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Evolution of the Microbioal Population in a Polluted Soil with Petroleum Hydrocarbons

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Abstract. Crude oil bioremediation of soils is limited by the bacteria activity in degrading the spills hydrocarbons. The aim of this study is to enhance the bioremediation of soils polluted with crude oil by adding the natural biodegradable product and bacterial inoculum. Biodegradation was quantified by total petroleum hydrocarbons (TPH) analyses.

Petroleum hydrocarbon pollution is one of the main environmental problems, not only by the important amounts released but also because of their toxicity. The main objective of this work is to accelerate the biodegradation processes. The enhancement of petroleum hydrocarbons degradation was achieved under natural product treatment and bacterial inoculum. The bacterial inoculum was used to enrich indigenous microbes to enhance biodegradation rate in the green house experiment.

Keywords: bioremediation, soils, pollution, crude oil, biodegradable product, bacterial inoculum

INTRODUCTION

Many developing countries face serious problems with soil pollution, but environmental concerns seem to be a luxury given the economic situation in most countries of our days. Common soil clean-up technologies, like, e.g. high-temperature thermal desorption, are often beyond financial possibilities, especially if large areas or volumes of soil are contaminated. Furthermore, soil structure and biology can be dramatically disturbed or even destroyed making the land useless for agricultural purposes. Crude oil is a complex mixture of hydrocarbons. It includes a saturate fraction, an aromatic fraction, asphaltenes, and resins (Atlas, 1981; Leahy and Colwell, 1990). Due to this complexity, petroleum hydrocarbons cannot be fully degraded by a single strain of microorganisms but its decomposition is achieved by microbial consortia and their broad enzymatic capacity (Leahy and Colwell, 1990).

The present greenhouse experiment intended to clarify if the treatment of the polluted soil with this natural biodegradable product named ECOSOL and microbial population enhancement by bacterial inoculum will accelerate the biodegradability rate of petroleum hydrocarbons from the polluted soil. This paper introduces some research results on natural attenuation and enhanced bioremediation of a polluted soil with a residual petroleum content of 5% and 10% dry soil weight.

MATERIALS AND METHODS

The main objective of this research is testing the natural hydrocarbon absorbent named ECOSOL. It is tested the capacity to increase the biodegradation of petroleum hydrocarbons by stimulating the bacteria. To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment. The soil used for this

experiment (calcic chernozems) was reaped from arable layer 0-20 cm (Teleorman). This type of soil was chosen because of its currency in our country, also, for its physical, chemical and biological properties favorable to plant growth.

The experiment was set up by artificial pollution of a cambic chernozem with different quantities of ECOSOL. After 21 days from pollution, the soil was inoculated with bacteria. The bacterial inoculum was developed from microorganisms that occur naturally in the soil like *Pseudomonas, Mycobacterium, Arthrobacter globiformis* and *Bacillus megaterium*.

ECOSOL is an absorbent natural product, meant to facilitate quick and efficient biodegradation of hydrocarbons from contaminated soils. Accelerates biostimulation and favors the development of existing bacteria from the soil, with strong effects in crude oil biodegradation. This natural biodegradable product is obtained from vegetal fibers from celluloid waste, all treated and with additives, being used in order to bring soils back to normal fertility levels.

The experimental variants are:

- ✓ V_1, control (unpolluted soil);
- ✓ V_2, polluted soil with 5% crude oil;
- ✓ V_3, polluted soil with 10% crude oil;
- ✓ V_4, polluted soil with 5% crude oil + 50 g ECOSOL;
- ✓ V_5, polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum;
- ✓ V_6, polluted soil with 5% crude oil + 100 g ECOSOL;
- ✓ V_7, polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum;
- ✓ V_8, polluted soil with 10% crude oil + 100 g ECOSOL;
- ✓ V_9, polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum;
- ✓ V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL;
- ✓ V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum.

RESULTS AND DISCUSSION

The polluted soil with petroleum hydrocarbons was treated with different quantities of the natural hydrocarbon absorbent – ECOSOL and bacterial inoculum determined variation of the total heterotrophic bacteria (THB). The enhancement of the total heterotrophic bacteria leads to a decrease of total petroleum hydrocarbons concentration in the polluted soil.

The evolution of bacterial community was determined in dynamic, in soil samples by quantitative and qualitative determinations of total heterotrophic bacteria in each experimental variant.

The ability of the heterotrophic bacteria to remediate the petroleum crude oil polluted soil samples was investigated by a greenhouse experiment during 5 months.

