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Method of aerobic-anaerobic bioremediation of a raised bog in Western Siberia affected by old oil pollution. A pilot test

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ABSTRACT

The aerobic-anaerobic method of bioremediation was used in Western Siberia on an extreme site of a raised bog polluted with oil 20 years ago. The area was covered with an asphalt crust that can withstand a weight up to 60 kg without being destroyed. The crust was up to 1.5 cm thick; the underlying heavily oil-polluted moss was 15–25 cm. This area was divided into three plots (25×30 m^2). The concentration of total petroleum hydrocarbons (TPH) was 446.7–526.8 g/kg DM. The oil-oxidizing preparation Rhoder and a fertilizer were applied three times to the surface of the first plot by sprinkling; the electron acceptor was introduced into the moss together with the Rhoder under pressure to 15 cm. On the second plot indigenous microorganisms were activated with a fertilizer three times. The third plot was used as a negative control. As a result TPH concentration decreased by 23.0% in the layer of 0–10 cm and by 72.6% in the layer of 10–25 cm on the first plot. On the second plot (the activation of indigenous microflora) the TPH decreased by 28.7% only in the layer of 10–25 cm. The HC concentration in the control plot did not decrease at all.

1. Introduction

Crude oil production in Russia is carried out mainly in the northern regions with harsh climate, where vast territories are contaminated with accidental crude oil spills. A considerable part of these territories has already been remediated, mainly using on-site bioremediation technologies. However, there are still a lot of impassable raised bogs polluted with oil for a long time. The asphalt-like crust on the surface of such old spills has remained unchanged for 20 years and is not covered by vegetation. In such bogs, it is technically difficult and economically disadvantageous to use the existing remediation technologies with the involvement of heavy equipment. In addition, it disturbs the integrity of bog landscape and biogeocenosis. Therefore, the development of novel “gentle” and low-cost bioremediation techniques for such extreme sites, which will cause no environmental damage to oil-polluted bogs, is a pressing problem.

The incentive for developing an aerobic-anaerobic bioremediation technique was our experience of reclamation in 2011 of a part of raised bog polluted in spring as a result of accidental crude oil spill in the Yamal-Nenets Autonomous District (Western Siberia). It was technically impossible to use milling or removing the heavily-polluted upper moss layer; therefore, the oil-oxidizing preparation Rhoder was introduced three times on the surface of the bog and, as a result, the concentration of oil was reduced by 32%–98% depending on the initial concentration of hydrocarbons in the moss. While performing this work, we revealed that oil degradation both on the bog surface and at a depth of 15–25 cm under actually anaerobic conditions was quite intensive. The gas chromatographic analysis of hexane fractions from polluted moss, corresponding to TPH, and the HPLC analysis of acetonitrile extracts from these hexane fractions confirmed the results obtained by gravimetric analysis (Gaydamaka and Murygina, 2013a).

It is known that electron donors and acceptors are used for polluted soil remediation on a surface of soil by on site or in situ techniques (Lebeau, 2011; Zaweierucha and Malina, 2011) or they are injected into wells (vertical or horizontal) to a depth of 10–60 m or into trenches together with a biopreparation degrading a xenobiotic that contaminates soil and/or groundwater by slurry or in situ techniques (Glenn Ulrich, 2014). The continuous or semi-continuous injections of slowly utilized nutrients and the long-term injections of electron acceptors (nitrate and/or sulfate salts) into the contaminated zone are used. During this process, there is a

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risk of salinization of this zone being purified and cessation of microbial activity (Glenn Ulrich, 2014).

The peculiar properties of HC degradation under consecutive aerobic and anaerobic conditions with different electron acceptors (CO_2, ferric iron, manganese sulfates, nitrates and oxides) and petroleum emulsifying agents used for bioremediation of contaminated soils and bottom sediments have already been studied by some researchers (Harris et al., 2001; Grishchenkov et al., 2000; LaRiviere et al., 2001; 2003). In Russia, the works being carried out at present (Tolpeshta et al., 2015) are dedicated to the study of petroleum degradation, the changes in pH, Eh and species composition, mainly of indigenous microorganisms, depending on the depth of contamination (i.e., consecutive aerobic and anaerobic conditions), in the experimental vessels filled with oil-polluted peat from raised bogs in laboratory scale.

Beginning from 2011 and during the following two years, we developed under laboratory conditions an aerobic-anaerobic bioremediation technique, which makes it possible to manage without heavy equipment and to perform bioremediation without the traditional agro technical stages of remediation (Gaydamaka and Murygina, 2013a).

