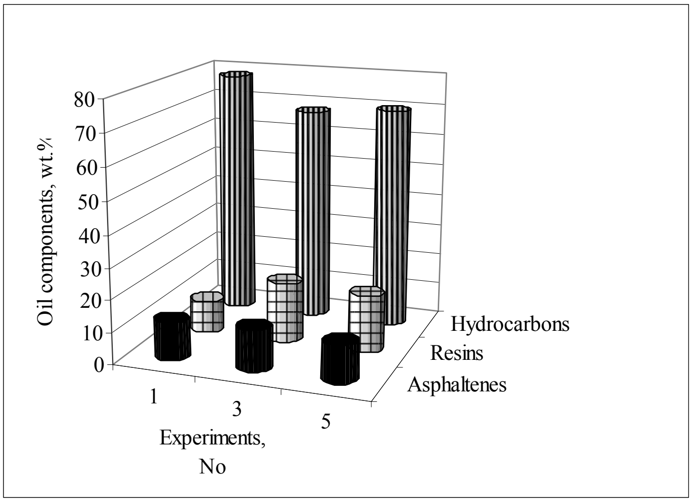

Fig. 3. Variation in the contents of hydrocarbons and asphaltenes+resins of the crude-contaminated soil samples within a six-month period (variants of experiments 1, 3 and 5)

Table. The effect of ameliorant composition on the microbial community of the PHC-contaminated soil samples

Microorganisms	Period (days)	Variants of tests				
		1	2	3	4	5
MPN*	3	31.7	35.0	16.7	6.6	62.5
	8	48.3	1303.3	1660.0	7.5	458.3
	30	63.3	4108.3	224.2	16.9	86.7
Hydrocarbon-oxidizing microorganisms	3	1.1	14.9	8.7	6.7	4.0
	8	0.3	100.0	435.0	1.4	88.3
	30	64.5	413.3	151.7	121.7	76.7
Fungi	3	$<10^4$	$<10^4$	$<10^4$	1,1	0,8
	8	$<10^4$	$<10^4$	$<10^4$	4,2	$<10^4$
	30	$<10^4$	$<10^4$	$<10^4$	33,0	17,0

Footnote: *MPN by 10^5 CFU per g of dry soil (here MPN is Most Probable Number and CFU is colony forming unit)

argument for the application of a carbamide fertilizer to achieve a significant enhancement of microbial activity (Table 1).

The enumeration of PHC-oxidizing bacteria was continued in the above two groups of tests. It was found that in the case of carbamide fertilizer, the microbial community continued to grow, while in the case of ammonium nitrate, the population of bacteria diminished dramatically beginning from the 8th day of observation. This might be attributed to an enhancement in the soil toxicity due to the presence of ammonium nitrate. This observation is supported by the abundance of microscopic fungi whose metabolism products are highly toxic for both soil microbial community and vegetation [11, 12].

It should be noted that the PHC-contaminated soil samples containing an ammonium nitrate fertilizer, were found to have high micromicete contents after 30 days, while the total number of

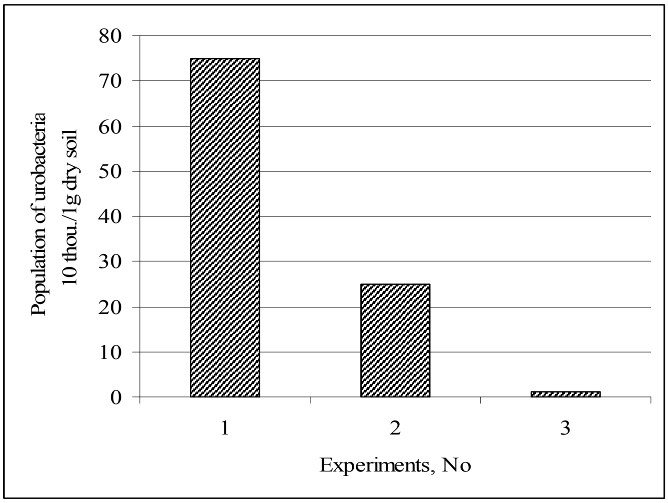

Fig. 4. The effect of PHC contaminant on the urobacteria population of soil (variants of experiments 1, 2 and 3)

microorganisms of the same samples tended to decrease (Table 1). In this case, high fungi contents might be attributed to the availability of nitrogen provided by ammonium nitrate mineralization, while carbamide mineralization is a very slow process so that nitrogen is virtually unavailable to the fungi occurring in soil. This is supported by a three-fold decrease in the urobacteria content of uncontaminated soil relative to that of PHC-contaminated soil, which is due urobacteria being involved in carbamide decomposition to give ammonia (Fig. 4).

Conclusion

The bioremediation technology developed for PHC-contaminated soil exploits naturally occurring environmentally appropriate peat ameliorants. It is found that the application of peat-based ameliorants containing a carbamide fertilizer provides for a significant enhancement of bioremediation due to the slow mineralization of carbamide in the PHC-contaminated soil samples. Moreover, the application of ameliorants containing carbamide affords a higher extent of petroleum hydrocarbon degradation relative to ameliorants containing ammonium nitrate.

The application of peat ameliorants containing high ratios of a carbamide or an ammonium nitrate fertilizer is liable to decrease the extent of PHC degradation. The highest extent of PHC degradation is achieved using C:N:P in the 863:1:1 ratio.

The use of peat ameliorant containing a carbamide fertilizer is found to enhance the activity of microbial community and fermentation processes, which causes an increase in the hydrocarbon-oxidizing microorganism content and a decrease in the microscopic fungi content of the remediated soil samples.

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Интенсификация биодеградации нефтяных загрязнений в почве с помощью мелиорантов

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Установлено, что мелиоранты на основе торфа, которые содержат азот и фосфор, способствуют увеличению роста микроорганизмов в нефтезагрязненных почвах, благодаря чему скорость и интенсивность биодеградации увеличиваются в 1,5-2 раза.

Изучено влияние мелиорантов, содержащих карбамид и нитрат аммония, на скорость и интенсивность ремедиации почв различной степени загрязненности нефтью.

Ключевые слова: нефть, нефтепродукты, почва, торф, мелиорант, биодеградация, биоремедиация.