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The Evaluation of Process of Bioremediation of Oil-Polluted Soils by Different Strains of Pseudomonas

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Abstract. The determination of content hydrocarbon of diesel fuels and individual hydrocarbons in oil-polluted soil before and after bioremediation by oil-oxidational microorganisms was carried out by method of chromato-mass-spectrometry. It has been shown that changes in composition hydrocarbons of diesel fuels and individual hydrocarbons in soil observe during growth of microorganisms on this soil.

Introduction

Biotechnological methods of soil's purification, polluted with oil and oil products, is based on the ability of some aerobic microorganisms to utilization of oil and oil products as only source of carbon for nutrition on oxidation its step-by-step to simple compounds – shot chain hydrocarbons, alcohols, acids and other. The bioremediation's technology are ecological safely, give a possibility for the degradation of pollutants to harmless intermediate products at fully retained structure of soil and without additional environment's pollution. Use of this technology is limited by large duration of the process and dependence it's on natural-climatic factors [1].

There are in Russia a various biopreparations for cleaning soil and waters polluted of oil and oil products such as Valentis, Olevorin, Ecodin, Devoroil, Bioset, Putidoil and etc. [2]. In our Republic also carried out investigations on creation and using biopreparations for cleaning oilpolluted soil [3], as microbial preparation "Munaibak" and others [4]. Nevertheless, a question about chemical mechanisms of conversion of different hydrocarbon's groups of during biodegradation of oil and oil products, methods of determination of intermediating products are still unsettled [5].

Material and methods

In the present work are given results of laboratory experiments on investigation of biodegradation's hydrocarbons of diesel fuels and individual hydrocarbons processes in soil before and after the bring into aerobic oiloxidative microorganisms by method of chromato-mass-spectrometry. Summer brands of diesel fuels of joint stock company "PetroKazakhstan Oil Products", Pavlodar petrochemical factory (PPCF) and Russian refinery "LukOil" were chosen as hydrocarbon raw materials. The choice of the given fuels is conditioned by that they are characterized by different chemical composition and properties. Bacterial cultures *Pseudomonas mendocina H-3* and *Pseudomonas alcaligenes H-15* were isolated from oil-polluted soil [6, 7]. Cultivation was carried out on liquid medium *E-8* with adding diesel fuel as a source of carbon and energy in concentration of 5 and 10 % per 100 ml of medium suspension. Analysis was carried out on Agilent 6890N gas chromatograph with Agilent 5973N mass-spectroscopic detector. Separation was carried out on DB-XLB 30m x 0.25 mm x 0.50 μm column in the regime of programming of the temperature: 40 .C – 10 min, 2 .C/min to 205 .C – 20 min. Ion range –10-300. Sample – 2 mkl with flow separation of 1:50. Gas-carrier – helium. Flow rate – 1 ml/min.

Results and discussion

Chromato-mass-spectroscopic analysis of soils in three samples: initial fuels, after 1st and 2nd months of interaction of microorganisms with oil hydrocarbons of soil has shown that spectra have long intensive picks of normal saturated hydrocarbons. Picks with lower intensity correspond to branchy saturated cyclic and aromatic hydrocarbons. In spectra of diesel fuel of Pavlodar petrochemical factory intensity of cyclic and aromatic hydrocarbons is high in comparison with other fuels.

