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Gas chromatographic investigation of oil biodegradation degree

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Abstract

The research of the degree of oil biodegradation by gas chromatography and individual classes of hydrocarbons by various strains of hydrocarbon-oxidizing microorganisms isolated from indigenous microflora of oil fields was carried out. It has been shown that some of the investigated strains of hydrocarbon-oxidizing microorganisms are 100% capable to biotransform naphthenes and olefins, showing high activity in the destruction of paraffins and isoparaffins. There are no signs of biodegradation of aromatic compounds due to the large duration of the process. All investigated strains of hydrocarbon-oxidizing microorganisms are largely able to reduce the total number of individual components of oil. The obtained data can be used to develop new biologics of the purpose.

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Keywords: biodegradation, hydrocarbon-oxidizing microorganisms, gas chromatography

1. Introduction

At various stages of production and operation of oil and gas processing complex (prospecting, exploration, mining, oil and gas) emergencies resulting polluting with petroleum hydrocarbons and their products are not excluded. The soil is especially affected, resulting in deterioration in its morphological and physicochemical properties, oppression of self-purification ability and negative changes in the development and functional activity of organisms of soil biocenosis. Emergency and chronic oil spills lead to a rapid loss of land productivity or total degradation of the landscape. Limited land resources put an urgent task of returning all disturbed and degraded soils to agricultural use[1,2].

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Intensification of microbiological methods of oil degradation takes an important place among the measures taken to clean the environment from oil pollution. It is based on application of microorganisms which are able to use petroleum hydrocarbons as a sole carbon source. This assumes not only the activation of indigenous microflora of contaminated sites, but also the introduction of biological products containing strains of active oil destructors. It is important to know the mechanisms and the degree of bio-oxidation of different classes of petroleum hydrocarbons by specific genera of hydrocarbon-oxidizing of microorganisms[3]. Analyzed changes occurred in microbial communities in contaminated soils by determining the number of microorganisms of different physiological groups, isolation and identification of microorganisms-destructors of hydrocarbons with a parallel chemical analysis of the residual oil content in it allow developing speedup pollutant conditions and efficiency of the microbial degradation process in oil-contaminated soils, in which soil disturbance returns to a steady state[2].

Variety of biologics market makes it necessary to study the rate and extent of degradation of hydrocarbon contaminant by strains of hydrocarbon-oxidizing microorganisms, and to select the most effective and adjusted to weatherization of recovering areas ones[4].

The aim of this study is to determine the amount of degradation of individual classes of petroleum hydrocarbons by various strains of hydrocarbon-oxidizing microorganisms isolated from indigenous microflora by the method of gas chromatography.

2. Materials and methods

In this work 10 strains of hydrocarbon-oxidizing microorganisms (HOM) isolated from oil-contaminated soil samples selected during the work on bioremediation oil-polluted soil and water in the fields of Strezhevoy (Tomsk region) together with "Ecoil" (Tomsk) were used. The laboratory of "Genotech" (Moscow) defined taxonomic affiliation of pure cultures of microorganisms on the basis of the analysis of gene fragments of 16S pRNA, their modification and primary bioinformatic analysis. HOM strains were assigned the following code titles: 3a, 33, 42, 131, 21, 32, 3b, 312, 6a, 61 for commercial confidentiality.

Pure cultures were seeded into liquid mineral medium of Adkins (Table 1) with the addition of oil as a sole carbon source. As a substrate oil from the Zapadno-Katylginskoye field (Strezhevoy, Tomsk region) was used.

Table 1. Composition of mineral medium of Adkins

Substance	Concentration g/l
KH_2PO_4	0.75
K_2HPO_4	1.50
NH_4Cl	1.00
$MgSO_4 \cdot 7\ H_2O$	0.20
KCl	0.10

Culturing was carried out on the model samples within 14 days by a stirred thermostatic device LAB PU-01 at the speed of 85-90 rev/min at the temperature of 30-32 ^{0}C. Oil of water surface was extracted with carbon tetrachloride and analyzed for the content of residual hydrocarbons by gas chromatography with Chromatec-Crystal device 5000 under the following conditions:

- Column length - 100 m;
- Column diameter - 0.25 mm;
- Evaporator temperature – 290 ^{0}C;
- Column oven temperature - from 0.2 to 290 ^{0}C;
- Carrier gas - helium;
- Carrier gas flow - 315 ml/min;
- Injection size - 0.2 μl;
- Division of the flow - 200:1;
- Detector - flame-ionization detector;
- Total time of analysis - 150 minutes.

3. Results and discussion

Oil is a complex multi-component system containing about 200 -250 individual components of all classes of hydrocarbons. The control of the oil degradation process by hydrocarbon-oxidizing microorganisms was carried out by sampling a liquid-liquid extraction with carbon tetrachloride preliminary acidification of the medium (until pH = 2) for a complete killing of micro-organisms and subsequent gas chromatography analysis. The residual content and the degree of degradation of components and oil separate hydrocarbon classes were judged according to the analysis. The results of the chromatographic analysis are shown in Table. 2.

