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Investigation of black oil and diesel biodegradation in water

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The effectiveness of biopreparations in destruction of black oil and diesel contaminated water was analysed. The destructive ability of the preparation GVT was compared with that of another biopreparation, K-2000, and of the enzymatic preparation Roebic K-47, as well as of GVT and Roebic K-47 mixture. The prevalence of oil oxidising microorganisms in the case of black oil biodegradation in water was estimated. The biopreparation GVT was the fastest in oil destruction. When water was contaminated with diesel all biopreparations were equally effective.

Key words: black oil, diesel, biodegradation, oil oxidising microorganisms

INTRODUCTION

Oil and its refined products, which are composed of hundreds of chemical compounds, are subject to great changes due to the environmental impact and are among the most frequently occurring and most dangerous pollutants of natural ecosystems (Čipinytė et al., 1995). Oil hydrocarbons after entering water reservoirs induce essencial changes in the functioning conditions of the biological systems. Changes also the pH of the medium, aeration, living organisms are exposed to a toxic effect, the stability of the communities is violated, and the species diversity gets impoverished (Ignatavičius et al., 2006).

The capacity of assimilating oil hydrocarbons as a source of energy and carbon is characteristic of the representatives of many groups of microorganisms. Microorganisms, especially bacteria and some microscopic fungi that are decomposing hydrocarbons are important groups of organisms participating in the carbon metabolic cycle. Low concentrations of those compounds may stimulate the abundance of microorganism populations, since hydrocarbons are used as a reliable source of their nutrition. In the event of the average-level pollution, the amount of microorganisms gets reduced, their species composition and qualitative indicators of microbiological processes, for example, enzymatic activity, undergo changes. In the case of extensive pollution, only microorganisms resistant to hydrocarbons survive in water reservoirs (Kalėdienė, 1999; Repečkienė et al., 1999).

Part of the microorganisms that survive in a contaminated environment are able to partly or fully utilize oil hydrocarbons. Different biodegradation qualities of hydrocarbons predetermine the fact that during oil biodegradation its various components are decomposed at a different rate. Oil hydrocarbons, after entering water reservoirs as pollutants, join the natural communities and create new ecological links and relations stimulating the biodestructive activity of microorganisms, which help the water to self-clean and provide conditions for the development of new biotechnological methods for the treatment of contaminated environments.

Black oil is one of the refined oil products most hazardous for the ecosystems in water reservoirs. Its composition contains resins, asphaltens, and polycyclic arenas with lateral chains, solid paraffin, sulphur, and unsaturated compounds. These are slowly biodegrading fractions, therefore black oil survives in the water environment for a long time. A. Boronin and co-authors (Boronin et al., 1997), in the presence of a 2% contamination with black oil, decomposed this pollutant by using pure cultures of microorganisms oxidising oil products and determined that substrate degradation was not quite efficient: the most active strains of the cultures decomposed only 16–18% of black oil in the water within 10 days.

Seeking to accelerate the decomposition of oil hydrocarbons, natural microorganisms are supplemented with pure cultures of other microorganisms species actively oxidising oil products or their mixtures. Seasonal temperature changes and too big concentrations of oil products have a significant effect on the activity of microorganisms effectively oxidising oil pollutants in natural ecosystems (Song et al., 1990; Čipinytė et al., 1995; Grigiškis, Žunda, 2001). The artificially created *Arthrobacterium* sp. N3, NJ9 and M1 association decomposed heavy hydrocarbon substrates (oil and black oil) in fresh and saline waters more efficiently than did the solitary strains of the association. This

Arthrobacterium association is recommended for depuration of hydrocarbon pollutants from the environment: the mixed association decomposes oil and black oil 1.1–2.3 times more efficiently than do the solitary *Arthrobacterium* strains (Čipinytė, Grigiškis, 2000).

