Biodegradation of Oil Hydrocarbons by Soil MicrofloraActivated with Photoluminescence Films

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Abstract

Stimulating effect of polyethylene photoluminescence films on the growth of autochthonous microflora population in oil-polluted soils has been determined under the laboratory conditions, where oil concentration in the soil was 50 g/kg. The increase in number of basic groups of microflora was accompanied with the increased activity of the enzymes, which catalyzed oxidizing processes. At the end of the experiment, on the 45-th day, oil content decreased to 15 g/kg. The analysis of the residual hydrocarbons by IR-spectroscopy has revealed absorption bands in the regions of 1710 cm^{-1} and 1600 cm^{-1}. At the same time spectral coefficients C_1, C_2, A_1 and A_2 increased 1.5-3 times, while C_3 and A_3 decreased 1.5-2.5 times indicating oxidation processes of oil hydrocarbons. Chromatographic analysis proved the intensity of hydrocarbon biodegradation. Hydrocarbons C_{11}-C_{14} completely eliminated in the test samples and the concentration of hydrocarbons with a high molecular weight decreased by 70-80%. At the same time hydrocarbon biodegradation index, determined as the ratio of the sum of isoprenoids (pristine + phytane) to the sum of n-alkanes (C_{17} + C_{18}), increased 5-6 times due to the use of photoluminescence films.

Introduction

Biotechnologies intended for environmental improvement are based on the ability of microorganisms for fermentative oxidation of oil hydrocarbons [1]. A degree of hydrocarbon degradation correlates with the growth in population of microorganisms and their oxigenase activity [2-5]. Oxidizing processes proceed in the presence of the following enzymes: dehydrogenase, urease, catalase, polyphenoloxidase and peroxidase [6]. Microbial oxidation of oil hydrocarbons occurs via a series of catalytic processes to yield intermediate products of metabolism – alcohols, aldehydes, ketones, fat and carboxylic acids, which undergo further oxidation to give CO_2 as the end product [1].

Naturally due to microbiological and physicochemical factors hydrocarbon destruction provides self-purification of oil-polluted soils. In the environment conditions such processes occur very slowly, over a long period of time especially in the regions with lower temperatures and a short vegetation period.

In order to regulate processes of hydrocarbon biodegradation one should first of all activate microbial associations and enhance the growth conditions for their existence.

Temperature and light are among the factors stimulating vital activity of soil microorganisms. Under the conditions of frigid and moderate climate photoluminescent polymer films are widely used as a covering material [7]. It has been determined that a sunbeam transmitted through a photoluminescent film transforms a part of ultraviolet spectrum into a red region with a peak at 615 nm [8]. The study on photosynthesis intensity using photoluminescent films was carried out with cucumber and tomato leaves. It demonstrated that carbon dioxide absorption per a unit of leaf area increased from 6 to 15% as compared to conventional films [9].

We have revealed the stimulating effect of photoluminescent films not only on plants but also on

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Printed in Kazakhstan

vital activity of soil microflora including microflora of oil-polluted soils.

Thus, it seems to be possible to use the factors activating vital functions of hydrocarbon-oxidizing microflora to develop environmentally appropriate methods intended to restore productivity of oil-polluted soils.

The objective of the present study is to investigate the effect of photoluminescence polymer films on oxigenase activity of microorganisms in oil-polluted soils.

Materials and Methods

Mixed samples of upper layers of hothouse and sod-podzol soils from a forest zone were used to simulate microbiological destructive processes. The soil was polluted with oil recovered from Las-Eganskoye oil field, West Siberia, at concentration of 5% (50 g/kg). Oil viscosity was 14.1 mPa·s and density amounted to 0.861 g/cm^3 at 20°C.

Number changes were investigated using three major groups of microorganisms providing soil fertility, *i.e.* heterotrophic bacteria, actinomycetes and fungic microflora (micromycetes). Their numbers were determined via inoculation on selective agar-like media [10].

