Biotechnology for Recultivation of Oil Polluted Soils

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Abstract

All the biotechnologies intended for improvement of environmental conditions are based on the ability of microorganisms for hydrocarbon oxidizing. In the development of biotechnological approaches for soil re-cultivation the extent of soil pollution has to be taken into account. Among the advantages of a technology employing mineral nutrient substrates for enhancing the oxygenizing activity of soil microflora its cost-effectiveness is very important. When dealing with highly polluted soils, more complex methods for soil restoration are called for; however, these are much more labour-extensive and time-consuming.

Experimental investigations have been performed to study the effect of oil pollutants on the ability of soil microflora to assist the destruction of hydrocarbons.

Variation in the activity of indigenous soil microflora was investigated using three major groups of bacteria, *i.e.* Heterotrophs, Actinomycetes and microfungus cultures.

The presence of oil in amounts constituting up to 5% is found to stimulate the growth and oxygenizing activity of the soil biocenosis. Thus the residual oil samples were exposed into the soil microflora for a time period of 60 days and then analyzed by gas-liquid chromatography (GLC). The results obtained reveal significant changes in the molecular-mass distributions of saturated hydrocarbons C_9-C_{32}.

An addition of stimulating nutrient substrates is found to enhance the biochemical activity of soil microorganisms. Thus a 60 days' contact with soil microflora resulted in destruction of 80 – 85% of the saturated oil hydrocarbons, mostly C_9-C_{15} and C_{20}-C_{31} compounds.

However, the occurrence of 10% oil pollutants exerts an inhibiting effect on the indigenous soil microflora; therefore, only an insignificant amount of hydrocarbons undergoes degradation.

Introduction

The basic challenge one is faced with by designing environmentally appropriate technologies for soil restoration is handling hazardous situations involving man-made pollution. The theoretical concepts embodied in biotechnology design meant for bettering environmental conditions are based on the hydrocarbon oxygenizing enzymatic activity of bacteria [1]. The physical and chemical processes involved play a significant role in the self-purification of oil polluted water and soil. The mechanism of chemical oxidation of hydrocarbons to yield low molecular oxygen-containing derivatives, *e.g.* alcohols, aldehydes, carboxylic and aliphatic acids, *etc.*, is disclosed in Engler's theory [2]. Oil oxidizing bacterial activity may also cause total hydrocarbon degradation.

The co-operative action of hydrocarbon oxidizing bacteria is the first link in the chain of processes to cause degradation of the organic substances of oil. Aerobic oxidation of hydrocarbons occurs via a series of catalytic enzymatic processes to give various derivatives, *e.g.* alcohols, aldehydes, ketones, aliphatic an carboxylic acids, phenols, *etc.*, which undergo further oxygenation to give CO_2 as the end product [1].

It has been found experimentally that in soil containing up to 1% oil pollutants, various hydrocarbon-oxidizing bacteria are liable to accumulate, which ensures the self-purification of the soil. In the environmental conditions, however, self-purification processes occur very slowly.

Biotechnology for soil restoration may be developed along two lines depending on the extent of soil contamination with oil. Biotechnologies based on the use of stimulating mineral nutrient substrates capable of sustaining bacteria proliferation are more advantageous due to their high cost-effectiveness [3].

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In the case of highly polluted oils, an alternative approach is called for. This envisages the use of more complex methods for soil restoration employing special compositions comprising active biomass and nutrient substrate, which makes them more expensive relative to the former.

Using simulation models of oil-polluted soils containing 5% and 10% pollutant, an experimental study was made of oil degradation processes and of oil-metabolizing soil microflora product accumulation. Mineral substrate composition was developed for stimulating soil microflora enzymatic activity.

Experimental

The oil-polluted soil samples were contacted with indigeneous soil microflora over a time period of 60 days to cause destruction of the organic matter of soil. The population of microorganisms was determined from three major groups of soil biocoenosis: heterotrophs, fungi and Actinomycetes [4].

Weighed portions of soil (300 g) containing the original microorganism population were placed into glass reservoirs to which oil was added in amounts constituting 5% and 10% of the soil. A control test using soil samples containing no oil pollutant served as a standard for evaluating the dynamics of microbial growth in the test samples. The oil-polluted soil was mixed carefully and soil samples were taken to determine the concentration of the original saturated hydrocarbons of oil. The control and test samples were subjected to thermal conditioning in an incubator for 60 days at 27°C.

