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THE EVOLUTION OF TOTAL HETEROTROPHIC BACTERIA IN A CRUDE OIL POLLUTED SOIL

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Abstract

Crude oil bioremediation of soils is limited by the bacteria activity in degrading the spills hydrocarbons. Petroleum hydrocarbon pollution is one of the main environmental problems, not only by the important amounts released but also because of their toxicity. It is known that the main microorganisms consuming petroleum hydrocarbons are bacteria, so in this paper are presented the results obtained in a bioremediation laboratory experiment. 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. A natural biodegradable product and bacterial inoculum was used for total petroleum hydrocarbon (TPH) removal from an artificial polluted soil. Soil polluted with 50000 mg/kg of TPH was treated with 0.25%, respective 0.5% and/or bacterial inoculum to increase the biodegradability rate. Also, the soil contaminated with 100000 mg/kg of TPH was treated with 0.5%, respective 1% and/or bacterial inoculum. 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. In soil excessively polluted with crude oil, bacterial population size in conditioned variant with Ecosol maximum dose (1%) presented values comparable to those of inoculated variants, demonstrating the protective and stimulation effect of soil bacteria, including those involved in the degradation of petroleum hydrocarbons exercised by organic compound applied Ecosol.At each phase of the study, the natural biodegradable product was found to significantly enhance the biodegradation of petroleum hydrocarbons.

Key words: *biodegradation, total heterotrophic bacteria, polluted soil, a natural biodegradable product.*

INTRODUCTION

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention. The interest in environmental pollution has increased for the entire population of the globe. Various institutions and organizations, some multidisciplinary other specialized publications, focused solely on pollution issues. There is no life without soil (Pepper et al., 1996; Alexander, 1997).

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 (Lee and Levy, 1989).

Crude oil is a complex mixture of hydrocarbons. It includes a saturate fraction, an aromatic fraction, asphaltenes, and resins (Atlas, 1992; Okoh and Trejo-Hernandez, 2006). 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 (Norris and Matthews, 1994).

There are many genera of known oil-degrading microorganisms, including bacteria such as *Achromobacter, Acinetobacter, Actinomyces, Bacillus, Microbacterium, Pseudomonas, Streptomyces* and *Vibrio*, and fungi or yeast such as *Allescheria, Aspergillus, Candida, Debayomyces, Penicillium, Saccharomyces* and *Trichoderma*. Under natural conditions, these microorganisms in most areas comprise very few, compared with the total number of identified microorganisms. However, at

petroleum hydrocarbon polluted soils, these populations may grow and increase because they use petroleum hydrocarbon as a carbon source (Alexander, 1997; Voiculescu et al., 2003).

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 study focused on the application of the two major technologies known in bioremediation method such as: soil biostimulation based on environmental conditions improvement for microorganisms multiplication and activity to degrade petroleum hydrocarbons, and bioaugmentation based on enriching the soil with specific biodegrading hydrocarbons microorganisms.

Biostimulation - the first technological link included a innovation element based on using an organic compound made from cellulose fibers for soil polluted conditioning with additives to optimize its structure, water and air circulation regime in soil, and not least achieving a protective interface between degrading microorganisms and pollutant. Ecosol compound was chosen for experiment by analysing a series of organic compounds suitable for conditioning soil contaminated with organic pollutants, especially because of its biodegradability properties.

Bioaugmentation - the second technology link was achieved by soil inoculation with bacterial bioproducts made from specific bacteria selected and tested in the laboratory for their ability to degrade petroleum hydrocarbons.

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/20 kg polluted soil (0.25%);
- V_5, polluted soil with 5% crude oil + 50 g ECOSOL/20 kg polluted soil (0.25%) + bacterial inoculum;
- V_6, polluted soil with 5% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%);
- V_7, polluted soil with 5% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%) + bacterial inoculum;
- V_8, polluted soil with 10% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%);
- V_9, polluted soil with 10% crude oil + 100 g ECOSOL/20 kg polluted soil (0.5%) + bacterial inoculum;
- V_{10}, polluted soil with 10% crude oil + 200 g ECOSOL/20 kg polluted soil (1%);

V_{11}, polluted soil with 10% crude oil + 200 g ECOSOL/20 kg polluted soil (1%) + bacterial inoculum.

