Hydrocarbon- and metal-polluted soil bioremediation: progress and challenges



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The problem of soil contamination with petroleum hydrocarbons and heavy metals is becoming particularly acute for large oil-producing countries, like the Russian Federation. Both hydrocarbon and metal contaminants impact negatively the soil biota and human health, thus requiring efficient methods for their detoxification and elimination. Bioremediation of soil co-contaminated with hydrocarbon and metal pollutants is complicated by the fact that, although the two components must be treated differently, they mutually affect the overall removal efficiency. Heavy metals are reported to inhibit biodegradation of hydrocarbons by interfering with microbial enzymes directly involved in biodegradation or through the interaction with enzymes involved in general metabolism. Here we discuss recent progress and challenges in bioremediation of soils co-contaminated with hydrocarbons and heavy metals, focusing on selecting metal-resistant biodegrading strains and biosurfactant amendments.

Environmental impacts of hydrocarbon and metal soil co-contamination

Contamination of soil environments with petroleum hydrocarbons (in the form of crude oil, fuels, organic solvents and other petroleum products) and heavy metals is becoming prevalent globally. Moreover, many contaminated industrial and municipal sites around the world are co-contaminated with organic and metal pollutants[1–3]. In fact, the largest emission sources for heavy metals are energy-related activities associated with oil extraction and refinery, as well as fuel combustion for heat and transport[4–6]. Trace metals most frequently found at oil-contaminated sites include arsenic, barium, cadmium, chromium, lead, mercury, nickel, vanadium, and zinc. For example, Russian heavy oils are enriched with metals, especially V and Ni, and to a lesser extent with Cd, Pb and Zn, which can contaminate soil along with hydrocarbons during accidental oil-spills or petroleum gas burning. Geological reserves of vanadium in heavy oils of the largest petroliferous basins of the Russian Federation are estimated at 1.3 million tonnes, of which 0.2 million tonnes are extracted along with oil[7]. Both hydrocarbon and metal contaminants impact negatively the soil biota and human health. Oil constituents (e.g. low molecular weight aliphatics, light aromatics, polycyclic aromatic hydrocarbons, and phenols) are highly toxic and carcinogenic. Also heavy metals present in oil can be accumulated by plants, thus leading to toxic reactions along the food chain. Moreover, trace metals occur in crude oil partly as organo-metallic compounds (e.g. geoporphyrins of V, Ni, Cu and Zn) and form stable complexes with asphaltenes, thus making their removal a difficult task[8,9]. Even more difficult is the assessment of environmental risks caused by simultaneous oil and heavy metal pollution. Software modelling is considered a powerful tool for integrating various elements in quantitative risk assessment, such as site characterisation, contaminant fate and transport, exposure assessment and risk calculation[10]. We previously developed a model that can be used in the site-specific risk assessment to evaluate potential human health and environmental risks from terrestrial oil spills. The software developed allows estimation of hydrocarbon and metal impacts through various exposure pathways from different media. It is important that the model was validated and tested in pilot scale projects on management and bioremediation of crude oil contaminated site under cold climate conditions in the Perm region of Russia[11].

Metal toxicity for hydrocarbon-degrading microorganisms

Bioremediation of soil co-contaminated with hydrocarbon and metal pollutants is complicated by the fact that, although the two components must be treated differently, they mutually affect the overall removal efficiency. Biodegradation is considered to be an environmentally friendly and cost-saving process for removing organic contaminants, particularly hydrocarbons. In contrast, the non-biodegradable metal component must be removed, detoxified or stabilised within the site using microbial biosorption and phytoremediation. Heavy metals are reported to inhibit biodegradation of hydrocarbons by interfering with microbial enzymes directly involved in biodegradation or through the interaction with enzymes involved in general metabolism[12]. It should be noted that metal toxicity is related to the concentration of bioavailable metals rather than to the total metal concentration as the latter may include soil-adsorbed, precipitated or complexed metal species[1]. Metal speciation and the resulting bioavailability are greatly affected by soil properties, such as pH and ion exchange capacity, clay type, mineral and organic matter content. Soil contamination with oil could further impact metal bioavailability through the complexing with heavy petroleum fractions, especially asphaltenes[9]. Therefore, the extent of the combined metal and hydrocarbon stress on soil microbiota cannot be estimated simply as a function of co-contaminant concentrations, it should be determined for each specific case of soil contamination, considering the physiology and ecology of hydrocarbon-degrading microorganisms.