In order to stimulate the microbial growth, 1% solution of mineral nutrient (nitrogen and magnesium salts) was added to the reservoirs containing the oil-polluted soil samples which had been contacted with indigeneous soil microflora for 10 and 20 days.

In the course of thermal conditioning, weighed portions of oil (10 g) were collected at regular intervals from the control and test samples to monitor the microbial proliferation and evaluate the dynamics of aldehyde accumulation.

Among the oil biodegradation products are aldehydes, which content was determined by the Feigel analytical procedure using measurement of sample's optical density with the aid of a photocalorimeter at wavelength of 570 nm [5].

A GLC analysis of the oil-polluted test samples was performed after 60 days' contact with the indigenous oil microflora to determine the original and residual contents of saturated oil hydrocarbons. The measurements were performed on a "Kristall-200" unit equipped with a flame ionization detector and a quartz capillary column having dimensions 25 m × 0.2 mm using SE-54 as mobile phase. The analyses were performed using temperature programming from 50° to 290°C at a rate of 4°/min.

Extraction of oil pollutant from the soil samples and preparation of samples for GLC analysis were performed using the following procedure. Equal volumes of sterile water were first added to the weighed portions of soil containing the original and residual oil and the samples were stirred for 20 min. with the help of a magnetic mixer. Then the water extract was extracted three times with chloroform to remove hydrocarbons and the chloroform extracts were combined and water and chloroform were removed from the resulting extract.

Using GLC analysis data, histograms of molecular mass distributions of saturated oil hydrocarbons were constructed. The extent of destruction of the oil organic matter was determined from the ratio of net n-alkanes C_{17}-C_{18} to net isoalkanes (pristine + phytane).

Results and Discussion

The experiments were conducted using gray forest soil; oil from Las-Eganskoe field, Western Siberia, having viscosity of 14.73 mPa×s and density of 0.872 g/cm³ at 20°C was employed as a pollutant.

The growth and activity soil biocoenosis is affected by a variety of factors; therefore, a study of these factors involves recording of the dynamics of variation in the population and make-up of the major groups of microorganisms, *i.e.* bacteria, fungi and Actinomycetes. The maximal populations of the above three groups of microorganisms for the control unpolluted samples were 25, 18 and 20 million cells/g, respectively. Out of the bacterial cultures isolated from the soil we selected over 20 strains of bacteria, which are able to proliferate by utilizing pentane, hexane, hexadecane and benzene. The soil microflora is represented by species of the following genera: Bacillus, Actinomyces, Proactinomyces, Sarcina, Mycobacterium, Pseudomonas, Micrococcus, yeast cultures of the genus Candida and mold microflora of the genera Penicillium and Aspergillus.

The population of the above microflora groups in the test oil samples containing 5% oil pollutant was found to increase by one order relative to the con-

trol. An example shown on Fig. 1 illustrates the kinetics of growth observed for Heterotrophs at 5% and 10% oil pollutant content. The maximal population of the latter group of bacteria was 25 million cells/g for the control relative to 650 and 12 million cells/g for the test samples at pollutant concentrations of 5% and 10%, respectively. The maximal population of the fungus microflora and of the group Actinomycetes in the test samples with 5% pollutant was found to exceed by one order the original population and constituted 140 and 250 million cells/g, respectively.
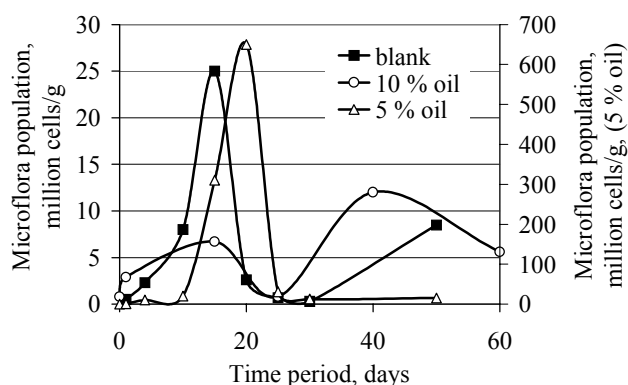

Fig. 1. The dynamics of heterotrophic soil microflora population at different concentrations of oil pollutant.