The values obtained by analyzing soil and plant samples were processed using more specific methods of mathematical statistics. Analysis of variance for establishing Fischer and Tukey tests determined for $\alpha = 0.05$, which shows the changes produced on soil and plant characteristics, the effects of treatments applied. ANOVA method provides information allowing the calculation of limit differences used in multiple comparison methods and the mean average for each graduation of studied factor. By correlation method was determined

the linear correlation coefficient or the correlation ratio (index), for assessing the intensity of the relationship between variables. For the estimation of a link between the two characteristics studied, stochastic experiments were conducted by achieving regression equations.

RESULTS AND DISCUSSIONS

Bacterial inoculum application after 21 days from the experiment beginning was reflected in very significant increases in the levels of total heterotrophic bacteria (THB) determined in polluted soil.

Immediately after controlled soil pollution with crude oil, at impact, bacterial populations have decreased the multiplication rate, and later, after an adaptation and selection process, the resistance component will extensively proliferate. For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration.

In soil excessively polluted with crude oil, bacterial population size in conditioned variant with Ecosol maximum dose (1%) presented values comparable to those of inoculated variants, demonstrating the protective and stimulation effect of soil bacteria, including those involved in the degradation of petroleum hydrocarbons exercised by organic compound applied Ecosol.

The quantitative evolution of bacterial communities from soil polluted with crude oil clearly demonstrated that the Ecosol application decreases the time needed for microorganisms involved in petroleum hydrocarbons biodegradation to adapt at substrate and conditions of pollution.

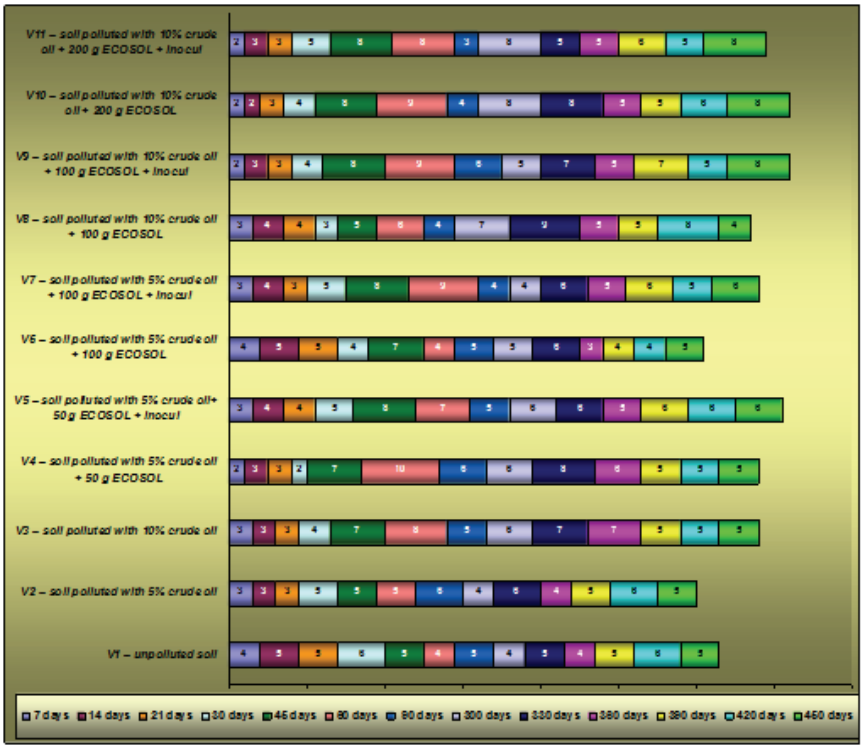

Figure 1. Diversity of heterotrophic bacteria in experimental variants at different determination moments