Within the experiment the weighed portions of oil-polluted soil (1000 g) were placed into sterile vessels sized 30×15×7 cm and covered with photoluminescence films L-50 and FE. The FE film contained organic luminophore – europium nitrate complex with phenanthroline. The L-50 film included yttrium phosphate vanadate activated with europium, as an inorganic luminophore. The soil was moistened up to 30% from the complete moisture capacity and this was kept constant to the end of the experiment. The soils of open reservoirs and under ordinary hothouse high-pressure polyethylene (HPPE) film served for control. All films used during the experiment were 0.12 mm thick. Ultraviolet lamp "PHILIPS-BLACK LIGHT" (9 W) was used as an irradiation source. Test samples were exposed to ultraviolet irradiation for 6 hours a day. The experiment was carried out for 40 days at 18-20°C.

Before the experiment oil-polluted soil was carefully mixed and a sample was taken to determine the initial pollution, microflora population and activity of oxygenase group enzymes (catalase, dehydrogenase, polyphenol oxidase, peroxidase, and urease) providing destructive processes in oil hydrocarbons [11].

Catalase activity was determined by a gasometric method based on the measuring of decomposition rate of hydrogen peroxide. Dehydrogenase, urease, polyphenol oxidase and peroxidase activities of the soil were determined by a photocolorimetric method [9].

Quantitative and qualitative assessments of oil hydrocarbons destruction were carried out by IR-spectroscopy and gas chromatography. Oil from polluted soil samples was extracted with chloroform in a Soxhlet's apparatus. Chloroform was removed on a rotary evaporator. The extracted oil was weighed using an analytical balance. The values obtained for experimental and control tests were compared with the initial pollution.

IR spectra for the residual hydrocarbons and of the initial pollution were recorded using IR-spectrometer Nicolet 5700 (FTIR) [10]. Each sample was scanned 64 times, the measurement continued for 76.5 seconds, resolution was 4.000 and interpolation level was equal to 0. The spectra were recorded ranging from 400 to 4000 cm^{-1} [12].

Chloroform extracts of polluting oil hydrocarbons were subjected to additional purification with hexane on a column packed with aluminum oxide. The residual content of individual saturated acyclic hydrocarbons (*n*-alkanes) was determined using gas chromatograph 3700 equipped with a flame-ionization detector and a capillary column 25 m and 0.25 mm in diameter using SE 54 as a fixed phase. The analyses were performed using temperature programming from 50 to 290°C, where helium was used as a carrier gas. Individual hydrocarbons were identified taking into account the known elution orders and the values of retention indexes [13,14].

Hexadecane, 5-10 times diluted with chloroform, was used as a standard. For quantitative determination chromatographic analyses were carried out twice – with and without hexadecane.

Based on the data of chromatographic analysis we plotted histograms of molecular-mass distribution of the saturated hydrocarbons. A destruction degree (K_{biod}) was calculated by the ratio of the sum of isoprenoids (pristan + phytane) to the sum of *n*-alkanes ($C_{17} + C_{18}$) [15].

Results and Discussion

In adaptive period after soil pollution with crude oil at oil concentration up to 50 g/kg the population of soil microflora (heterotrophs, actonomyces and

micromyces) increased by an order of magnitude. The population increased mainly due to reproduction of hydrocarbon-oxidizing microflora, which used oil hydrocarbons as energy and nutrition sources [16]. Due to the use of FE and L-50 photoluminescent films microflora of oil-polluted soils increased by 2 orders of magnitude (Fig. 1).

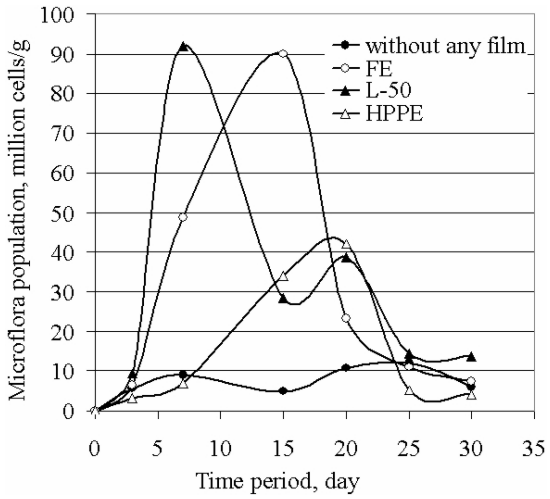

Fig. 1. Dynamics of heterotrophic hydrocarbon-oxidizing microflora growth in oil-polluted soils due to the use of photoluminescent films.