Aldehydes are oxidation products of saturated and cyclic oil hydrocarbons; therefore, the yield of these compounds depends exclusively on the accumulation of biomass. Figure 2 presents the dynamics of accumulation of aldehydes as a function of microorganism population stimulated by the nutrient substrate. As is seen from Fig. 2, the nutrient substrate is capable of sustaining microflora proliferation; and the aldehyde content has increased by one order, which is an indication of a significant enhancement in the microbial oxygenizing activity.

Thus, the presence of 5% oil hydrocarbons in the soil samples tested is found to stimulate the growth and oxygenizing activity of the major groups of soil microflora. The extent of degradation of oil hydrocarbons and the accumulation of metabolism products depend on the population of microorganisms. Thus the rate of oil hydrocarbon biodegradation occurring in soil can be judged from the presence of aldehydes and the kinetics of their accumulation.

The GLC data on the original and residual contents of oil pollutant obtained for the test soil samples subjected to thermal conditioning over a time period of 60 days reveal significant changes in the concentra-
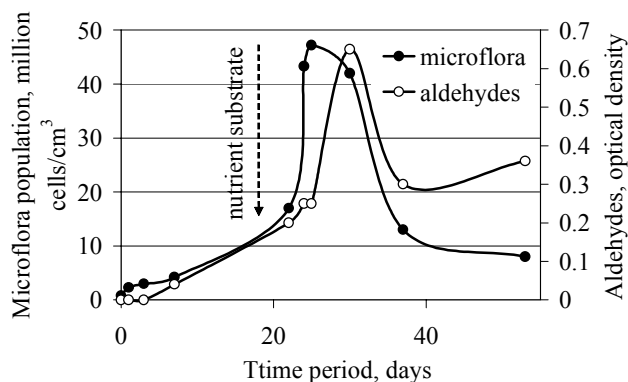

Fig. 2. The dynamics of microflora growth and aldehyde accumulation by the soil microflora-assisted oil degradation under experimental conditions.

tion of saturated hydrocarbons.

The molecular-mass distribution of n-alkanes observed for the original oil-polluted soil samples has the shape of unimodal curve (see Fig. 3a), with the maximum corresponding to low-molecular homologues C_9-C_{15}. Among isoprenoids, iC13-iC20 compounds have been identified, with higher isoprenane homologues occurring in minor amounts. The pristane/phytane ratio (Pr/Ph) is 0.88 and the net n-alkanes/net isoprenanes (Pr+Ph) ratio is 1.8.

Variation in the composition of all the saturated hydrocarbons is found to occur in the test soil samples containing 5% oil pollutant, which were subjected to soil microflora-assisted biodegradation (see Fig. 3b). These changes are the result of proliferation and stimulation of oxygenizing bacteria, which is supported by metabolism product (aldehyde) accumulation. As is seen from Fig. 3b, the relative content of low-molecular homologues C_9-C_{13} has decreased by 50-100%, with the maximum of molecular-mass distribution corresponding to n-alkanes C_{15}-C_{16}. Among the higher-molecular homologues, variation is found to occur in the composition of C_{18}-C_{30} compounds. The isoprenoid hydrocarbons content is almost twice the original one. The pristane/phytane ratio remains practically the same (0.8). The net n-alkane/net isoprenane (Pr+Ph) ratio is 1.07.