Biological and enzymic preparations are more and more frequently used for the decomposition of oil hydrocarbons. The basis of the majority of preparations is microorganisms isolated from the ground contaminated with oil or its products. The same preparations are used not only to purify water and ground from oil pollutants (Žukauskaitė et al., 2003), but also in the biological treatment of wastewater effluents (Skaisgirienė, Lapinskienė, 2003).

The objective of the present work was to study the effectiveness of the GVT preparation in the treatment of fresh and saline water contaminated with black oil and diesel and to compare it with other preparations – K-2000 and Roebic K-47 – as well as a mixture of GVT and the enzyme Roebic K-47.

MATERIALS AND METHODS

The biopreparation GVT is composed of seven strains of oil-oxidising microorganisms and aggregate. The total amount of microorganisms in this biopreparation is 1.5×10^8 col/cm^3. Suspension was produced from the biopreparation GVT by using a mineral medium (g/l): NH_4Cl – 2.0; NaCl – 5.0; Na_2HPO_4 – 3.,0; KH_2PO_4 – 0.1; $MgSO_4 \times 7H_2O$ – 2.0; $CaCl_2 \times 6H_2O$ – 0.01; $MnSO_4 \times 5H_2O$ – 0.02; $FeSO_4 \times 7H_2O$ – 0.01; black oil – 10.0; 1 liter of the suspension is inoculated with ten grams of the biopreparation. It was grown in a shaker at 25–27 °C for seven days. Additionally, 100 ml of suspension was introduced every 30 days.

The amount of microorganisms in the biopreparation K-2000 was 1.5×10^{11} col/cm^3. In the enzyme Roebic K-47, which is designed for aerobic degradation of organic pollutants, the total number of microorganisms was 3.5×10^7 col/cm^3. In all cases, biopreparation suspensions were produced in an analogous way as in the case of GVT (the same mineral medium).

Water under study in each case was contaminated with 10 g/l of oil products (black oil or diesel). Distilled water mixtures with the specific oil products were aerated in a shaker. Biopreparation suspensions used 50 ml/l water. The total volume of each sample was 2 liters. 24 hours after contamination of water with black oil or diesel, the concentration of these oil products, which was taken as a starting point of experimental investigation, was evaluated. The course of destruction of oil products was controlled by analysing the samples under examination every 30 days (total duration 150 days) and by the spectrophotometric method. The samples were taken by 5 ml, with the previous stirring of the liquid under study, using sterile instruments and keeping them in sterile vessels.

While preparing samples with saline water, 3% NaCl was additionally introduced into distilled water mixtures with black oil and diesel. Such salt concentration was selected for an experiment since higher NaCl concentrations inhibit the reproduction of many microorganisms: they are sensitive to the changes of osmotic pressure (Skaisgirienė et al., 2004).

Inoculations were performed in the MPA (meat–peptone agar) medium (28 g of powder diluted with 1 liter of distilled water). The solution obtained was well stirred and heated to boiling, then bottled into sterile bottles with corks and sterilised in an autoclave for 15 min (medium pH 7.2–7.4) at 121 °C. It was poured by 15–20 ml into sterile Petri dishes.

The Chapek medium was used for the isolation of oil-oxidizing microorganisms (OOM). It was prepared on analogy with the MPA (meat-peptone agar) medium, but after hardening its surface was covered with a sterile black oil layer (1% of the oil product in the volume of agar). Inoculated Petri dishes were incubated for 2–3 days in a thermostat at 27 °C.

Experimental investigations (duration 150 days) were carried out according to the diagram indicated in Table 1. Each experimental sample (20 variants in total) was tested in two dishes, and the data were assessed as the averages of tests performed in parallel.

RESULTS AND DISCUSSION

Scientific literature presents a description of several hundreds of microorganism species (bacteria, actynomycetes, micromycetes, etc.) capable of decomposing and assimilating oil hydrocarbons (Kosaric, 1993). However, search and creation of effective oil hydrocarbon-decomposing biopreparations, research of their operating mechanism and suitability for decomposition of specific substrates remain an urgent scientific and applied problem.

