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EFFECT OF A NATURAL BIODEGRADABLE PRODUCT AND BACTERIAL INOCULUM IN A CRUDE OIL POLLUTED SOIL

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Abstract

Petroleum hydrocarbons pollution of soils has become a big problem with the development of petrochemical industry and installation of numerous petrol stations and underground pipes. Physical, chemical and biological technologies have been developed to remove petroleum hydrocarbon pollutants from soils and restore environmental quality. However, costs are high, and many techniques are difficult to use for in-situ remediation. It still remains necessary to study the natural attenuation of hydrocarbons in soil and to develop simple cost-effective techniques for enhanced remediation. 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.

INTRODUCTION

Bioremediation is based on the capacity of microorganisms to degrade organic pollutant compounds, such as hydrocarbons. The "time-consuming" bioremediation of hydrocarbon polluted soils can be improved by the increase of hydrocarbon availability [5].

These compounds are important soil pollutants because of the high toxicity of the polycyclic aromatic hydrocarbon (PAH) fraction. According to the Environmental Protection Agency (EPA), 16 PAHs have been reported as carcinogenic and mutagenic compounds [7], so it is necessary to remove them from the contaminated/polluted sites.

Recent studies have reported several bacteria species with the capacity to mineralize or to degrade petroleum hydrocarbons [2]. Several different bioremediation techniques have been developed, but biostimulation is the most often used [4]. This consists of the activation of native soil microorganisms through the addition of nutrients.

The addition of commercial microbial cultures (as bioaugmentation) to contaminated soil concluded that it is not superior to just simply adding nutrients (as biostimulation) to the contaminated soil [1].

It is possible that, even if the native microorganism population is large enough, it does not have the ability to degrade components of high molecular weight or to emulsify insoluble compounds. Bioaugmentation could be used for the latter case. This technique is defined as the addition of pre-grown microbial cultures to perform a specific remediation task in a given environment [3].

The microbial cultures must have the ability to withstand different soil environmental conditions and to survive in the presence of other microorganisms [6].

MATERIAL AND METHODS

The main objective of this research is to enhance the biodegradation processes using a treatment with the natural hydrocarbon absorbent named ECOSOL. It 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, a greenhouse experiment was realized. The soil used in this experiment was calcic chernozems.

The experiment was set up by artificial pollution of a cambic chernozem with 5% and 10% petroleum and treated with different quantities of ECOSOL and bacterial inoculum. The bacterial inoculum was developed from microorganisms that occur naturally in the soil like *Pseudomonas, Mycobacterium, Arthrobacter globiformis* and *Bacillus megaterium*.

The polluted soil with petroleum hydrocarbons was treated with different quantities of the natural hydrocarbon absorbent and bacterial inoculum determined variation of the chemical characteristics of the soil. It was analysed the following chemical characteristics using standard methods: soil reaction (pH), organic carbon (Walkley and Black method), total azote (Kjeldahl method), phosphorous and potassium.

The total petroleum hydrocarbons were quantified by a gravimetric method, following previous solid–liquid extraction in a Soxhlet system. The extraction was carried out with methylene chloride in 1-2 g soil samples, which had been previously dried and grounded.

RESULTS AND DISCUSSION

As it can be observed in table 1, the pH values increase with the quantity of ECOSOL in each experimental variant. In the treatments with 50 g ECOSOL, the soil reaction could reach a value around 8.25, with 100g ECOSOL a value around 8.36 and with 200 g ECOSOL a value around 8.58.

The total petroleum hydrocarbons concentration is proportionally with the artificial pollution of the soil in each experimental variant.

The organic carbon content increases with crude oil concentration in the experimental variants where the soil was polluted with 5% crude oil, respectively 10% crude oil, comparatively with the control.

Table 1

Chemical characteristics of soil in the experimental variants at the beginning of the experiment

Experimental variant	pH	TPH (mg kg^{-1})	Organic C (%)	Total N (%)	C/N Ratio	P (mg kg^{-1})	K (mg kg^{-1})
V_1 – unpolluted soil	8.10	0	3.40	0.330	12.09	165	3220
V_2 – polluted soil with 5% crude oil	8.13	39566	7.31	0.313	27.26	158	3220
V_3 – polluted soil with 10% crude oil	8.14	90341	8.54	0.288	35.08	153	3380
V_4 – polluted soil with 5% crude oil + 50 g ECOSOL	8.21	54533	7.91	0.305	31.20	149	3540
V_5 – polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum	8.28	42233	7.56	0.327	27.05	175	3540
V_6 – polluted soil with 5% crude oil + 100 g ECOSOL	8.33	43225	7.84	0.341	26,79	151	3380
V_7 – polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum	8.36	40491	7.69	0.342	26.20	144	3380
V_8 – polluted soil with 10% crude oil + 100 g ECOSOL	8.34	90092	7.37	0.311	27.68	135	3220
V_9 – polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum	8.39	91500	9.50	0.284	39.42	131	3380
V_{10} – polluted soil with 10% crude oil + 200 g ECOSOL	8.60	91050	8.88	0.285	36.41	146	3540
V_{11} – polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum	8.56	90867	8.26	0.286	33.83	122	3220