It is seen from Fig. 3c that the activity of the indigenous microflora is suppressed in the test soil samples containing 10% oil pollutant so that the oil organic matter undergoes degradation to an insignificant extent. The aldehydes content is by one order lower relative to the test soil samples with 5% oil pollutant. The Pr/Ph ratio is 0.8. The level of hydrocarbons destruction is 1.2. The most significant changes in the molecular-mass distribution of satu-
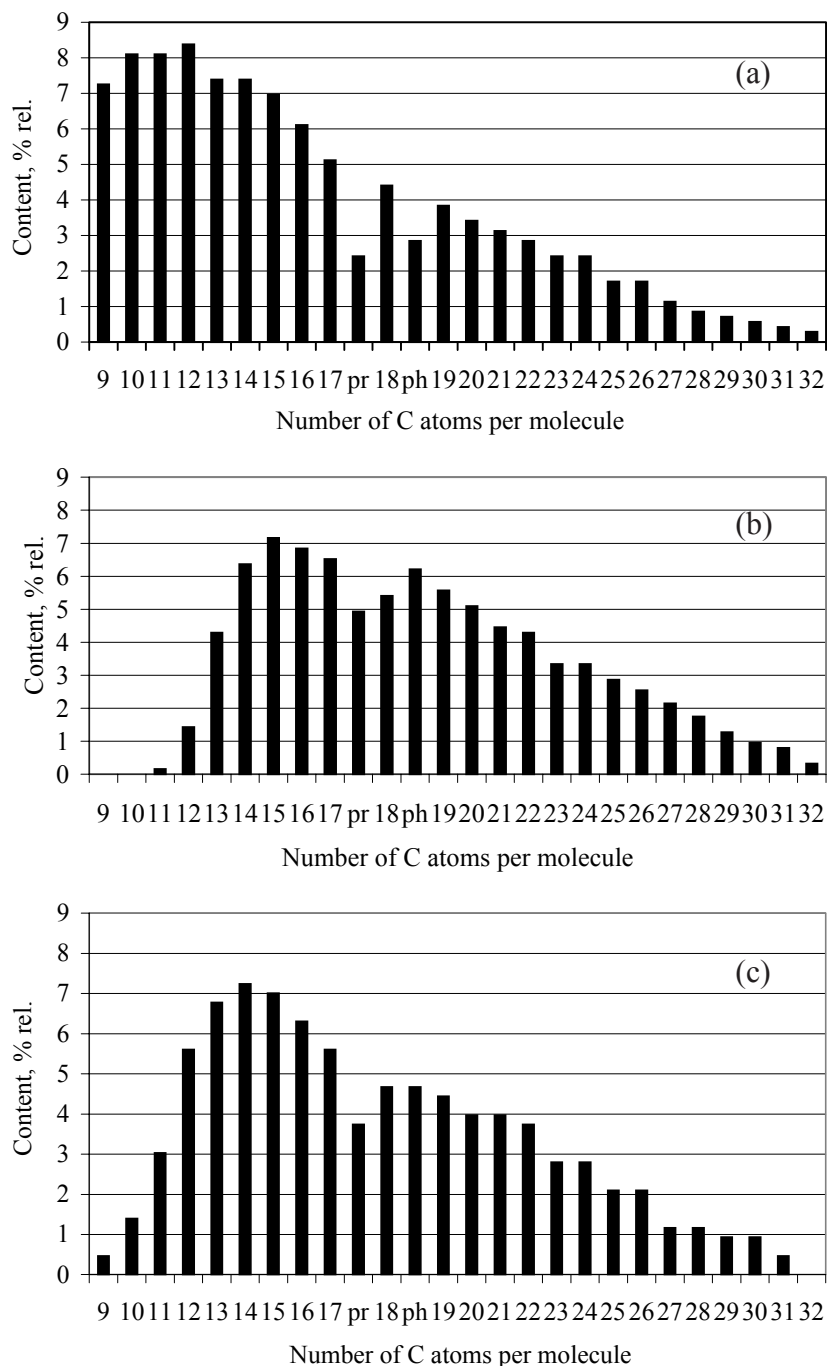
Fig. 3. The molecular-mass distribution of saturated hydrocarbons in the original oil from Las-Eganskoe field (a) and in the biodegraded oil from the test soil samples at oil pollutant concentrations of 5% (b) and (10%) (c).

rated hydrocarbons are found to occur in the region C_9-C_{12} and C_{18}-C_{26}.

Thus the addition, in experiment, of 1% stimulating nutrient substrate to the test soil samples with 5% oil pollutant is found to cause a four- to five-fold increase in the population of indigenous microflora relative to the original one. When the same substrate was added to the test soil samples containing 10% oil pollutant, the population of microorganisms in-

creased by no more than one order.