The increase in total number of microorganisms correlated with their destructive activity and metabolism processes. Redox processes proceeding in the presence of different ferments are known to promote general destruction of oil hydrocarbons in the oil-polluted soils.

Oxidoreductases including catalase, dehydrogenase, peroxidase and polyphenol oxidase are the main enzymes affecting oil hydrocarbons destruction. Dynamics of their activities in the test and control soil samples is presented in Table 1. As is seen from this table, catalase and peroxidase exhibited their maximum activity on the 30-th day of the experiment. The level of their activity is a dynamic parameter of self-purification capacity of soils from oil pollution. Dehydrogenase directly participated in the decomposition of the saturated acyclic hydrocarbons. On the 30-th day of cultivation its activity increased from 0.4 to 1.1 mg/g due to the effect of FE and L-50 photoluminescence films, while it was 0.45 mg/g in the control (Table 1).

By the end of the experiment the activity of catalase, providing oxidizing processes of oil biodegradation in the test soil samples with highly active oxygen, increased from 0.18 to 8.2 mL/g, while that in the control variants it did not exceed 2-4 mL/g (Table 1). Peroxidase and polyphenol oxidase enzymes participated in the conversion of hydrocarbon compounds of aromatic series into humus components. As affected by photoluminescent films their activity increased up to 0.6 mg/g and 0.41 mg/g, respectively, that was 1.5-2 times higher as compared with the control values (Table 1).

Urease plays a significant role in the nitrogen balance of soils. Urease activity is related to the processes of hydrolysis and nitrogen conversion into a form available for plants and microorganisms. One can use the increased urease activity as a diagnostic indicator at the estimation of self-purification capacity of soils from oil pollutions.

Table 1 presents the dynamics of urease activities in the test and control variants. Urease activity increased from 0.3 to 2.2-3.0 mg/g and correlated with the growth of heterotrophic microorganisms. In the experiments without any film and when HPPE film was used the activity of this enzyme did not exceed 0.7 mg/g.

The use of photoluminescence films increased the activities of redox and hydrolytic enzymes and thereby stimulated general oxygenase activity of microorganisms, providing soil remediation from oil pollution. The estimation of biodegradation processes demonstrated that in the test variants oil utilization in 40 days amounted to 30-35 g per 1 kg of soil and in the control variants it was 8-13 g/kg (Fig. 2).

IR-spectrometry is of great importance for petrochemical analyses. It is one of the most informative and sensitive methods for the analysis of composite organic mixtures. IR-spectra reveal practically

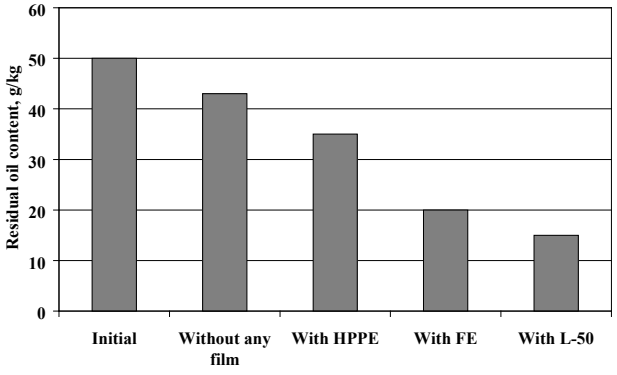

Fig. 2. Residual oil content (g/kg) of polluted soil at the beginning (column "initial") and after 40 days in the different batchers of the experiment.