Table 2. Contents of individual classes of residual hydrocarbons and the degree of biodegradation of oil in 14 days

Sample		Oil	3a	33	42	131	21	32	36	312	6a	61
Contents of hydrocarbons classes, % wt.	Number of components	200	30	30	35	35	38	24	46	46	32	70
	Paraffins	26.21	29.15	21.39	18.89	20.45	14.79	28.07	52.19	20.79	25.46	8.35
	Isoparaffins	19.93	1.19	9.48	12.38	10.71	11.41	11.65	8,02	4.60	9.21	14.17
	Aromatics	34.82	64.18	66.24	65.98	60.66	65.37	56.41	39,79	68.57	59.07	73.46
	Naphthenes	14.95	0	0	0.94	0	0	0	0	2.01	0	2.30
	Olefins	4.09	5.48	2.89	1.81	8.18	8.43	3.87	0	4.03	6.26	1.72
Degree of degradation, %	Number of components	–	85.0	85.0	82.5	82.5	81.0	88.0	77,0	77.0	84.0	65.0
	Paraffins	–	–	18.3	27.9	22	43.6	–	–	20.7	3	68
	Isoparaffins	–	94	52.4	37.8	46.2	42.7	41.5	59,8	76.9	53.8	28.9
	Aromatics	–	–	–	–	–	–	–	–	–	–	–
	Naphthenes	–	100	100	93.7	100	100	100	100	86.5	100	84.6
	Olefins	–	–	29.3	55.7	–		5.3	100	1.5	–	57.9

Table 2 shows that all studied microbial strains demonstrate high activity of naphthenes destruction because of the simple structure of the carbon skeleton of this class of hydrocarbons. Strains 3a, 3b, and 312 are the most active in the destruction of isoparaffins, in the process of destruction they reduce concentration of isoalkans by 94%, 59.8% and 76.9%, respectively. The strain of hydrocarbon-oxidizing microorganisms codenamed 3b is the only one of all strains capable to 100% olefin hydrocarbons destruction. Besides, it promotes minimum accumulation of aromatic hydrocarbons in the biotransformation, which is important due to the arenes toxicity.

Strain 42 showed the highest activity in the destruction of all classes of hydrocarbons in general. Strain 61 is also effective in degradation of all classes of hydrocarbons, but the process of biotransformation in this case takes place with the formation of a greater proportion of aromatic compounds and a smaller decrease in the total number of individual components. Strains 3a, 33, 32 and 6a are able to realize biotransformation of different hydrocarbon classes to a smaller number of common individual components in the mixture. Strains 61, 21 and 42 possess the highest activity to degrade paraffins.

In the case of aromatic hydrocarbons, only their complete accumulation and no signs of degradation were observed. This is explained by a short process period (14 days) because microbiologic destruction of aromatics is very slow and has different intensity for different arenes. The experimental oil originally contained mono-, di-, tri- and tetra-substituted benzene, indane and naphthalene belonging to slowly erodible hydrocarbon; their resistance to biodegradation was the higher, the greater the number of rings was in a molecule.

The standard deviation (SD) was calculated by 6 parallel values of the content of individual classes of residual petroleum hydrocarbons in the process of biodegradation. The following formula was used:

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

x_i — i-th element of the sample; n — sample number; $\bar{x}$ — sample arithmetical mean

Results of calculations are presented in Table 3.

Table 3. Standard deviation (%) for indexes of the content of individual classes of residual petroleum hydrocarbons in the process of biodegradation

Sample	The standard deviation, %					
	Number of components	Paraffins	Isoparaffins	Aromatics	Naphthenes	Olefins
Oil	0.22	0.03	0.03	0.02	0.03	0
3a	6.32	1.47	14.75	0.79	0	13.70
33	4.71	4.24	9.87	2.40	0	83.10
42	4.78	1.74	4.50	0.52	33.32	17.77
131	4.78	4.36	8.19	3.73	0	38.47
21	4.99	9.56	9.22	1.89	0	10.02
32	4.99	7.88	5.62	2.01	0	57.33
3б	3.89	2.87	7.91	4.17	0	0
312	4.35	7.38	10.42	0.70	21.54	23.63
6a	7.13	3.90	11.36	0.95	0	19.36
61	1.81	6.50	6.66	1.18	10.71	44.13

The resulting SD values are acceptable. Large values of the standard deviation (in some cases up to 83%) for indicators of residual content of individual classes of petroleum hydrocarbons in the process of biodegradation can be explained by extraordinary complexity of creating ideal conditions for the same living organisms to produce identical results.

4. Conclusion

Thus, the investigation of the degree of oil biodegradation by gas chromatography and individual classes of hydrocarbons by various strains of hydrocarbon-oxidizing microorganisms isolated from indigenous microflora of oil fields was carried out. 100% activity of all strains demonstrates the ability to degrade naphthenes. High activity of some of them can lead to the destruction of isoparaffin hydrocarbons. One of the strains showed olefins selective biotransformation. Lack of the biodegradation aromatics process can be explained by short time of the experiment. As a result of the study it can be assumed that the strains of hydrocarbon-oxidizing microorganisms with codenames 3a, 42, 61 and 3b in complex will promote the greatest degree of oil degradation.

The findings of the experiment data will be used to develop new biological products containing studied strains of hydrocarbon-oxidizing microorganisms oriented to the specific physico-chemical parameters of pollution.

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