Most literature data suggest that inhibition of biodegradation increases progressively with the increasing concentration of bioavailable metal. However, in several studies, low metal concentrations stimulated biodegradation and after reaching the threshold concentration, the metal toxicity began to increase with increasing their concentration. Such stimulatory effect of low metal concentrations could be due to reducing the competition between metal-resistant degraders and metal-sensitive non-degraders in soil populations, thus stressing the importance of ecological impacts of toxic metals[1]. Alternatively, high metal concentrations in co-contaminated soil create a selective pressure for

Table 1. Heavy metal resistance and emulsifying activity of actinobacterial strains from the Regional Specialised Collection of Alkanotrophic Microorganisms, Perm, Russia.

Genus, species (number of strains)	Minimal inhibitory concentration (mM)									Emulsion index E_{24} (%)
	Cd^{2+}	Cu^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}	CrO_4^{2-}	MoO_4^{2-}	VO_3^{-}	VO_4^{3-}	
Dietzia maris (5)	0.1 ± 0.02	6.0 ± 2.0	6.0 ± 2.0	8.0 ± 2.5	6.0 ± 3.4	20.0 ± 4.0	10.0 ± 2.0	>250	90 ± 20	26.2 ± 5.1
Gordonia rubripertincta (5)	0.8 ± 0.4	14.0 ± 7.4	12.0 ± 4.0	7.0 ± 2.5	4.5 ± 2.9	10.0 ± 2.0	14.5 ± 7.1	>150	80 ± 24	41.0 ± 3.9
Gordonia terrae (6)	0.4 ± 0.3	7.5 ± 2.5	4.3 ± 1.6	4.7 ± 2.9	1.7 ± 0.9	3.9 ± 3.0	3.8 ± 1.3	>200	58 ± 31	25.7 ± 9.1
Rhodococcus erythropolis (10)	0.3 ± 0.1	13.0 ± 9.0	6.3 ± 2.6	14.0 ± 6.2	4.6 ± 2.9	90 ± 83	8.0 ± 4.6	>200	48 ± 7.5	44.7 ± 3.3
Rhodococcus fascians (5)	0.6 ± 0.3	11.5 ± 7.4	10.0 ± 5.5	10.0 ± 5.5	5.6 ± 7.4	9.1 ± 6.5	13.0 ± 8.6	>250	40 ± 12	36.4 ± 10.2
'Rhodococcus longus' (8)	0.1 ± 0.03	6.0 ± 2.5	6.9 ± 2.4	6.3 ± 2.2	2.9 ± 1.8	15.0 ± 5.0	7.3 ± 3.6	>200	62 ± 21	42.5 ± 3.7
Rhodococcus opacus (7)	0.2 ± 0.1	4.3 ± 1.6	5.0 ± 0.6	7.9 ± 5.3	3.8 ± 1.5	14.3 ± 5.0	4.6 ± 2.7	>250	34 ± 15	45.7 ± 4.5
Rhodococcus rhodochrous (3)	0.1 ± 0.01	5.8 ± 1.9	10.0 ± 5.0	10.0 ± 2.0	4.2 ± 1.2	20.0 ± 4.0	8.3 ± 2.4	≥250	67 ± 24	41.2 ± 8.1
Rhodococcus ruber (12)	0.2 ± 0.1	10.4 ± 3.2	7.0 ± 3.3	12.1 ± 6.9	3.2 ± 1.3	12.9 ± 6.3	7.1 ± 6.4	≥250	83 ± 24	38.9 ± 7.3

metal-resistant oil-degrading microorganisms. We previously isolated and characterised a large number of vanadium-tolerant bacterial strains from soils contaminated with crude oil and refinery wastes[13]. The vast majority of isolated strains were resistant to high concentrations of vanadium salts and appeared to be capable of biosorption of the metal from the medium. Moreover, a non-specific resistance of selected actinobacterial cultures to heavy metals correlated positively with their growth on hydrocarbons and biosurfactant production (estimated as emulsifying activity) (Table 1)[14]. Thus, microorganisms exposed to metal- and hydrocarbon-polluted environments developed several mechanisms to tolerate a cumulative stress caused by metal ions (using efflux, complexation, or reduction) and toxic hydrocarbons (using modifications of the cell envelope and degradation[15]). Our recent findings suggest the involvement of proton- and sodium-dependent efflux pumps in the organic solvent tolerance of *Rhodococcus* actinobacteria[16], so these efflux systems could serve as cell adaptation mechanisms to both hydrocarbons and heavy metals.