With the biodegradation of oil products, a general increase in the amount of microorganisms not always proportionately increases the decomposition of oil pollutants (Žukauskaitė et al., 2003). Therefore, the change in the amount of microorganisms oxidising oil products during the biodegradation process was investigated, and it was compared with the general amount of microorganisms.

Table 1. **List of samples**

No.	Sample	No.	Sample
1	Water + black oil (control)	11	Water + NaCl + black oil (control)
2	Water + black oil + GVT	12	Water + NaCl + black oil + GVT
3	Water + black oil + K-2000	13	Water + NaCl + black oil + K-2000
4	Water + black oil + Roebic K-47	14	Water + NaCl + black oil + Roebic K-47
5	Water + black oil + GVT + Roebic K-47	15	Water + NaCl + black oil+ GVT + Roebic K-47
6	Water + diesel (control)	16	Water + NaCl + diesel (control)
7	Water + diesel + GVT	17	Water + NaCl + diesel + GVT
8	Water + diesel + K-2000	18	Water + NaCl + diesel + K-2000
9	Water + diesel + Roebic K-47	19	Water + NaCl + diesel + Roebic K-47
10	Water + diesel + GVT + Roebic K-47	20	Water + NaCl + diesel + GVT + Roebic K-47

The greatest amounts of microorganisms in fresh water contaminated with black oil were determined at the beginning of the degradation process (30 days after the beginning of the experiment) in the control samples and reached 274.8 ± 12.8 mill. col/ml. In other samples, the total amount of microorganisms fluctuated from 142.7 ± 6.3 mill. col/ml (using the biopreparation GVT) to 171.8 ± 7.9 mill. col/ml (using a mixture of GVT and Roebic K-47). During the biodegradation process, the total concentration of microorganisms in the control vessels within 120 days reduced to 6.8 ± 0.8 mill. col/ml. In vessels where the biopreparations were used, the amounts of microorganisms reduced much slower. Oil-oxidising microorganisms constituted a considerably greater portion in the total amount of microorganisms in the samples where the biopreparations under study were used. It is notable that microorganisms present in the composition of the biopreparations excrete emulsifying materials that assimilate oil hydrocarbons (Giedraitytė et al., 2001) and increase the solubility of oil hydrocarbons themselves in water. Microorganisms also excrete glycolipids, phospholipids, saturated acids, lipopeptides, and biopolymers (Ang, Abdul, 1991) improving the nutritional conditions of the organisms and accelerating the process of biodegradation.

In the control samples, part of the oil-oxidising microorganisms during the biodegradation process reduced proportionately from 42 ± 2.1% to 0.7 ± 9%, using biopreparation K-2000 from 39.6 ± 1.8% to 36.1 ± 1.5%, using the biopreparation GVT increased from 23.0 ± 1.6% to 51.1 ± 2.6% and the enzyme Roebic K-47 from 16.8 ± 1.1% to 29.2 ± 1.7%, whereas the mixture of GVT and Roebic K-47 the number of microorganisms almost did not change and constantly reached approximately 32.0 ± 1.8%.

During analysis of the black oil degradation process, the residual black oil concentrations (Table 2) were determined and the splitting rate of black oil was calculated (Fig. 1). The results correlated with the amount of oil-oxidising microorganisms. Biopreparation GVT was noted for decomposing black oil at the highest rate: at the beginning of investigation, the decomposition rate was 127.5 ± 1.8 mg/day and at the end 52.8 ± 1.1mg/day. Other biopreparations decomposed black oil effectively as well, but at a lower rate; the biopreparation K-2000 decomposed black oil at the lowest rate. If biopreparations were not used, the maximum rate of black oil decomposition was 26.0 ± 0.6 mg/day (after 60 days).

The results of the effectiveness of the biological treatment of water contaminated with black oil within 150 days are presented in Table 3. Biopreparation GVT was most effective in decomposing this pollutant of the complex chemical structure in fresh water (81.5 ± 1.4%) and Roebic K-47 least was effective, (65.0 ± 1.8%).