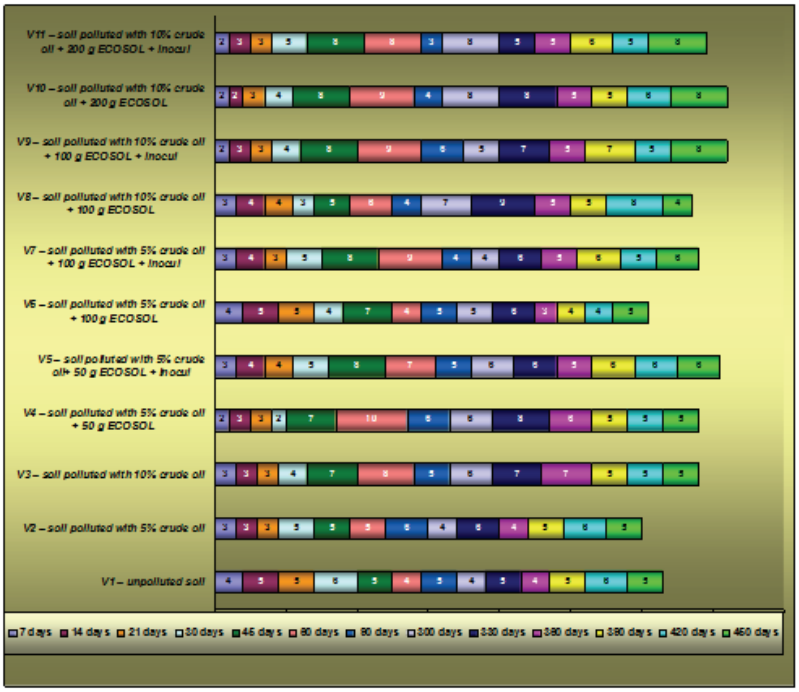

Figure 2. The presence of bacterial species with degradation potential in experimental variants at different determination moments

Frequent bacterial isolated from experimental variants, in most determination stages were: *Pseudomonas, Arthrobacter, Mycobacterium*, bacterial of the most reputable in terms of species with high capacities in hydrocarbon biodegradation.

Isolates of *Pseudomonas*, *Arthrobacter* and *Mycobacterium* led to the establishment of inoculum applied to the soil, and very high frequency showed a better survival rate and their adaptability to environmental conditions.

Bacterial isolated as *Pseudomonas* and *Arthrobacter* dominated the heterotrophic bacteria population in the majority of experimental variants, occasionally accompanied by species belonging to the genera: *Micrococcus*, *Enterobacter* and *Flavobacterium*.

Dynamics of microorganisms inoculated showed a slight adaptability in soil polluted with 5% crude oil, but complete inhibition in the first 30 days of experiment at 10% crude oil. After the acclimatization period by 30 days, the excessive concentration of pollutants has been massive multiplication of bacteria in inoculated variants, especially in the conditioned variant with Ecosol maximum dose. At 45 days, there was a real explosion in variants of bacteria inoculated with THB values double, triple compared with uninoculated variants. At 60 days, bacterial populations have begun to reduce their size, as the value of soil polluted with 10% crude oil and untreated. This demonstrates that the application reduces the time needed to adapt to Ecosol substrate for microorganisms involved in petroleum hydrocarbons biodegradation.

The value of the total heterotrophic bacteria (THB) in soil is a response to a large number of factors, including crude oil content, which causes new ecological conditions with a strong impact on microbial evolution. These conditions affect not only the size, but also the diversity of bacterial communities. The soil is mainly colonized by bacterial genera species able to use hydrocarbons from crude oil in its metabolism (Voiculescu et al., 2003).

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.

CONCLUSIONS

For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration.

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