Figure 4 shows chromatograms of saturated oil hydrocarbons obtained for the original oil-polluted soil samples (A) and for the same samples, which have been in contact with the stimulated microflora (B). In the latter case, the destruction of saturated oil hydrocarbons constitutes 80-90%. It follows from the figure that 100% n-alkanes C_9-C_{32} has also undergone biodegradation. The Pr/Ph ratio is the same

(0.8). The level of destruction of saturated oil hydro-carbons is 0.08, which indicates a high rate of oxidation of oil hydrocarbons.
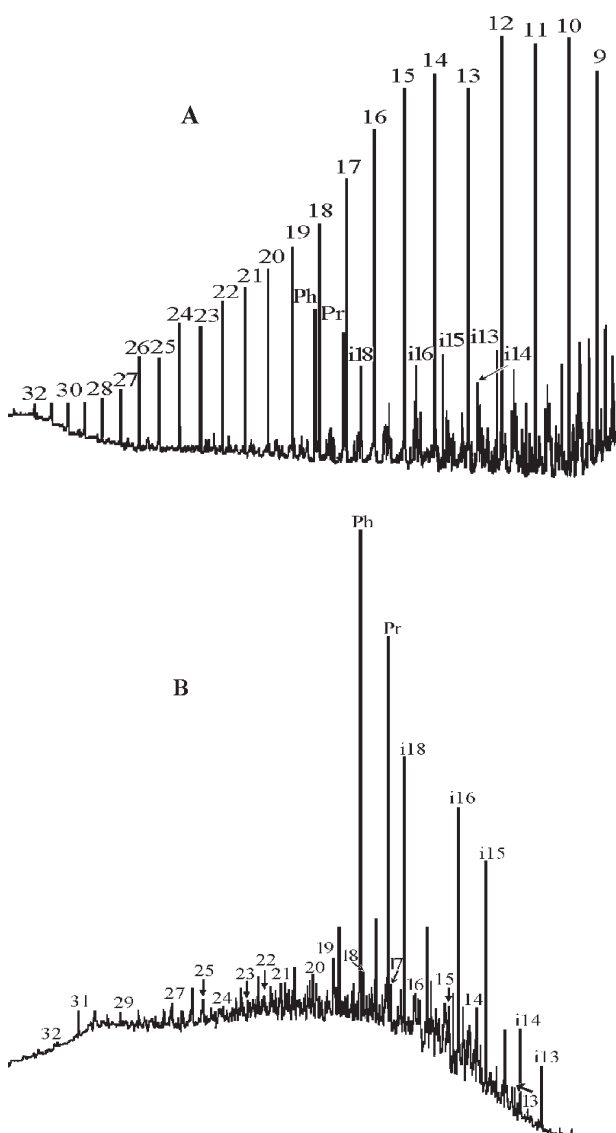

Fig. 4. Chromatograms of n-alkanes of the original oil from Las-Eganskoe field (A) and of the oil-polluted soil samples subjected to degradation by indigenous soil microflora (B).

Conclusions

In conclusion, the presence of oil pollutant in soil in concentrations of up to 5% is found to stimulate the growth and oxygenizing activity of the indigenous soil microflora; however, the population of microorganisms increases by no more than one order.

The biooxidation processes occurring in the soil samples with 5% oil pollutant over a time period of 60 days cause destruction of 20-30% higher-molecular homologues relative to the original oil. The destruction rate of saturated hydrocarbons is 1.07 relative to 1.8 of the original oil.

In the soil samples with 10% oil pollutant, the growth of microflora is suppressed, so that only an insignificant portion of hydrocarbons undergoes biodegradation.

The addition to oil-polluted soil of stimulating nutrient substrates in amounts of 5% by mass results in a three- to five-fold increase in the population of microorganisms. The biooxidation processes cause 80-90% reduction in the saturated hydrocarbon content of the soil samples tested, the level of hydrocarbons destruction being 0.08.

On the base of the above evidence, environmentally appropriate and cost-effective biotechnology for polluted soil restoration is being developed. This involves the use of mineral nutrient substrate (domestic product) for stimulating indigenous microflora.

The dynamics of aldehyde accumulation in oil-polluted soil may be an indication of the rate of biodegradation of the organic matter of oil.

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