Bioremediation for recycling of polluted soil - our experience with petroleum hydrocarbons

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Introduction

Bioremediation is a (bio)technology which is compatible with principles of green chemistry and engineering. This technology is sustainable since it conserves and generates the soil as a "non-renewable" resource [1]. A consortium of zymogenous microorganisms can conduct complex processes of degradation, while at the same time, being more resistant to changes in the ecosystem than just a single microbial species [2]. This paper present results of our multi-year work on microbiological biodegradation of oil and oil derivatives through the application of selected and naturally adapted consortia of zymogenous microorganisms, staring from the laboratory level to *in situ/ex situ* bioremediation procedures applied to thousands of tons.

Objectives

The object of this study was bioremediation on industrial scale of the soil polluted with petroleum hydrocarbons by consortia of zymogenous microorganisms isolated from petroleum contaminated soil, with the main aim to conduct polluted soil recycling.

Material and methods

The biopile for bioremediation was prepared on a waterproof asphalt surface of approximately 1500 m^2. The biopile consisted of:

- **270 m^3** of soil heavily polluted with oil hydrocarbons
- **60 m^3** of softwood sawdust as the additional carbon source and bulking component
- **300 m^3** of un-graded river sand added as a bulking and porosity increasing material.

The biopile (approximately **600 m^3** of material, around **1000 tons**) components were mixed with a front-end loader, and finally, raked using a tractor fitted with a harrow. To increase the rate of biodegradation biostimulation, bioaugmentation and re-inoculation followed by intensive mixing/aeration were applied every 15 days.

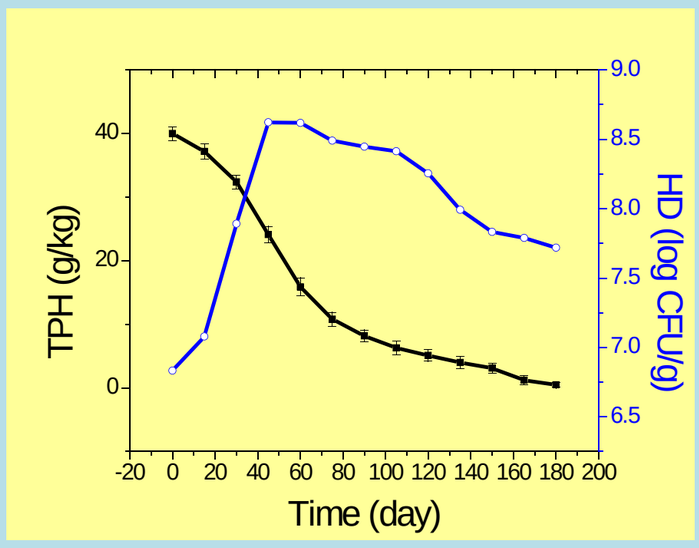
Total petroleum hydrocarbons (TPH) were analyzed in accordance with ISO 16703 and DIN EN 14345. For qualitative analysis, comprehensive two dimensional gas chromatography – mass spectrometry **(GC×GC-MS)** performed on GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan) and GC×GC thermal modulator (Zoex Corp.). A Rtx®-1 (RESTEK) and BPX50 (SGE Analytical Science) columns, thermal modulation of 6 sec. Software GCMS Solution (Shimadzu), ChromSquare Ver.2 and GCImage.

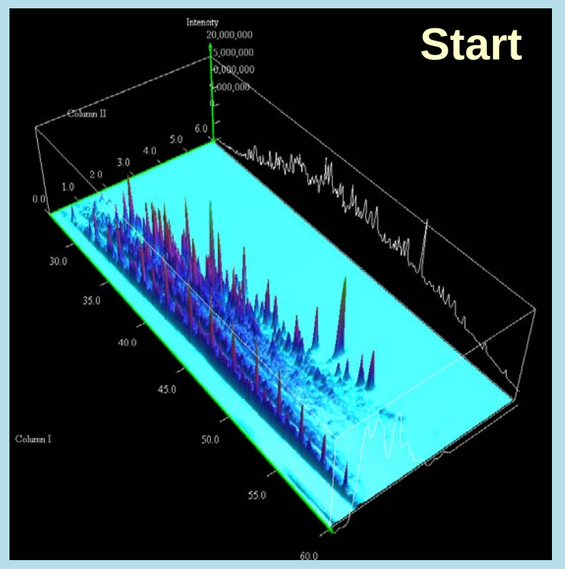
Results and Discussion

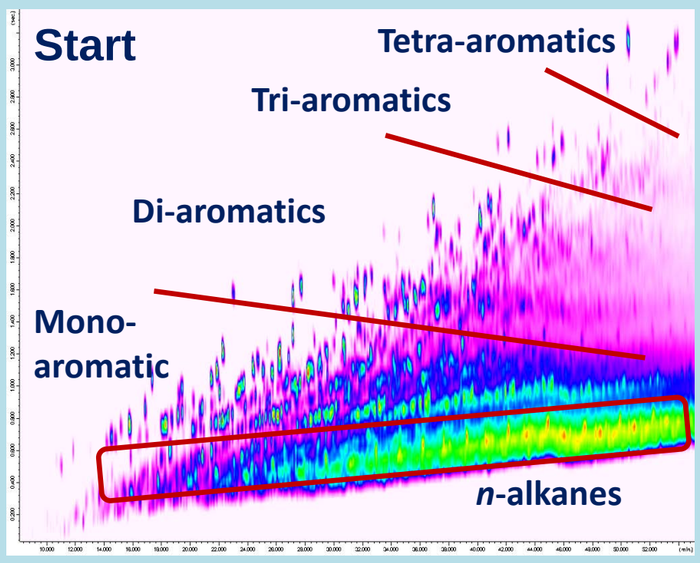
The composition of the consortium of microorganisms changed, depending on the bioremediation stage, but the total number amounted to **10^7-10^9 CFU/g** all the time. The dominant hydrocarbon degrading (HD) bacteria belonged to the following species: ***Pseudomonas***, ***Achromobacter***, ***Bacillus***, ***Micromonospora***, ***Rhodococcus***.