Bioremediation approaches to hydrocarbon- and metal-polluted soils

Recent advances in bioremediation of co-contaminated environments have focused on the use of metal-resistant hydrocarbon-degrading bacteria (indigenous or bioaugmentation cultures) and different treatment amendments to mitigate metal toxicity. Of the latter, clay minerals, pH modifiers and chelating agents are widely used to reduce heavy metal mobility and bioavailability in soil[1]. Biosurfactants, microbially produced surface-active compounds, show promise for enhancing soil bioremediation due to their possible dual action (Figure 1): (i) micelle solubilisation of hydrocarbons; and (ii) metal detoxification via complexation[1,17,18]. The possibility of *in situ* biosurfactant production by oil-degrading microorganisms, indigenous or introduced during bioaugmentation, can be advantageous for soil bioremediation[19]. However, despite numerous laboratory demonstrations of positive biosurfactant effects on hydrocarbon biodegradation, there are still many gaps in our understanding of mechanisms of their action in soil co-contaminated with hydrocarbons and heavy metals.

Bioremediation strategies currently applied to soils affected by different types of pollutants include natural attenuation, biostimulation and bioaugmentation, phytoremediation and vermicomposting[20,21]. These strategies can be also used in combinations, thus increasing the efficiency of complementary approaches. For example, bioaugmentation-assisted phytoremediation resulted in the highest removal of petroleum hydrocarbons from soil co-contaminated with diesel and heavy metals (Cu, Pb and Zn) followed by bioaugmentation, phytoremediation and natural attenuation applied separately[20]. While the synergistic effect of plants and rhizosphere microorganisms has been demonstrated in many bioremediation experiments, more research is required to confirm such effects for the simultaneous treatment of organic and metal contaminations.

To date, only a few field trials have been performed on bioremediation of environments co-contaminated with organics and heavy metals. In the Northeastern Bulgaria oil deposit, waters contaminated with crude oil and toxic heavy metals (Zn, Cd, Cu, Pb, Mn, and Fe) were treated using the constructed wetlands[22]. Both oil and heavy metal concentrations decreased significantly due to hydrocarbon biodegradation and metal reduction/biosorption by indigenous microorganisms. Similarly, in our experiments, the oilfield wastewater treatment in a fluidised-bed bioreactor using *Rhodococcus* cultures co-immobilised on sawdust resulted in the efficient removal of hydrocarbons (68%) and heavy metals (75–96%)[23]. Using pilot bioreactors, the dual-bioaugmentation strategy was evaluated for soil co-contaminated with cadmium and 2,4-dichlorophenoxyacetic acid (2,4-D)[24]. A dual-bioaugmentation procedure involved the addition of cadmium-resistant *Pseudomonas* strain H1 in reactors inoculated with a cadmium-sensitive 2,4-D degrader

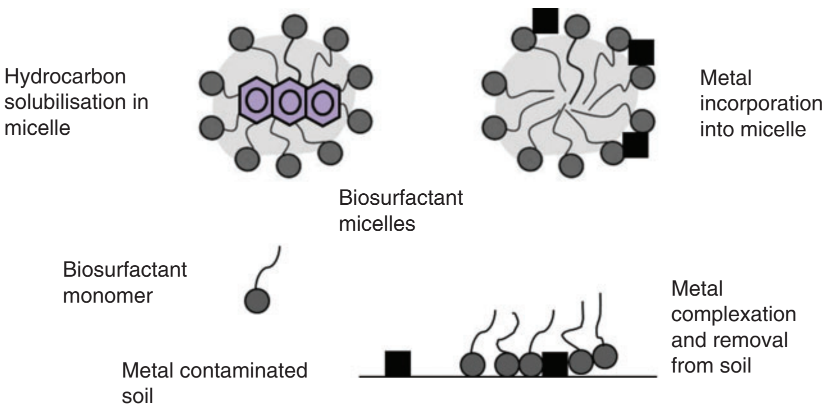

Figure 1. Mechanisms of hydrocarbon and metal removal from soil by biosurfactants (modified from Mulligan[17]).

Ralstonia eutropha JMP134, resulting in the enhanced biodegradation of the organic fraction. Overall, bioaugmentation with metal-resistant and oil-degrading bacterial cultures appears to be a viable approach in the remediation of co-contaminated soil and water.

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