Chapter 27
Regularity of Transformations of Oil-Contaminated Microbial Ecosystems by Super-Oxidation Technology and Subsequent Bio-remediation

Elena Maklyuk, Ganna Tsygichko, Ruslan Vilnyy and Alex Mojon

Abstract Super-oxidation technology uses chemical reagents to break down heavy residues of oil hydrocarbons. Its effects on the soil microbial flora were investigated for potential recovery of soil biotic activity. The dynamics of microbial ecosystems in oil-contaminated soils under application of the patented bio-remediation technology of the Man Oil Group (Switzerland) were investigated in the field and laboratory in turf and sand with different degrees of contamination by oil products over 5 years. Neither oil contamination nor super-oxidation technology sterilized the soil, but the structure of the microbial populations of peat and sandy soil was much altered, depending on soil type, characteristics of pollutant, period and pollution intensity.

Keywords Soil contamination · Bio-remediation · Microbial flora

Introduction

Nowadays, there are many of different bio-remediation technologies. Their major advantage over physico-chemical treatments of oil-contaminated soil is that they are environment-friendly; they depend on bio-preparations or activation of the native soil microbes (Rogozina et al. 2010; Golodyaev et al. 2009). But rapid and effective removal of oil and oil products from soil is difficult using only bio-remediation because of physiological constraints on the main object of bio-remediation—the

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microbial flora itself—under certain soil and climatic conditions (Kochergin et al. 2009; Filler 2008). Therefore, improving the effectiveness of bio-remediation is our goal (Karpenko et al. 2009; Belkov 2000).

Oil in the soil has a selective effect on the native microbial flora, inhibiting sensitive groups but promoting oil-pollution-resistant microorganisms that can utilize *n*-alkanes and benzene hydrocarbons as an energy substrate. However, thanks to bio-degradation of the hydrocarbons, the effect passes over the years. Therefore, we tested a combined method of the remediation in which, first, the patented super-oxidation technology developed by Man Oil Company (Switzerland) acts upon the long-chain hydrocarbons, tearing the chain into smaller lengths, followed by a further bio-remediation stage. Choice of chemical reagents is important because they must be non-toxic to the environment and should not create harmful residues that may accumulate in the soil. The rate of application is also important to allow the recovery of the native soil microbial flora.

Materials and Methods

We investigated oil-contaminated samples of raised-bog peat and sand (contaminated for 5 years), from the Western Siberian oil slurry pits (Yuganskneftegaz) remediated by the Man Oil Group. The level of contamination in the samples was different: a mean of 12% in the peat but 68% (1%—10,000 ppm) in the sand. The little-decomposed *Sphagnum* peat had an ash content of 6.6% and elementary composition of: N 2.58%, C 55.8%, H 5.99%, S 0.35% and O 35.3%. The composition of turf characterized by the low consists of bitumens 3.1%, the total composition of water- soluble and easy hydrolysable substances is 47%. According to Arkhipov (1998), the typical peat of the region is low in bitumens (3%); the total content of water-soluble and easily hydrolysable substances is 47%; the content of humic and fulvic acids is—17.0% and 16.4%, respectively; and combined cellulose and lignin 15.5%. The total composition of cellulose and lignin is 15.5%. The sandy soil of the area is characterized by available nitrogen (nitrate and ammoniacal) 30–40 mg/kg; available phosphorus and potassium (Kirsanov) 120–170 and 150–200 mg/kg, respectively; humus 2.0–2.2%; cation exchange capacity 4–8 meq/100 g of soil; and pH_{water} 5.7 and pH_{CaCl_2}—4.2 (Tseh and Hintermayer-Erhard 2009).

It is generally accepted that pollution by oil leads to sterility of the soil; it is for this reason that remediation is required. Employment of chemical reagents has two effects: the first, negative—even greater damage to the soil biome; the second, positive—break down of petroleum into lighter fractions of hydrocarbon that are readily available for nutrition of microorganisms. Therefore, the task is to choose technological conditions that do not reduce the numbers of the resistant microbial flora that can thrive within an environment of active hydrocarbon degradation. The MOG technology was carried out with different concentrations of reagents (Fig. 27.1).