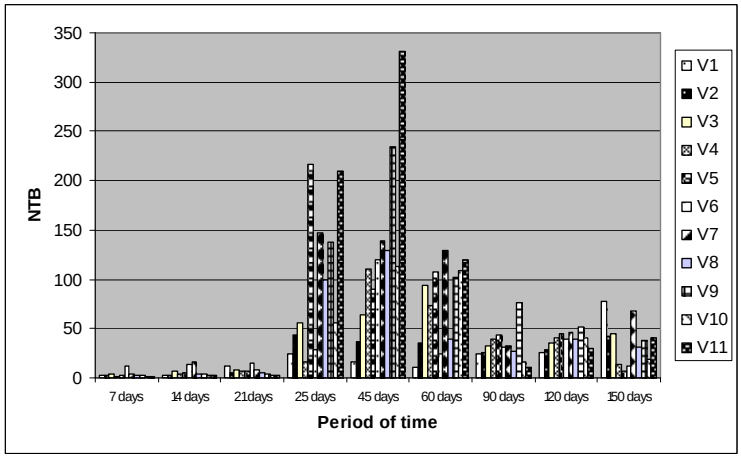

Fig. 1 The evolution of total number of heterotrophic bacteria (THB) in all experimental variants

In Fig. 1 is presented the evolution of total heterotrophic bacteria (THB) in all experimental variants. As it can be observed, in the beginning of the experiment, between 7 and 21 days, the numbers of heterotrophic bacteria are very low, being a period of accomodation. The activity of microorganisms is very low because of the pollutant in high concentrations.

After this period, between 25 and 60 days, the total numbers of heterotrophic bacteria are very high, especially in the inoculated experimental variants. The bacterial microflora addapted to the pollution conditions and the effect of the ECOSOL could be observed. ECOSOL accelerates biostimulation and favors the development of existing bacteria from the soil, respectively from bacterial inoculum increasing the crude oil biodegradation.

Between 90 and 150 days, in all experimental variants can be observed an uniformization of microbial population with a decrease of the total heterotrophic bacteria. In consequence, microbial population shortened the dimensions, but it specialized in pollutant metabolism in order to eliminate from soil.

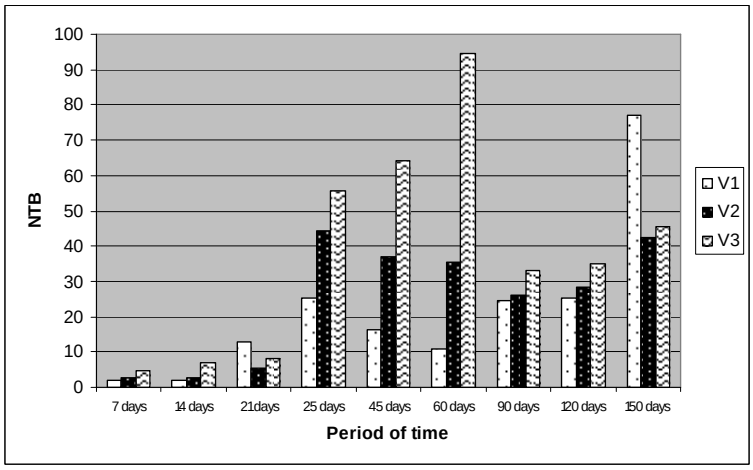

Fig. 2 The evolution of total number of heterotrophic bacteria (THB) in unpolluted soil, polluted soil with 5% crude oil and polluted soil with 10% crude oil (V_1,V_2, V_3)

The evolution of total number of heterotrophic bacteria with time in experimental variant V_1 - unpolluted soil, V_2 - polluted soil with 5% crude oil and V_3 - polluted soil with 10% crude oil is presented in Fig. 2. The figure shows that the total numbers of heterotrophic

bacteria were lower in the experimental variants in the begining of the experiment. The high number of heterotrophic bacteria is reached after 60 days having a value by 94,55 x 10^6 ufc (colony forming units) /g dry matter in the experimental variant were the soil was polluted with 10% crude oil. At the end of the experiment can be observed a easy decrease of the total numbers of heterotrophic bacteria.

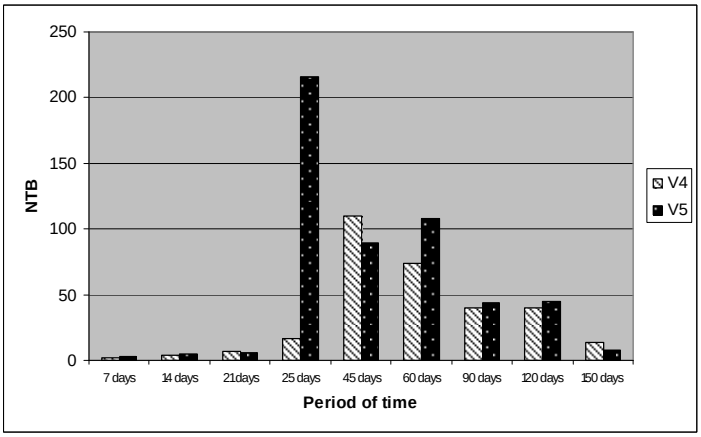

Fig. 3 The evolution of total heterotrophic bacteria (THB) in the polluted soil with 5% crude oil conditioned with 50 g ECOSOL (V_4 andV_5)

