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Bioremediation of oil polluted aquatic systems and soils with novel preparation 'Rhoder'

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Abstract

This paper summarises the experience accumulated during the field application of biopreparation 'Rhoder' (solely or in a combination with preliminary mechanical collection of free oil) for remediation of oil polluted aquatic systems and soils in the Moscow region and Western Siberia during 1994–1999. It was demonstrated that 'Rhoder' had a very high efficiency (>99%) for bioremediation of the open aquatic surfaces (100 m^2 bay of the River Chernaya, two 5,000 m^2 lakes in Vyngayakha) at initial level of oil pollution of 0.4–19.1 g/l. During remediation of the wetland (2,000 m^2) in Urai (initial level of oil pollution of 10.5 g/l), a preliminary mechanical collection of oil was applied (75% removal) followed by a triple treatment with 'Rhoder'. It resulted in an overall treatment efficiency of 94%. Relatively inferior results of bioremediation of the 10,000 m^2 wetland in Vyngayakha (65% removal) and the 1,000 m^2 marshy peat soil in Nizhnevartovsk (19% removal) can be attributed to the very high initial level of oil pollution (24.3 g/l and >750 g/g dry matter, respectively) aggravated by the fact that it was impossible to apply a preliminary mechanical collection of oil on these sites. A possible strategy for remediation of such heavily polluted sites is discussed.

Introduction

Due to systematic accident spills, an annual release of oil into the environment in Russia accounts for 25 millions tonnes according to the estimations of 'Greenpeace' (Zhanovich et al. 1995). Among a variety of approaches proposed for the elimination of these spills (Rosenberg & Ron 1996; Scragg 1999), three main methods (mechanical, physico-chemical and microbiological are being applied both separately and in various combinations) are currently considered as the most perspective methods for Russian conditions (Murygina et al. 1999). Each of these methods has its advantages and drawbacks. Under fresh and abundant spills, the mechanical methods of oil collection are usually applied as a principal treatment. However, oil pollution is not eliminated completely. The physico-chemical methods using special reagents (detergents, emulsifiers, solidifiers, adsorbents etc.) can efficiently eliminate or concentrate oil pollution, but frequently they themselves are not fairly irreproachable from the ecological point of view, e.g., collection of oil-saturated adsorbents as well as their subsequent utilisation becomes sometimes troublesome. The microbiological methods using both external introductions of oil-degraders cultivated *ex situ* (bioaugmentation) and stimulation of indigenous microorganisms (if they are present in necessary concentrations) are usually quite efficient for treatment of low polluted water surfaces and soils. However, their effects are frequently not so pronounced at a high level of oil pollution. Besides, the low average annual temperatures on the largest area of territory, especially where the principal oil fields are located, is another critical bottleneck for the application of these methods in Russia because bacterial oil-degrading activity drops dramatically under temperatures below 10 °C. Despite the above-mentioned limitations, microbiological methods are drawing more and more attention in our country, especially as post-treatment or polishing steps, due to their economic attractiveness and ability to fulfil with the stringent legislation requirements concerning a permissible level of oil pollution. It should be noted that the renewed interest in bioaugmentation was also recently emphasised on an international level (Vogel 1996; Alleman & Leeson 1999).

The objective of this paper is to summarise the experience accumulated during the field application of biopreparation 'Rhoder' (solely or in a combination with preliminary mechanical collection of free oil) for remediation of oil polluted aquatic systems and soils in various regions of Russia throughout 1994–1999.