Table 2

Chemical characteristics of soil in the experimental variants at the end of the experiment

Experimental variant	pH	TPH (mg kg^{-1})	Organic C (%)	Total N (%)	C/N Ratio	P (mg kg^{-1})	K (mg kg^{-1})
V_1 – unpolluted soil	8.02	0	3.22	0.340	11.53	167	3380
V_2 – polluted soil with 5% crude oil	8.13	36278	7.23	0.290	29.67	175	3220
V_3 – polluted soil with 10% crude oil	8.13	81144	7.77	0.326	27.78	95	2580
V_4 – polluted soil with 5% crude oil + 50 g ECOSOL	8.26	46734	7.56	0.284	31.10	127	3220
V_5 – polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum	8.24	38967	7.69	0.298	30.33	120	3380
V_6 – polluted soil with 5% crude oil + 100 g ECOSOL	8.33	42878	7.57	0.324	27.95	110	3380
V_7 – polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum	8.35	38417	7.81	0.369	25.60	96	3220
V_8 – polluted soil with 10% crude oil + 100 g ECOSOL	8.40	81867	10.57	0.357	34.79	128	3220
V_9 – polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum	8.42	81550	7.99	0.304	30.72	118	3220
V_{10} – polluted soil with 10% crude oil + 200 g ECOSOL	8.53	83067	8.29	0.314	31.43	85	3060
V_{11} – polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum	8.56	80134	8.00	0.343	27.87	105	2580

The total nitrogen contents decrease with the hydrocarbon concentrations increase reaching a value around 0.330 in the control, a value around 0.325 in the experimental variants polluted with 5% crude oil, and a value around 0.290 in the experimental variants polluted with 10% crude oil.

The C/N ratios are higher than the control in all experimental variants according to organic carbon increase and nitrogen decrease.

The phosphorous contents fluctuate in the experimental variants polluted with crude oil, treated with ECOSOL and bacterial inoculum.
The potassium contents fluctuate in the experimental variants polluted with crude oil, treated with ECOSOL and bacterial inoculum.

As it can be observed in table 2, the pH values increase with the quantity of ECOSOL in each experimental variant, being lower than the values obtained in the beginning of the experiment, probably, because of the crude oil biodegradation in soil.

The total petroleum hydrocarbons concentration is proportionally with the artificial pollution of the soil in each experimental variant. The TPH concentration dropped because of the bioremediation treatment applied. In the end of the experiment, the total petroleum hydrocarbons are lower than in the beginning of the experiment because of the degradation process.

The organic carbon content increases with crude oil concentration in the experimental variants where the soil was polluted with 5% crude oil, respectively 10% crude oil, comparatively with the control. There was a drop in soil organic carbon comparing to the initial values because of the crude oil biodegradation in soil.

The total nitrogen contents decrease with the hydrocarbon concentrations increase. Comparatively with the initial values from the beginning, could not be observed a fluctuation in the end of the experiment.

The C/N ratios are higher than the control in all experimental variants according to organic carbon increase and nitrogen decrease. In the end of the experiment, comparatively with the values obtained in the beginning of the experiment, there was a drop also in the C/N ratios due to the remediation treatment.

The phosphorous contents fluctuate in the experimental variants polluted with crude oil, treated with ECOSOL and bacterial inoculum. In the end of the experiment, comparatively with the values obtained in the beginning of the experiment, it was not observed a significantly difference during the remediation treatment.
The potassium contents fluctuate in the experimental variants polluted with crude oil, treated with ECOSOL and bacterial inoculum. In the end of the experiment, comparatively with the values obtained in the beginning of the experiment, it was not observed a significantly difference during the remediation treatment.

CONCLUSIONS

1. Some chemical characteristics of soil differ during the bioremediation treatment.
2. The soil reaction, the total petroleum hydrocarbons, the organic carbon and the C/N ratios are proportionally with the artificial pollution of the soil in each experimental variant. In the end of the experiment, these parameters dropped because of the bioremediation treatment applied comparatively with the values obtained in the beginning of the experiment.
3. The total nitrogen contents decrease with the hydrocarbon concentrations increase. Comparatively with the initial values from the beginning, could not be observed a fluctuation in the end of the experiment.
4. The phosphorous and potassium contents fluctuate in the experimental variants polluted with crude oil, treated with ECOSOL and bacterial inoculum. In the end of the experiment, comparatively with the values obtained in the beginning of the experiment, it was not observed a significantly difference during the remediation treatment.
5. The treatment with the natural biodegradable product and the addition of bacterial inoculum recorded the highest rate of degradation, as it can be observed from the total petroleum hydrocarbons concentrations.
6. The experimental research will continue in the greenhouse on the same artificial polluted soil.

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