Table 1
Enzymatic activity of oil-polluted soil with use of photoluminescence films

Test days	Enzymes	Control (without film)	Control (with HPPE film)	FE film	L 50 film
Initial	Catalase, mL/g	0.18±0.01			
	Dehydrogenase, mg/g	0.41±0.01			
	Urease, mg/g	0.28±0.02			
	Peroxidase, mg/g	0.27±0.03			
	Polyphenol oxidase, mg/g	0.14±0.01			
10	Catalase, mL/g	1.58±0.08	1.65±0.06	3.64±0.05	4.3±0.04
	Dehydrogenase, mg/g	0.29±0.02	0.31±0.01	0.39±0.01	0.41±0.01
	Urease, mg/g	0.52±0.09	0.52±0.09	0.81±0.02	0.89±0.03
	Peroxidase, mg/g	0.26±0.01	0.28±0.04	0.49±0.02	0.51±0.04
	Polyphenol oxidase, mg/g	0.17±0.03	0.18±0.02	0.27±0.01	0.31±0.02
20	Catalase, mL/g	2.01±0.15	2.79±0.21	5.35±0.17	5.96±0.09
	Dehydrogenase, mg/g	0.31±0.01	0.38±0.03	0.67±0.03	0.68±0.02
	Urease, mg/g	0.67±0.15	0.72±0.19	1.51±0.02	1.62±0.05
	Peroxidase, mg/g	0.22±0.02	0.26±0.02	0.52±0.03	0.58±0.03
	Polyphenol oxidase, mg/g	0.15±0.01	0.17±0.01	0.31±0.01	0.32±0.04
30	Catalase, mL/g	2.1±0.14	4.01±0.13	8.24±0.32	7.74±0.21
	Dehydrogenase, mg/g	0.39±0.02	0.43±0.01	0.98±0.01	1.01±0.02
	Urease, mg/g	0.71±0.01	0.81±0.02	2.18±0.05	2.72±0.41
	Peroxidase, mg/g	0.23±0.01	0.28±0.03	0.61±0.05	0.62±0.02
	Polyphenol oxidase, mg/g	0.16±0.03	0.17±0.02	0.39±0.03	0.41±0.04
40	Catalase, mL/g	1.29±0.04	1.99±0.02	6.89±0.04	6.81±0.06
	Dehydrogenase, mg/g	0.40±0.01	0.44±0.02	1.10±0.01	1.10±0.02
	Urease, mg/g	0.70±0.02	0.8±0.02	2.15±0.05	3.00±0.03
	Peroxidase, mg/g	0.19±0.02	0.25±0.01	0.58±0.03	0.59±0.01
	Polyphenol oxidase, mg/g	0.15±0.02	0.16±0.02	0.37±0.04	0.39±0.04

all typical absorption bands of the main functional groups of oils.

Figure 3 demonstrates that in the experiments with photoluminescent films one has revealed considerably greater amount of oxygen-containing compounds (alcohols, aldehydes and carboxylic acids), which are considered as intermediate products of metabolism at the oxidation of *n*-paraffins. It is evident from absorption bands observed in the regions of 1710 cm^{-1} and 1600 cm^{-1}, indicating intensification in the processes of microbial oxidation of oil (Fig. 3).

The spectral coefficients C_1 and C_2 reflect the ratio between aromatic and *n*-paraffin hydrocarbons (Table 2). The CH_2/CH_3 value in paraffin chains in the absorption regions 1380 and 720 μm determines a degree of hydrocarbon oxidation. As a result of oil oxidation in the presence of photolumiscent films the coefficients C_1 and C_2 increase by a factor of 2.5-3 and the coefficients A_1 and A_2-by a factor of

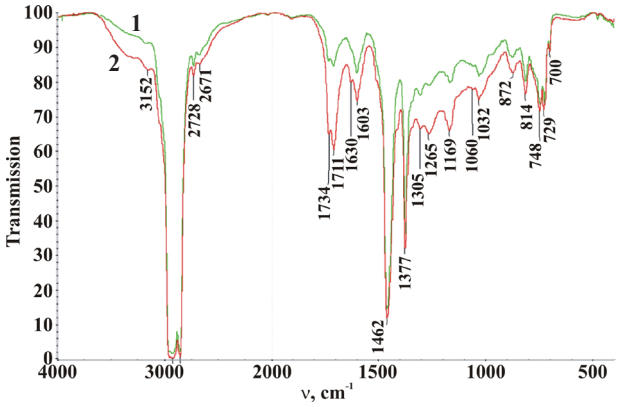

Fig. 3. IR spectra of the residual oil components extracted from the soil: under HPPE (1) film and under photoluminescence film FE (2).