Materials and methods

Biopreparation

The biopreparation known under the commercial name 'Rhoder' and recently developed in All-Russian Research Institute of Oil and Gas together with Moscow State University (Patent RF No. 2090697) consists of two hydrocarbon-degrading bacteria – *Rhodococcus ruber* and *Rhodococcus erythropolis* revealing a synergistic action on oil degradation under a joint application. The individual strains were isolated from oil-water mixture originating from the Bondyuzhskoye oil field (Tatarstan, Russia), and the corresponding pure cultures were characterised (growth and morphological characteristics, nutritional needs, biodegradation rates for various hydrocarbons etc.) and then deposited to the All-Russian Collection of Microorganisms (ARCM indexes are 1513-D and 1514-D, respectively) and patented (Patents RF Nos. 2069492 and 2069493, respectively). The biopreparation 'Rhoder' is now fully certificated for production, delivery and application in the territory of Russia (Certificate No. 77.99.11.515.P.4865.8.99 issued 17.08.99 by the Russian Ministry of Health). For scaling up, the both Rhodococcus strains were separately cultivated in the aerated fermentors (16 or 100 l, liquid oxygen concentration around 1 mg/l) during 18–36 hours on the liquid medium of the following composition (% weight): feed yeast concentrate – 3.0; corn extract – 1.0; tap water- till 100 (pH – 6.8–7.2). The cells were then harvested by centrifugation of the cultivation broth and mixed together (1 : 1 w/w). This concentrated wet suspension of cells of both bacteria with a titre of hydrocarbon-degrading bacteria of 10^8–10^{10} cells/ml is the usual delivery form of 'Rhoder'. The working solutions were prepared on site by dilution of concentrated suspension with water (1 : 1000 and 1 : 100 for treatment of open water surfaces and soils, respectively) followed by addition of some nutrients and biostimulators. As a source of nutrients, the Russian commercial fertiliser 'Nitroammophoska' (potassium, nitrogen and phosphorous content is 16% each) was usually used in the concentrations of 2 and 20 g/l of working solution for treatment of open water surfaces and soils, respectively.

Sites and remediation methods used

The following oil polluted sites were used for field testing of biopreparation 'Rhoder' in 1995–1999: the bay of the River Chernaya (Lukhovitsy, Moscow region), lakes and wetland (Vyngayakha, Western Siberia), lake and wetland (Urai, Western Siberia), marshy peat soil (Nizhnevartovsk, Western Siberia). Some characteristics of these sites are listed in Table 1. When necessary and possible, preliminary mechanical collection of spill oil (using special devices – skimmers) on the site was undertaken before application of bioremediation technology. The latter include a spraying of the working solution on the polluted areas using pump equipment. Usually the treatment with biopreparation was repeated twice or three times with a time interval of 2 weeks. The impact of activity of indigenous hydrocarbon-degrading bacteria was assessed by a spraying of the working solution lacking 'Rhoder' on the control areas having a similar oil pollution level.

Analysis

Throughout all the field trials, a state of the treated areas was visually monitored and photographed. Sampling was periodically undertaken using a so-called 5-points method (Murygina et al. 1999) taking into account the recommendations of Hogg et al. (1994). Analysis of total oil contamination was performed by the gravimetric method (Bogomolov 1984; Lurie & Rybnikova 1984). Chemical analysis of water was carried out according to the recommendations of Lurie & Rybnikova (1984). Microbiological control of bioremediation process was monitored by the most probable number (MPN) method accounting for total and hydrocarbon degrading bacteria titres in

Table 1. Some characteristics of oil polluted sites used for 'Rhoder' field tests

Site (ambient temperature during the field tests)	Area, m^2	Initial oil pollution in the upper (10 cm) layer, g/l	(Pre)-treatment
Bay of the River Chernaya (15–25 °C)	100	0.44	'Rhoder' (twice)
Vyngayakha (15–34 °C):			
Lake 1	5,000	15.1	'Rhoder' (triple)
Lake 2	5,000	19.1	'Rhoder' (triple)
Wetland	10,000	24.3	'Rhoder' (triple)
Urai (5–30 °C):			
Lake	1,900	11.0	PMC* + 'Rhoder' (twice)
Wetland	2,000	10.5	PMC* + 'Rhoder' (triple)
Nizhnevartovsk (0–27 °C):			
Marshy peat soil	1,000	758–828 (g/kg)	ploughing + 'Rhoder' (triple)

*PMC – preliminary mechanical collection of free oil.

the samples described by Nazina et al. (1988) and Mesarch & Nies (1997).

Results and discussion

The results of bioremediation field tests are presented in Figures 1–4. It is seen that 'Rhoder' demonstrated a very high efficiency for treatment of open water surfaces, especially at low initial level of oil pollution as in the case of the bay of the River Chernaya (Figure 1a). It should be noted that the residual oil concentration after 1 month of bioremediation of this site was only 0.04 mg/l, i.e., lower than the Russian permissible level of oil pollution – 0.05 mg/l. It should be noted that this Russian standard is rather low and close to the majority of international standards (e.g., the Dutch standard for mineral oil is also 0.05 mg/l). The concentrations of both HDB and heterotrophs firstly increased by 1–2 orders of magnitude at day 14 and then returned back to the initial level after an exhaustion of organic substrates in the river water (Figure 1b). Thus, an addition of the external bacteria did not seem to result in substantial changes of microbial community existing in the river water. Analogously, the initial dosage of nutrients was chosen in such a way that the residual level of phosphate and nitrate after treatment was low enough to prevent a possible eutrophication of this bay (Figure 1c).