The general tendencies of diesel biodegradation were very similar to the course of black oil decomposition (Table 3). The maximum amount of microorganisms was determined in the samples where a mixture of GVT and Roebic K-47 was used, whereas the largest portion of oil-oxidising microorganisms (which changed during biodegradation from 32 ± 1.8% to 67 ± 2.9%) was determined when using biopreparation K-2000. Using all biopreparations, the highest total amount of microorganisms was determined after 60 days from the beginning of the experiment (up to 872.4 ± 51.8 mln col/ml when using a mixture of GVT and Roebic K-47; in control vessels, without using biopreparations, the maximum was determined after 60 days (2 15.4 ± 18.6 mill. col/ml).

Table 2. **Residual amounts of pollutants in the system black oil-contaminated water (percent)**

Day of measurement	Control	GVT	K-2000	Roebic K-47	GVT + Roebic K-47
15	98.8 / 99.0	81.4 / 82.5	90.2 / 91.5	86.4 / 87.7	87.1 / 88.3
30	97.4 / 98.2	56.2 / 58.8	74.6 / 76.7	66.8 / 69.0	69.4 / 71.2
45	88.6 / 89.9	45.4 / 47.9	64.1 / 66.2	56.7 / 58.8	54.6 / 56.5
60	82.3 / 84.1	33.1 / 36.2	53.6 / 55.1	46.5 / 49.5	39.3 / 41.2
75	79. 3 / 80.9	28.3 / 30.0	44.3 / 46.8	41.9 / 43.7	34.7 / 36.8
90	76.1 / 78.6	22.6 / 24.2	34.8 / 37.0	37.6 / 39.7	29.3 / 32.5
105	72.9 / 74.2	21.0 / 22.7	32.5 / 34.4	36.9 / 38.9	27.9 / 30.4
120	69.1 / 70.9	19.0 / 20.8	30.1 / 32.5	36.1 / 38.1	26.1 / 28.7
135	67.4 / 69.9	18.6 / 20.3	29.0 / 30.4	35.2 / 37.2	25.3 / 27.4
150	67.8 / 69.6	18.4 / 20.0	28.6 / 30.2	34.9/ 36.8	24.9 / 26.7

Note. The first number – fresh water, the second number – salt water.

Table 3. **Residual amounts of pollutants in the system diesel – contaminated water (percent)**

Day of measurement	Control	GVT	K-2000	Roebic K-47	GVT + Roebic K-47
15	94.4 / 95.7	85.2 / 86.8	87.0 / 88.5	81.8 / 83.7	83.7 / 85.2
30	85.0 / 86.7	64.0 / 66.4	70.0 / 73.0	59.9 / 62.2	63.1 / 66.4
45	82.4 / 84.0	55.1 / 56.9	47.6 / 49.1	52.5 / 54.7	56.3 / 59.2
60	79.4 / 81.0	44.1 / 46.3	32.6 / 35.1	46.2 / 49.3	50.0 / 53.8
75	74.1 / 75.6	34.6 / 36.9	26.8 / 29.0	37.0 / 39.4	39.3 / 41.0
90	70.0 / 71.0	24.7/ 27.0	21.2 / 24.1	27.2 / 29.9	29.8 / 31.0
105	66.9 / 68.6	19.2 / 21.8	19.5 / 21.7	26.7 / 29.6	28.3 / 30.2
120	63.7 /65. 8	14.8 / 16.9	17.7 / 19.8	26.2 / 29.2	27.3 / 29.6
135	62.5 / 65.4	14.4 / 16.8	17.3 / 19.3	25.4/ 38.3	25.9 / 29.1
150	62.1 / 64.9	14.0 / 16.6	16.8 / 19.0	24.8 / 27.9	25.1 / 28.8

Note. The first number – fresh water, the second number – salt water.