Table 1 shows results of chromato-massspectrometric analysis of biodegradation of the fuel of Pavlodar petrochemical factory. According to the obtained data a distinctive feature of microorganisms' action is that they promotes chemical change of liquid saturated light alkanes. For example, due to biodegradation amount of normal saturated paraffines to tetradecane (C14H30) in diesel fuels of Pavlodar petrochemical factory and joint stock company "PetroKazakhstan Oil Products" and amount of *n*-alkanes to cetane (hexadecane C16H34) in diesel fuel of Russian refinery decrease, and amount of heavy solid *n*-paraffines increases. Due to action of *Pseudomonas alcaligenes H-15* a total amount of saturated hydrocarbons decreases, and *Pseudomonas mendocina H-3* contributes to decreasing amount of individual paraffines. In initial diesel fuels a total amount in diesel fuel of Pavlodar petrochemical factory decreases, and in diesel fuel of Russian refinery increases. During interaction of microorganisms' strains isoparaffineous hydrocarbons with large amount of carbon atoms increase and amount of light isoalkanes to 2-methyltridecane (iso-C14H30) decreases after their biodegradation. Increase in a total amount of cycloparaffinaceous hydrocarbons in diesel fuel of Pavlodar petrochemical factory and also their decrease in diesel fuel of joint stock company "PetroKazakhstan Oil Products" is a quite important result. Here one should say that content of cycloalkanes to hepthylcyclohexane (C13H26) decreases and mass fraction of higher cyclic hydrocarbons increases. Diesel fuel of Pavlodar petrochemical factory in comparison with others is different with high content of aromatic hydrocarbons where mass fraction of arenes amount to 11 % and in two other fuels – 4 %. As a result of biodegradation content of aromatic hydrocarbons increases in all diesel fuels. It should be noted that fraction of 1- and 2- methylnaphthalenes decreases and amount of other aromatic hydrocarbons increases and new aromatic hydrocarbons as 1,6,7-trimethylnaphthalene and 1,3-dimethylbenzene appear. At large, data obtained by chromato-massspectroscopic analysis have showed that content of n-alkanes decreases and content of iso-, cycloalkanes and aromatic hydrocarbons increases under influence of hydrocarbon oxidation microorganisms in the composition of diesel fuels. For more detail investigation of chemization of biodegradation there were carried out experiments with individual hydrocarbons: *n*-decane and toluene. The choice of the data of hydrocarbons is conditioned by that they belong to different classes of hydrocarbons – paraffines and aromatic hydrocarbons. Bacterial culture *Pseudomonas mendocina H-3* was extracted from oil-polluted soil substrates. Cultivation was carried out on liquid synthetic medium E8 with adding diesel fuel as a source of carbon and energy of hydrocarbons with concentration of 1 % (solution № 1), 5 % (solution № 2) and 10 % (solution № 3) per 100 ml medium suspension. After strains' interaction for 5 and 10 days samples of solutions with microorganisms and check solution (without microorganisms) with a volume of 10 ml were shaken *(mixed)* with 5 ml hexane for 10-15 min and centrifuged for 30 min at 4000 rev/min. Aliquot of upper hexane layer was selected in tightly closed test tubes for carrying out chromato-massspectroscopic analysis. Table 2 shows results of chromato-massspectroscopic analysis of n-decane biodegradation. In the first solution content of *n*-decane is 1.15 %, and in the third one is 11.2 %. The main part of the solution consists of hexane (solvent), its isomers (2-and 3-methylpentanes) and *n*-heptane. *N*pentane, 2,4-dimethylpentane, 2- and 3-methylhexane, methylcyclopentane, 2-pentanol and 2-pentanon are contained in small quantities in solutions. After interaction of *Pseudomonas mendocina H-3* strain for 5 days *n*-decane wasn't discovered in the first solution, in the third one its content decreases from 11.22 % to 2.72 %. At that, content of *n*hexane increases in both solutions. Amount of 2- and 3-methylpentanes in the solution № 1 decreases and in the solutions № 3 increases. In 10 hours n-decane was again discovered in the first solution in amount of 0.37 %, at that, content of *n*-hexane and its isomers, 2-methylhexane is more than in the initial solution. Content of *n*-heptane decreases.

All these data testify about that destruction of normal paraffines with a large amount of carbon atoms (C10 and C7) occurs with formation of hydrocarbons and their isomers with a small amount of carbon atoms (C6).

Table 1. Results of chromato-mass-spectrometric analysis of diesel fuel of Pavlodar petrochemical factory (PPCF) before and after interaction of *Pseudomonas mendocina H-3* (1 month) and *Pseudomonas alcaligenes H-15* (2 months)

Compounds and classes of compounds	Primary DF PPCF	In 1 month with H-*3*	In 2 month with H-*15*
NORMAL ALKANES			
Decane	0.979	0.620	0.955
Undecane	2.466	2.237	2.472
Dodecane	3.456	3.402	3.520
Tridecane	4.792	4.769	4.957
Tetradecane	5.321	5.742	6.014
Pentadecane	4.482	4.505	4.704
Hexadecane	4.616	4.647	4.933
Heptadecane	7.156	7.153	7.519
Oktadecane	5.885	5.923	2.721
Nonadecane	2.816	2.874	3.036
Eikosane	2.372	2.419	2.576
Heneicasane	1.615	1.600	1.702
N-ALKANES TOTAL	45.956	45.891	45.109
ISOALKANES			
Methyl alkanes			
4-methyldecane	0.332	0.225	0.254
2-methylundecane	0.450	0.440	0.447
4-methylundecane	0.427	0.389	0.383
5-methylundecane	0.905	0.796	0.808
4-methyldodecane	0.446	0.411	0.433
2-methyltridecane	0.535	0.567	0.601
3-methyltridecane	0.248	0.263	0.267
4-methyltridecane	0.499	0.549	0.417
7-methyltridecane	1.636	1.660	1.686
3-methyltetradecane	0.463	0.462	0.499
2-methylpentadecane	0.573	0.540	0.617
3-methylpentadecane	0.255	0.250	0.296
4-methylpentadecane	0.957	0.814	0.858
2-methylhexadecane	0.772	0.769	0.840
3-methylhexadecane	0.404	0.613	0.483
2-methylheptadecane	0.691	0.722	0.758
Methylalkanes total	9.593	9.470	9.647
Di-, tri- and tetramethyl alkanes			
2,4-dimethylundecane	0.332	0.326	0.338
2,6-dimethylundecane	2.278	2.103	2.133
2,6,10,14-tetramethylhexadecane	0.844	0.837	0.863
Total of di-, tri- and tetramethylalkanes	3.454	3.266	3.334
ISOALKANES TOTAL	13.047	12.736	12.981
CYCLOALKANES			
Butylcyclohexane	-	0.224	0.279
Hexylcyclohexane	0.332	0.320	0.329
Oktylcyclohexane	0.704	0.727	0.721
2-butyl-1,1,3-trimethylcyclohexane	0.233	0.236	0.235
2-methyldecahydronaphthalene	0.297	0.253	0.271
Cyclododecane	-	0.232	0.247
Cyclotetradecane	0.332	0.313	0.340
CYCLOALKANES TOTAL	1.898	2.305	2.422
AROMATIC HYDROCARBONS			
1,3-dimethylbenzene and p-xylol	-	0.467	-