1.5-2. The coefficients C_3 and A_3 decrease by a factor of 2.5-1.4, respectively. Such changes are due to oil aromaticity increase and a decrease in the amount of *n*-alkanes during a biodestruction process (Table 2).

Comparative chromatographic analysis of the saturated acyclic hydrocarbons (*n*-alkanes) in the initial and test samples of oil-polluted soil also proved significant changes in their contents and compositions (Fig. 4). The Figure 4 presents histogram reflecting the concentrations of individual oil hydrocarbons nC_{11}-nC_{30}. Iso-alkanes are presented with pristane (iC_{19}) and phytane (iC_{20}), as biomarkers required in determining a coefficient of oil hydrocarbons biodestruction. As is seen from Figure 3 during biodegradation n-alkanes with a chain length of C_{11}-C_{14} were completely eliminated from the test soil samples and the content of hydrocarbons with greater molecular weight decreased by 70-80%. The concentration of *n*-alkanes under HPPE film is decreased. Against the background of significant decrease in the content of *n*-alkanes relative concentration of phytane (iC_{20}) and pristane (iC_{19}) peaks as if it increases. However their ratio (iC_{19}/iC_{20}) remains nearly unchanged in all the experiments. At the same time a biodegradation coefficient for the initial pollution amounts to 0.6, in the control soil samples (without any film)-0.8, in the test with HPPE film-1.0, with FE film-4.8 and with L-50 film-5.2 indicating deep oxidation processes in oil hydrocarbons.

Conclusions

Summarizing the experimental results obtained on the use of FE and L-50 photoluminescent films as a covering material one can conclude about stimulating effect of the radiation, transformed by film luminophors, on vital and oxygenase activities of microflora in oil-polluted soils.

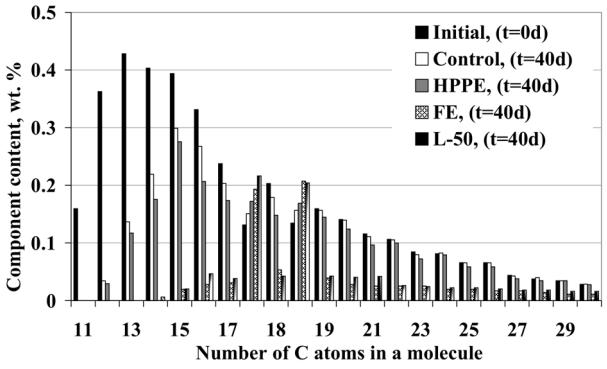

Fig. 4. Content (wt.%) of individual hydrocarbons (*n*-alkanes and pristine and phytane) in the different batches of the lab experiments.

Table 2
Spectral characteristics of oil recovered from Las-Eganskoye oil field after biodegradation in the presence of photoluminescence films

Spectral coefficients	Initial (t = 0 d)	Control (t = 40 d)	HPPE film (t = 40 d)	FE film (t = 40 d)	L 50 film (t = 40 d)
C_1 = D1610/D720	0.33	0.5	0.62	1.68	1.76
C_2 = D750/D720	0.62	0.8	0.88	1.84	1.88
C_3 = D720/D1380	0.45	0.4	0.37	0.2	0.22
A_1 = D815/D750	0.86	0.92	0.97	1.3	1.32
A_2 = D875/D750	0.45	0.6	0.64	1.1	1.07
A_3 = D815/D875	1.9	1.54	1.53	1.1	1.2

IR-spectrometric and chromatographic analyses proved photoluminescent films to accelerate biodestruction of soil-polluting hydrocarbons by a factor of five.

The revealed effect of photoluminescent enzymatic activation of microorganisms can be used for the development of environmentally safe methods for remediation of oil-polluted soils in relatively small areas.

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Received 12 February 2007