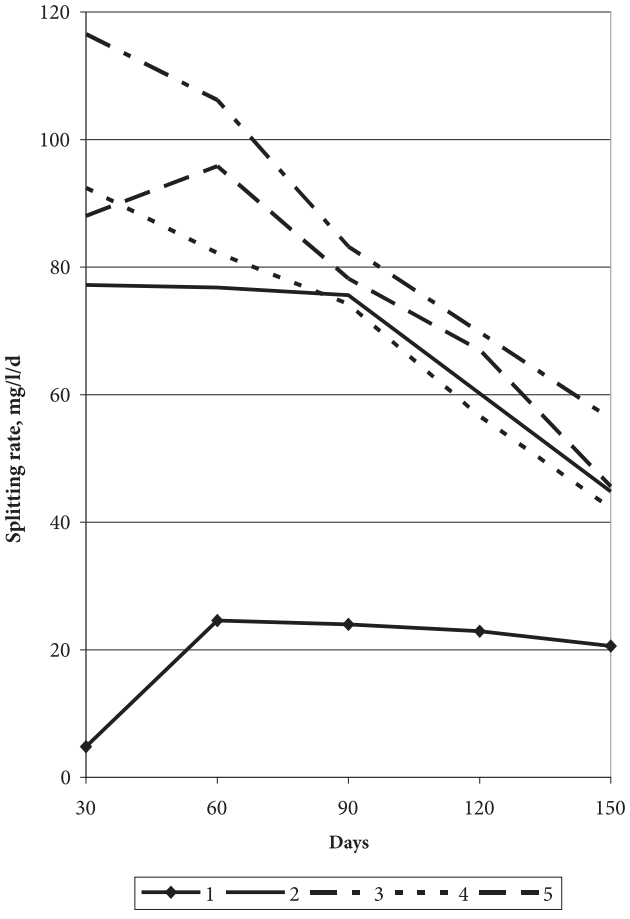

Fig. 1. Dependence of black oil splitting rate in fresh water on time (*1* – control; *2* – K-2000; *3* – GVT; *4* – Roebic K-47; *5* – GVT + Roebic K-47)

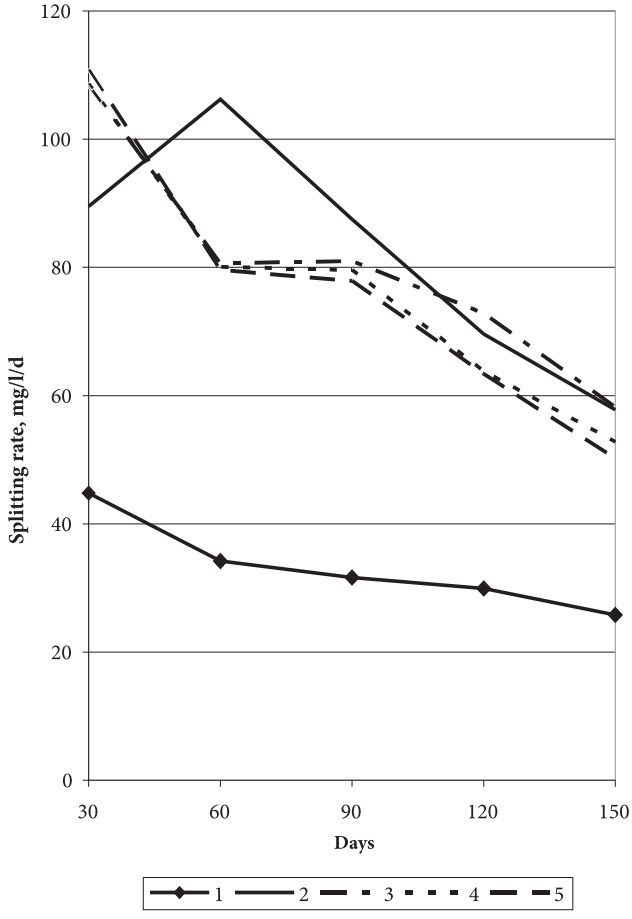

Fig. 2. Dependence of diesel oil splitting rate in fresh water on time (*1* – control; *2* – K-2000; *3* – GVT; *4* – Roebic K-47; *5* – GVT + Roebic K-47)

Diesel, as compared to black oil, is noted for better biodegradation qualities because its composition contains a lesser amount of slowly biodegrading fractions. Only with the application of a mixture of GVT and Roebic K-47 identical results were obtained (approximately 75%). In the control samples, with no biopreparations used, only 32.2 ± 1.8% black oil and 37.4 ± 2.0% diesel underwent biodegradation.