1-methylnaphthalene	2.021	1.950	1.965
2-methylnaphthalene	1.672	1.609	1.632
1,4-dimethylnaphthalene	1.227	1.219	1.265
1,5-dimethylnaphthalene	1.128	1.565	1.616
1,6,7-trimethylnaphthalene	0.437	0.403	0.473
2,3,6-trimethylnaphthalene	0.508	0.487	0.558
1,4,6-trimethylnaphthalene	-	0.346	-
2-ethyl-1,4-dimethylbenzene	0.481	0.365	0.393
1-ethylnaphthalene	0.402	0.402	0.443
1,2,3,4-tetrahydronaphthalene	2.142	2.019	2.134
Hepthylbenzene	0.455	0.438	0.486
TOTAL of AROMATIC HYDROCARBONS	10.473	11.270	10.965

Table 2. Results of chromato-mass-spectrometric analysis of *n*-decane biodegradation

Compound	Initial solutions		In 5 hours		In 10 hours
	№ 1	№ 3	№ 1	№ 3	№ 1
n-pentane	0.08	0.07	-	-	-
n-hexane	90.24	80.79	95.70	89.36	91.72
n-heptane	1.88	1.73	-	0.71	0.73
n-decane	1.15	11.22	-	2.72	0.37
2-methylpentane	1.37	1.27	0.25	1.49	1.47
3-methylpentane	4.45	4.12	0.79	4.71	4.67
2,4-dimethylpentane	0.05	0.05	-	-	-
2-methylhexane	0.32	0.32	-	0.34	0.34
3-methylhexane	0.17	0.16	-	-	-
methylcyclopentane	0.15	0.15	-	-	-
3-methyl-2-pentene	-	0.02	-	-	-
Ethanol	-	-	-	0.67	0.70
2-pentanol	0.02	-	-	-	-
2-pentanon	0.03	0.03	-	-	-
Dichlormethane	-	-	2.67	-	-
Total	99.91	99.93	99.41	100.0	100.0

Table 3 shows results of chromato-massspectrometric analysis of toluene biodegradation. In 5 and 10 hours toluene wasn't discovered in the solutions being investigated although in initial solutions its content was 1.73; 7.62 and 14.42 %. It testifies about its full destruction at interaction by *Pseudomonas mendocina H-3* strain.

Table 3. Results of chromato-mass-spectrometric analysis of toluene biodegradation.

Compound	Initial solutions			In 5 hours	In 10 hours		
	№ 1	№ 2	№ 3	№ 1	№ 1	№ 2	№ 3
n-pentane	0.08	0.07	0.06	-	-	-	-
n-hexane	89.48	84.02	77.88	92.58	90.36	92.55	92.67
n-heptane	1.84	1.86	1.73	0.74	0.71	0.73	0.71
n-decane	0.24	-	-	-	2.57	0.28	0.25
2-methylpentane	1.37	1.30	1.19	1.53	1.44	1.46	1.44
3-methylpentane	4.47	4.23	3.87	4.80	4.57	4.64	4.58
2,4-dimethylpentane	0.05	0.05	0.05	-	-	-	-
2-methylhexane	0.34	0.33	0.30	0.36	0.34	0.35	0.34
3-methylhexane	0.17	0.17	0.15	-	-	-	-
1-ethyl-1-methylcyclopropane	-	-	0.07	-	-	-	-
methylcyclopentane	0.16	0.15	0.14	-	-	-	-
3-methyl-2-pentene	-	0.11	-	-	-	-	-
2-hexen	-	0.03	0.02	-	-	-	-
Toluene	1.73	7.62	14.42	-	-	-	-
2-pentanon	0.03	0.03	0.03	-	-	-	-
Total	99.96	99.97	99.91	100.01	99.99	100.01	99.99

In the given case small quantities of cyclic (1-ethyl-1-methylcyclopropane, methylcyclopentane) and unlimited hydrocarbons (3-methyl-2-penten, 2-hexen) which disappear as a result of biodegradation are present in initial solutions. In 5 and 10 hours content of *n*-hexane, *n*-decane, 2- and 3-methylpentanes and 2-methylhexane increases and content of *n*-heptane decreases. It is explained by that there is also presumably taken place full destruction of toluene and *n*-heptane with formation of hexane and its isomers.

Summary

Thus, reached results show that microorganism's strains may use individual hydrocarbons in the composition of diesel fuels as a feeding substrates. It means that cells of *Pseudomonas alcaligenes H-15* and *Pseudomonas mendocina H-3* may be recommend for including in biopreparation for cleaning soils from hydrocarbons of oil and oil products. Method of chromato-mass-spectrometry is suitable for evaluation of bioremediation's efficacy on results of determination content of various hydrocarbons in soil during bioremediation's process.

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