		Concentration of Reagent A, 1 kg/2 l H_2O			
Concentration of reagent B, %		**0**	**0.1**	**0.3**	**1.0**
	0	V1	V2	V3	V4
	1	V5	V8		
	10	V6		V9	
	30	V7			V10

Fig. 27.1 Experimental design

Laboratory analyses were carried out in the Soil Microbiology Laboratory of Sokolovskyi Institute for Soil Science and Agrochemistry Research. The structure of the microbial ecosystem before and after application of the MOG super-oxidation technology was assessed according to the numbers of microorganisms of ecological and trophic groups that actively participate in the destruction of the oil hydrocarbons and reconstituting the biological activity of soil in these specific conditions. Data were obtained by seeding on selective culture media: for fungi (th/g) on the

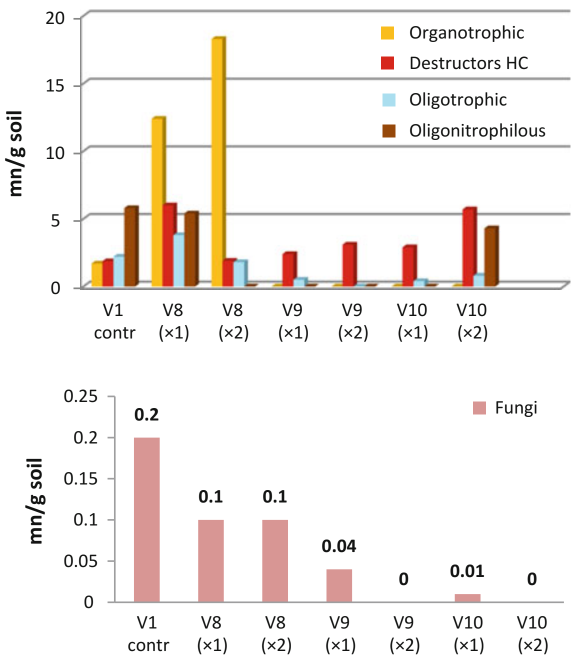


Fig. 27.2 Impact of the reagents and their concentration on the main ecological—trophic groups of microorganisms, including bio-destructors, in the peaty soil

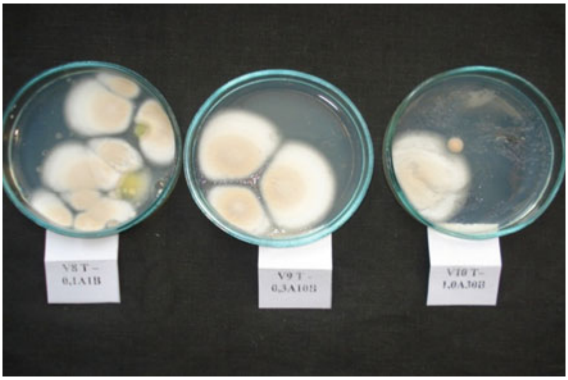
Richter medium; for m/o, which assimilate organic forms of the nitrogen (colony mn/g) on agar-meat infusion; destructors HC on the medium for hydrocarbon destructors (patent № RU2390555); oligotrophic microbes on starvation agar; and oligonitrophilous microbes on the Ashby medium (Iutyns'ka 2006).

Results and Discussion

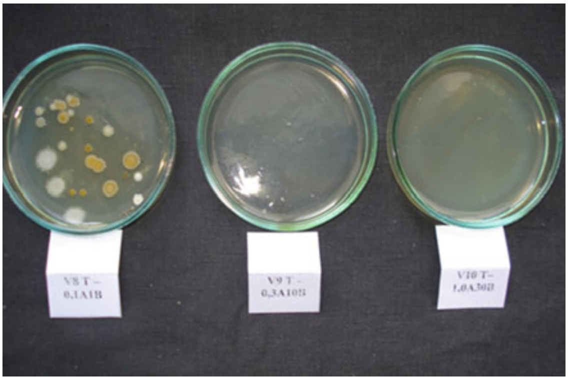
Microbiological analysis revealed colonies of all microorganism groups in the contaminated peat before application of the technology—variant control. In the soil, over a long time, stable microbial ecosystems formed where the number of groups of microorganisms is almost the same—but the dominant bacteria are oligonitrophilous (adapted for the nitrogen content) and fungi.