Diesel showed a higher rate of splitting (Fig. 2). With the use of biopreparations, the splitting rate of diesel at the beginning of biodegradation was about three times (biopreparation K-2000 was especially distinguished for the rate after 60 and 90 days from the beginning of the experiment) and at the end two times higher than in control samples with no biopreparations used. Most effective in the decomposition of diesel in fresh water were biopreparations GVT (85.9 ± 1.2%) and K-2000 (83.1 ± 1.3%), and the established differences between various biopreparations were less as compared to the course of the black oil degradation process. The capacity of microorganisms to decompose various oil hydrocarbons in black oil and diesel is predetermined not only by their physiological and chemical systems, but also by the physical, chemical and toxic properties of the hydrocarbons (Kalėdienė, 1999).

Biodegradation of black oil and diesel in saline water did not differ in the essence from the results in fresh (distilled) water: an insignificant slow-down in the splitting rate of the pollutants and biodegradation processes was established. This may be explained by the use of only 3% NaCl concentration, since it was not intended to hamper the processes of reproduction of the organisms present in the biopreparations (Skaisgirienė et al., 2004). There are data in the literature that oil hydrocarbons are decomposed in fresh water more effectively than in saline water (Boronin et al., 1997), but in our case water of low salinity was investigated.

CONCLUSIONS

1. Decomposition of black oil and diesel by biopreparations GVT, K-2000, enzyme Roebic K-47 and a mixture of biopreparation GVT and enzyme Roebic K-47 was investigated in fresh and saline water contaminated with the said pollutants.

2. Biopreparation GVT within 150 days decomposed 81.5 ± 1.4%, biopreparation K-2000 71.3 ± 1.5%, enzyme Roebic K-47 65.0 ± 1.9%, a mixture of biopreparation GVT and enzyme Roebic K-47 75.1 ± 1.8% of black oil contained in the fresh water. The biopreparation GVT decomposed black oil most efficiently.

3. Biopreparation GVT in the same period decomposed 85.9 ± 1.2%, biopreparation K-2000 83.1 ± 1.3%, enzyme Roebic K-47 75.1 ± 1.8%, a mixture of GVT and Roebic K-47 74.9 ± 1.7% of diesel contained in fresh water. Diesel, owing to the properties of the chemical compounds composing it, was biodegraded more rapidly.

4. For the treatment of water contaminated with black oil and diesel, it is most expedient to use the biopreparation GVT, and the biopreparation K-2000 is recommended for the decomposition of diesel in water. The application of GVT for three to two months may reduce the treatment time of water reservoirs contaminated with oil products.

5. The presence of 3% NaCl has no considerable effect on the process of black oil and diesel degradation in water.

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MAZUTO IR DYZELINO BIODEGRADACIJOS TYRIMAI VANDENYJE

S a n t r a u k a

Tirtas biopreparato GVT efektyvumas valant mazutu ir dyzelinu užterštą vandenį. Preparato galimybė skaidyti teršalus lyginta su biopreparatu K-2000, fermentiniu preparatu Roebic K-47 bei su biopreparato GVT ir fermento Roebic K-47 mišiniu. Nustatyta, kad mazuto biodegradacijos metu bandiniuose naftą oksiduojančių mikroorganizmų buvo daugiausia ir efektyviausias skaidymas – naudojant biopreparatą GVT. Dyzelinu užterštą vandenį visi naudoti preparatai valė iš esmės vienodai efektyviai.

Raktažodžiai: mazutas, dyzelinas, biodegradacija, naftą oksiduojantys mikroorganizmai