We have constructed a set of plastic tubes (5 models), 100 cm in length and 10 cm in diameter, attached vertically to a mounting stand. In each model, two 2 cm holes were made at a distance of 40 and 90 cm from the model top for taking samples and introducing electron acceptors. Each model was filled with oil-polluted moss from the real polluted raised bog. The TPH concentration in the polluted moss varied from 370 g/kg to 550 g/kg of DM. Model No. 1 was a negative control with no exposure. In model No. 2, the indigenous microflora was activated by applying mineral fertilizers to the surface layer of polluted moss, while a gaseous acceptor of electrons (CO_2) was introduced under pressure to the depth of 40 cm. In models No. 3, No. 4 and No. 5, the Rhoder and mineral fertilizers were applied to moss surface, with either the gaseous electron acceptor or liquid acceptors as nitrate solution introduced under pressure to the depth of 40 cm. In model No. 5 the Rhoder solution was injected together with the liquid acceptor. As a result, a liquid electron acceptor, its concentration, and the method of its introduction along with the Rhoder were selected (Gaydamaka and Murygina, 2013b). The periodic injections of the electron acceptor in physiologically relevant concentrations together with the Rhoder make it possible to avoid salinization of moss layers, where they are injected to.

Thus, the proposed method of aerobic-anaerobic bioremediation envisages the simultaneous application of the Rhoder and fertilizers to bog surface by sprinkling (aerobic process). The electron acceptor with the Rhoder is injected under pressure with a special device into the oil-polluted moss layer (anaerobic process) to ensure oil degradation by the Rhoder bacteria and indigenous microflora on the depth of 15–25 cm. This gentle method of bioremediation does not disturb the bog landscape and biogeocenosis that had been developed over centuries. It was necessary to test this bioremediation technique under field conditions.

The goal of this work was to test the developed method for aerobic-anaerobic bioremediation of oil-polluted raised bogs under field conditions.

2. Materials and methods

2.1. Background

The raised bog area with a 20-year old oil pollution chosen for the field experiment was an irregular polygon (25×90 m^2) located at the oil field near the town of Megion in Western Siberia (Fig. 1). The asphalt-like crust (up to 1.5 cm), was formed on the surface of the bog over 20 years, and withstood the weight up to 60 kg, without being destroyed. When the crust was broken, crude oil emerged on its surface; the moss under the crust was black from percolated oil. The crust surface was uneven; in the center of the site there was a “mirror” of crude oil (about 1.5 m in diameter) and puddles of atmospheric water with oil floating on their surface. The water for the working solutions of the Rhoder and fertilizers was taken from a small pond near the site.

Fig. 1. The general view of the bog area with 20-year-old oil spill before the treatment.

2.2. Pilot-scale experiment

The area was divided by stakes into 3 approximately equal plots of 25×30 m^2.

The first plot was used for bioremediation by the developed aerobic-anaerobic method; the second plot was used for activation of the indigenous microorganisms by applying the fertilizer and lime to its surface; the third plot was a negative control with no exposure.

The sampling points for chemical, agrochemical and microbiological analyses were marked with sticks. The scheme of the site with the indicated sampling points is shown in Fig. 2.

The first ten samples were taken by the “envelope” scheme (150–200 g) from the depth of 0–10 and 10–25 cm from plots No. 1 and No. 2 before starting the work. The HC concentrations in the first 10 samples were subsequently used as the initial level of pollution for the first two plots. Two more samples were taken from the middle of plot No. 3 (0–10 cm and 10–25 cm) considered as a negative control. In addition, the samples of clean moss were taken from the same depth in an unpolluted territory, about 50 m from the polluted site.

The reagents were the Azofoska fertilizer (N_{16}:P_{16}:K_{16}) and the Rhoder developed in the mid-90s of the past century in Russia and permitted to be extensively used in the environment. The Rhoder consists of two Rhodococcus strains (R. ruber Ac-1513 D and R. erythropolis Ac-1514 D) isolated from oil-polluted soils. The strains are not pathogenic for humans, animals and plants and do not cause mutations in bacteria. The Rhoder was more than once successfully used for cleaning of water surfaces (Murygina et al., 2000) and bioremediation of soils polluted with oil (Murygina et al., 2005), black oil fuel, diesel fuel, as well as railway sludge (Murygina et al., 2011) and petroleum refinery sludge (De-Qing et al., 2007).

The method of aerobic-anaerobic bioremediation includes the