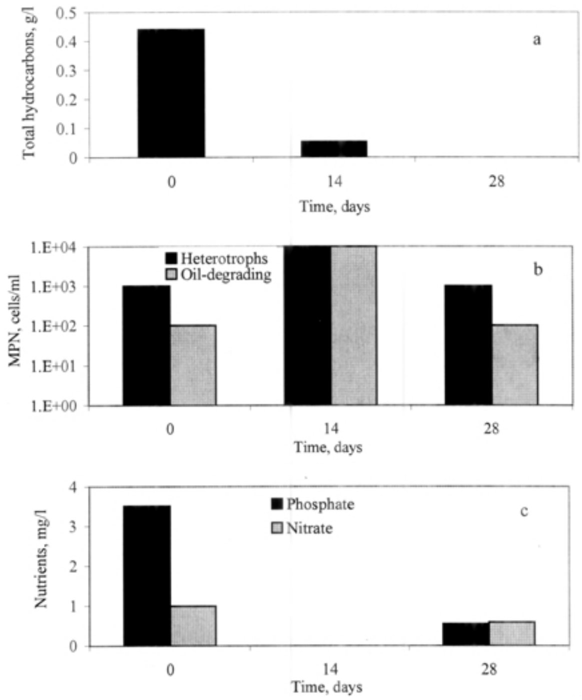

Figure 1. Dynamics of concentrations of total hydrocarbons (a), hydrocarbon-degrading and heterotrophic bacteria (b), phosphate and nitrate (c) during the 'Rhoder' bioremediation of bay of the River Chernaya.

Both lakes in Vyngayakha had a high initial level of oil pollution (Table 1) and the thick (till 1–2 cm) oil film was clearly seen on their surfaces. In spite of rather tough conditions, the triple treatment with 'Rhoder' accompanied by unusual warm weather in that period (till 34 °C) resulted in an almost complete elimination of oil pollution – the residual oil concentrations were 5 and 190 mg/l in lakes 1 and 2, respectively (Figure 2). Moreover, after bioremediation, both these lakes were certificated by the local ecological authorities as "the objects almost free of oil pollution". During the treatment of the lake in Urai

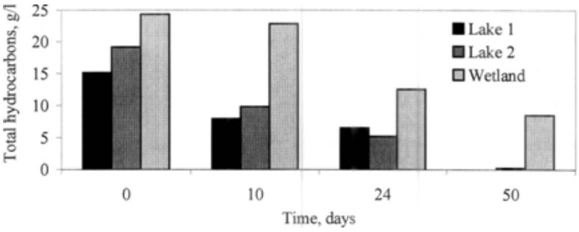

Figure 2. Dynamics of total hydrocarbons decrease during the 'Rhoder' bioremediation of polluted sites in Vyngayakha.

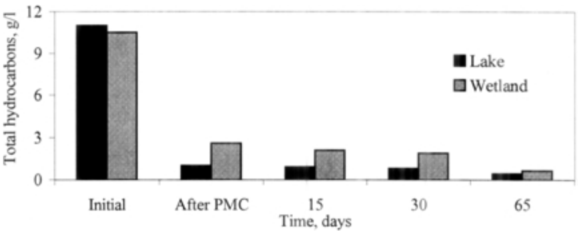

Figure 3. Dynamics of total hydrocarbons decrease during the remediation of polluted sites in Urai (PMC – preliminary mechanical collection of spilled oil).