The evolution of total heterotrophic bacteria in the polluted soil with 5% crude oil conditioned with 50 g ECOSOL is presented in Fig. 3. As it can be observed, the total numbers of heterotrophic bacteria were lower in the experimental variants in the begining of the experiment. The high number of heterotrophic bacteria is reached after 25 days having a value by 216,3 x 10^6 ufc /g dry matter in the experimental variant were the soil was inoculated.

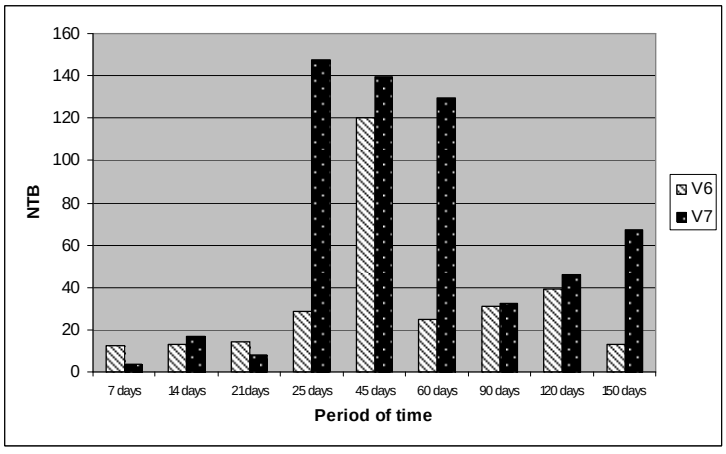

Fig. 4 The evolution of total number of heterotrophic bacteria (THB) in the polluted soil with 5% crude oil conditioned with 100 g ECOSOL (V_6 and V_7)

The evolution of total heterotrophic bacteria in the polluted soil with 5% crude oil conditioned with 100 g ECOSOL is presented in Fig. 4. The total numbers of heterotrophic bacteria were lower in the experimental variants in the beginning of the experiment. The high number of heterotrophic bacteria is reached after 25 days having a value by 147,42 x 10^6 ufc /g dry matter in the experimental variant were the soil was inoculated.

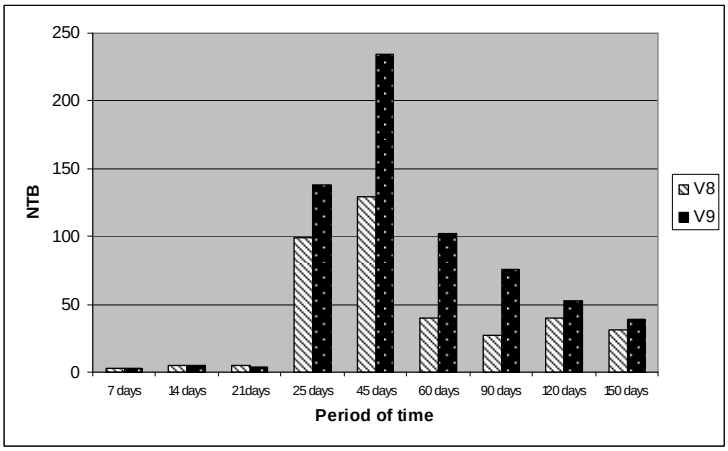

Fig. 5 The evolution of total number of heterotrophic bacteria (THB) in the polluted soil with 10% crude oil conditioned with 100 g ECOSOL (V_8 and V_9)

The evolution of total heterotrophic bacteria in the polluted soil with 10% crude oil conditioned with 100 g ECOSOL is presented in Fig. 4. total numbers of heterotrophic bacteria were lower in the experimental variants in the beginning of the experiment. The high number of heterotrophic bacteria is reached after 45 days having a value by 234,3 x 10^6 ufc /g dry matter in the experimental variant were the soil was inoculated.