After using the MOG oxidation technology at low concentrations of reactants (V8) and with only a single treatment, the number of microorganisms in the soil was increased, but fungi lost their ecological niche. We observed significantly

Fig. 27.3 Influence of strengthening concentration of oxidizing reagents on the turf microflora



(a) Fungal colonies



(b) Colonies of m/o which assimilate organic forms of the nitrogen

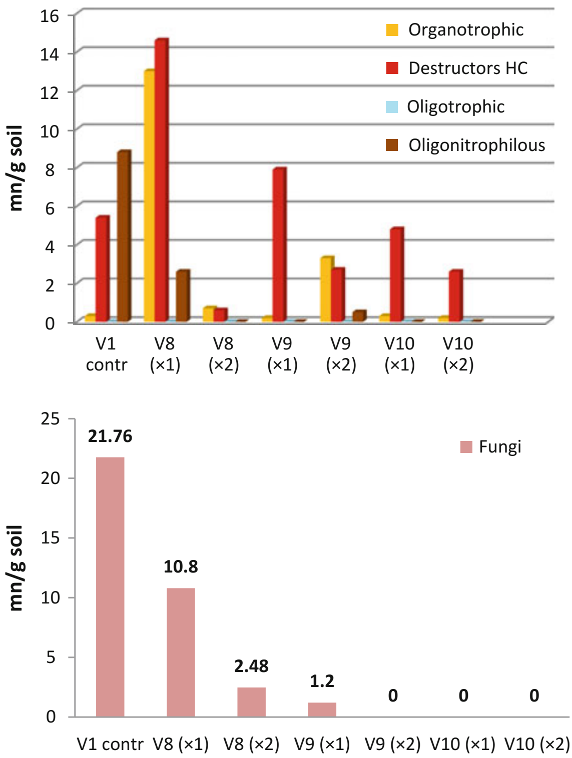


Fig. 27.4 Impact of the reagents and their concentration on the main ecological—trophic groups of microorganisms, including bio-destructors, in the sandy soil

greater numbers of organotrophs—destructors of oil hydrocarbons—and high increase in organotrophs was promoted by the double treatment of the turf (V8 × 2). With strengthening of the concentration of reagents (V9, V10), soil life was significantly decreased (Fig. 27.2).

Even so, the turf, as an organic soil, maintains conditions suitable for bio-destructors, even with increased concentration of reagents. In short, use of the technology can keep the resistant microflora of turf, but high reagent concentrations destroy microflora (Fig. 27.3). That is why we need to choose such norms of reagents that will be most beneficial for subsequent bio-remediation—where the treatment will activate oil-destructors within the native soil microbial flora.

At the same time, we need to consider that each type of soil has its specific soil microbiota—soil characteristics have great impact on the effectiveness of the bio-remediation. Sand is characterized by poor microflora, and oil contamination contributes to the selection of hydrocarbon destructors and oligonitrophilous microbes in the microbial ecology (Fig. 27.4). With strengthening of the concentration of reagents, we observed a decrease in microbial and fungal numbers, but the number of bio-destructors was at the high level, suggesting a significant opportunity for the use of this group in bio-remediation.

Conclusions

Our data suggest a possible solution for intensification of bio-remediation by combining technologies—where scientifically grounded norms of chemical components in the oxidation of the heavy hydrocarbon components increase the amount of light hydrocarbons available for bio-remediation.

At the same time, we must consider soil characteristics, period of pollution and pollution intensity to arrive at the scientifically based norm for using chemical reagents. Further research is needed on other impact factors such as characteristics of pollutant and application methods.

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