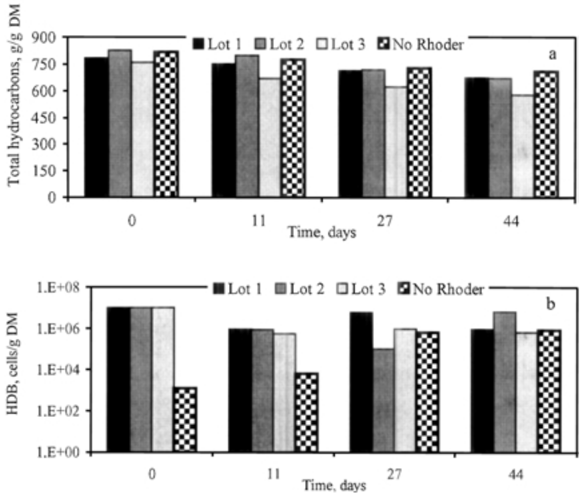

Figure 4. Dynamics of total hydrocarbons decrease (a) and concentrations of hydrocarbon-degrading bacteria (b) during the 'Rhoder' bioremediation of marshy peat soils in Nizhnevartovsk.

(Figure 3), most of the oil pollution was removed by mechanical collection (90%), i.e., the oil contamination level decreased from 11 to 1.01 g/l after this step. The subsequent treatment by 'Rhoder' (twice) led to the residual oil contamination of 0.43 g/l resulting in an overall treatment efficiency of 96% (Figure 3). A relatively high level of residual contamination could be mainly related to the presence of oil polluted sediments accumulated in this lake. These sediments served as a continuous source of oil emission to the lake water.

Relatively inferior results of remediation of the wetland in Vyngayakha (Figure 2) can be attributed to the fact that due to specific local geological conditions, it was very difficult to apply a preliminary mechanical collection of free oil on this site. However, taking into account a high initial level of oil pollution (>24 g/l) and age of spill (4 years old; ageing decreases a bioavailability of substrates for hydrocarbon-degrading bacteria), the results look quite satisfactorily – approximately 65% removal of oil contamination (Figure 2). On the contrary, application of preliminary mechanical collection of spilled oil (75% removal) followed by triple treatment with 'Rhoder' has resulted in much higher overall treatment efficiency (94%) in the case of remediation of the wetland in Urai (Figure 3).

The worst results of application of 'Rhoder' obtained on the marshy peat soils in Nizhnevartovsk (Figure 4) were not surprising taking into account an extremely high initial level of oil pollution (>750 g/kg of dry matter) and age of spill (6 years). Since an overwhelming majority of the spilled oil was adsorbed by peat, it was not economically reasonable to apply a preliminary mechanical collection of spilled oil. The pre-treatment used included only a ploughing of upper layer of the contaminated area (to increase a bioavailability of oil) accompanied by an addition of lime (to increase pH) and nitrogen and phosphorous fertilisers. An average (for 3 lots) reduction of oil pollution was 19% under application of 'Rhoder', while in control (without 'Rhoder' addition) it was 13% (Figure 4a). The latter fact manifested about a high activity of indigenous hydrocarbon-degrading bacteria already developed on the contaminated site during 6 years (though the diversity of the microorganisms could be reduced (Bossert & Compeau 1995; Mesarch & Nies 1997)) and substantially stimulated by pH adjusting and addition of nitrogen and phosphorous. This supposition was further confirmed by direct counts of MPN of hydrocarbon-degrading bacteria from the control lot, which were 10^3 and almost 10^6 cells/ml in the beginning and in the end of experiments, respectively (Figure 4b). The MPN of hydrocarbon-degrading bacteria on the 'Rhoder' treated lots were also around 10^6 cells/ml (Figure 4b).

Conclusions

The following conclusions can be drawn from the results presented above.

1. Field tests showed a very high efficiency of biopreparation 'Rhoder' for remediation of aquatic systems moderately contaminated by oil (<20 g/l).
2. However, for treatment of heavily polluted aquatic systems (thickness of oil film >3 mm) as well as oil spills on wetlands and grounds, the best strategy should include a preliminary mechanical collection of free oil, or application of adsorbents, or other pre-treatment methods followed by microbiological polishing step.
3. If for some reasons it is impossible to apply pre-treatment, the possible strategy can include multiple microbiological treatment with ploughing (to increase a bioavailability of oil), pH adjusting and supplementing by nutrients throughout several years.
4. On the aged spills (>5 years), the oil-degrading activity of indigenous microflora is frequently high enough to omit an addition of biopreparations produced *ex-situ* (bioaugmentation procedure). The economically reasonable strategy can include a stimulation of indigenous hydrocarbon-degrading bacteria already developed on the site and adapted to the site environment.

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