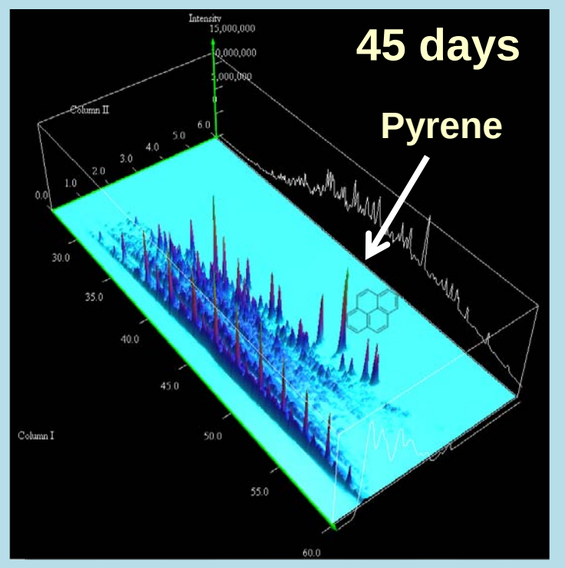
	Hydrocarbon degraders	GenBank No.
1.	Pseudomonas sp.	JF826528.1; JQ292806.1
2.	Achromobacter sp.	JF826529.1
3.	Bacillus sp.	JF826526
4.	Micromonospora sp.	JF826530.1
5.	Rhodococcus sp.	JQ065876.1; JX965395.1

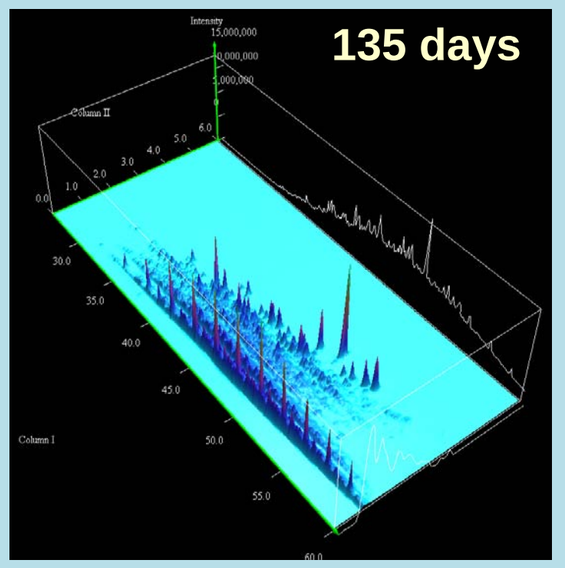
The initial levels of soil pollution were up to cc. **40 g/kg** dry matter of total petroleum hydrocarbons and after maximum 6 months the concentration was reduced to cc. **0.5 g/kg** dry matter (**99% efficacy**) with the disintegration of *n*-alkanes, mono and di-aromatics, persistent steranes and triterpanes (hopanes) and a segment of the NSO-fraction!

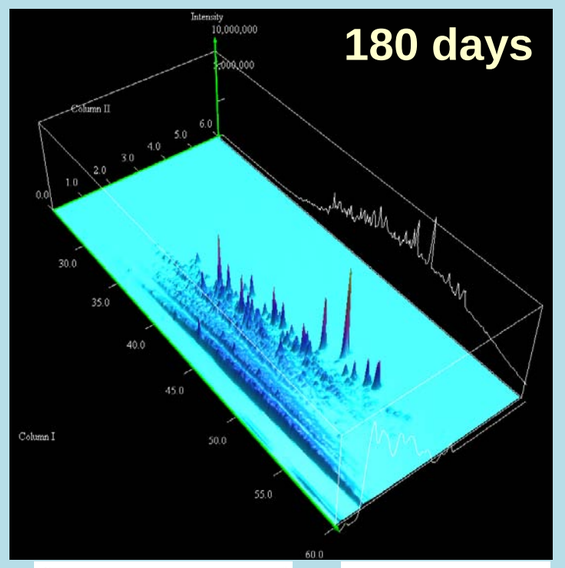












Conclusion

All fractions of petroleum hydrocarbons were reduced except tetra-aromatics such as pyrene. This study shows that successful industrial scale bioremediation can be conducted using consortia of zymogenous microorganisms. With the reduction of contamination, selective pressure of the environment and abiotic factors were reduced, which resulted in increased soil biodiversity.

References

1. Kaushik, G., Applied Environmental Biotechnology: Present Scenario and Future Trends, Springer, 2015.
2. Bertrand et al., Environmental Microbiology: Fundamentals and Applications, Springer, 2015.