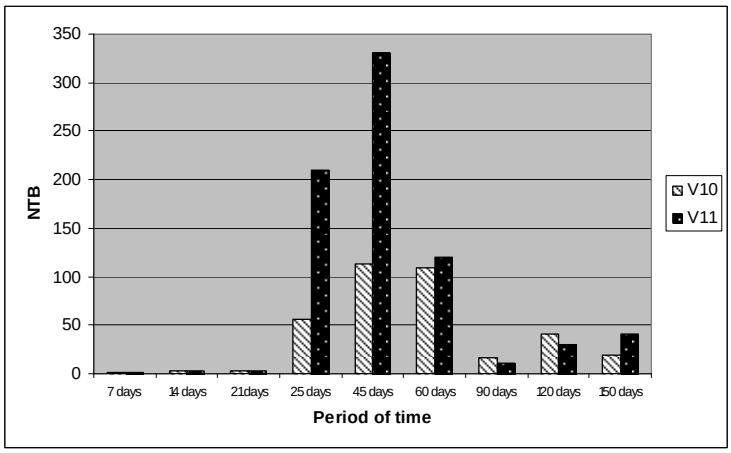

Fig. 6 The evolution of total number of heterotrophic bacteria (THB) in the polluted soil with 10% crude oil conditioned with 200 g ECOSOL (V_{10} andV_{11})

The evolution of number of heterotrophic bacteria in the polluted soil with 10% crude oil conditioned with 200 g ECOSOL is presented in Fig. 5. total numbers of heterotrophic bacteria were lower in the experimental variants in the begining of the experiment. The high number of heterotrophic bacteria is reached after 25 days having a value by 330,28 x 10^6 ufc /g dry matter in the experimental variant were the soil was inoculated.

The highest numbers of the heterotrophic bacteria were obtained in the inoculated variants, after 25 days from the begining of the experiment.

CONCLUSIONS

In the beginning of the experiment, between 7 and 21 days, the numbers of heterotrophic bacteria are very low, being a period of accomodation. The activity of microorganisms is very low because of the pollutant in high concentrations.

After this period, between 25 and 60 days, the total numbers of heterotrophic bacteria are very high, especially in the inoculated experimental variants. The bacterial microflora addapted to the pollution conditions and the effect of the ECOSOL could be observed. ECOSOL accelerates biostimulation and favors the development of existing bacteria from the soil, respectively from bacterial inoculum increasing the crude oil biodegradation.

Between 90 and 150 days, in all experimental variants can be observed an uniformization of microbial population with a decrease of the total heterotrophic bacteria. In consequence, microbial population shortened the dimensions, but it specialized in pollutant metabolism in order to eliminate from soil.

Survival of microorganisms in petroleum hydrocarbons medium in uninoculated variants and after bacterial inoculation in the inoculated variants is a key deciding factor in the rate of biodegradation of hydrocarbons in soils.

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REFERENCES

1. Alexander, M. (1997). Sequestration and Bioavailability of Organic Compounds in Soil in Linz D. G., and Nakles D. V. (eds.) Environmentally Acceptable Endpoints in Soil, American Academy of Environmental Engineers, USA.

2. Atlas, R. M., R. Bartha (1992). Hydrocarbon biodegradation and oil spill, Bioremediation.In: Advances in Microbial Ecology, Marshall K. C (editor), Vol 12. Plenum. New York,.USA, pp 287-338.

3. Dumitru, M, C. Răuță, M. Toti, Eugenia Gamenț (1994). Evaluarea gradului de poluare a solului. Măsuri de eliminare a efectului poluant, Conferinţa Naţională pentru Ştiinţa Solului, Bucureşti.

4. Lee, K., E. M. Levy (1989). Bioremediation of petroleum in the marine environment and its enhancement, Aquatic Toxicology Manager, J. A. Nriagu ed., John Wiley and Sons, New York, pp. 217-243.

5. Norris, R. D., J. E. Matthews (1994). Handbook of Bioremediation, Lewis Publishers, Boca Raton, Florida.

6. Okoh, A. I., M. R. Trejo-Hernandez (2006). Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies, African Journal of Biotechnology Vol. 5 (25), pp. 2520-2525.

7. Pepper, I. L., Gerba, Charles P., Brusseau, L. Mark (1996). Pollution Science, Academic Press.

8. Salanitro J. P. et al. (1997). Crude oil hydrocarbons bioremediation and soil ecotoxicity assessment, Environmental Science and Technology 31(6):1769-1776.

9. Toti M., M. Dumitru, V. Căpitanu, Maria Dracea, Carolina Constantin, C-tin Crăciun (1999). Poluarea cu petrol şi apă sărată a solurilor din România, Ed. RISOPRINT, Cluj-Napoca.

10. Toti M., M. Dumitru, M. Mihalache, A. R. Voiculescu, G. Mihalache, Carolina Constantin (2003). Metodologia de bioremediere a solurilor poluate cu ţiţei cu ajutorul microorganismelor specifice selecţionate din microflora autohtonă, Ed. GNP MINISCHOOL.

11. Voiculescu Anca-Rovena, M. Dumitru, M. Toti (2003). Decontaminarea Solurilor Poluate cu Compuşi Organici, Ed. SITECH, Craiova, ISBN 973-657-939-5.