Journal of Agri-Food and Applied Sciences
Available online at jaas.blue-ap.org
©2014 JAAS Journal. Vol. 2(7), pp. 191-195, 31 July, 2014
E-ISSN: 2311-6730

NATURAL ATTENUATION, BIOSTIMULATION AND BIOAUGMENTATION ON REMEDIATION OF BIODIESEL CONTAMINATED SOILS

Rios Ruth Paola[1*],Giuffré Lidia[1] and Romaniuk Romina[2]

1-Edafología, Facultad de Agronomía, Universidad de Buenos Aires. Buenos Aires, Argentina.
2-Instituto de Suelos, INTA. Buenos Aires, Argentina

Corresponding Author: Rios Ruth Paola

Received: 15 June, 2014 *Accepted: 10 July, 2014* *Published: 31 July, 2014*

ABSTRACT

Biodiesel has emerged as an interesting energy option to the shortage of petroleum products, the main source of energy for mankind, so it is relevant to focus the study of the life cycle of biodiesel on the ground because it acts as an acceptor and final deposit of the waste generated by the biofuel industry. The objectives of this study are: to compare natural attenuation, biostimulation and bioaugmentation as bioremediation techniques in biodiesel contaminated soils in a pot trial and evaluate the impact of such practices on some soil properties. The design consisted of 5 treatments with 3 replications; soil without pollution (L), soil + biodiesel (LB), soil + biodiesel + inorganic fertilizer (LBF), soil + compost + biodiesel (LBC), soil + biodiesel + hydrocarbon degraders (LBM). Single samples were taken in the first 20 cm depth, at baseline T0, T1 (1 month), T3 (3 months), T6 (6 months), and a year (T12). Edaphic variables studied were TPH (total petroleum hydrocarbons), pH, organic carbon (% C), nitrate (NO_3) and phosphorus (P). Organic biostimulation treatments (LBC) and bioaugmentation (LBM) were more efficient in the degradation of biodiesel. The contamination resulted in a decrease in the pH value of the soil. Contamination with biodiesel produced a decrease in soil pH and an increase in the content of organic carbon

Keywords: *biodiesel ; bioremediation ; edaphic properties.*

INTRODUCTION

Biodiesel emerged from a technology that can take advantage of various raw materials. By low environmental pollution generated by its combustion is a promising renewable energy source especially for countries where fossil fuel reserves are scarce. Despite careful handling, transport and storage, there is always the possibility that at some point it could accidentally enter the ground. Therefore, it is relevant to focus the study of the life cycle of biodiesel on the ground because it acts as an acceptor and final disposal of the waste generated by the biofuel industry (Cardona Villada, 2007).

Biodiesel has proven advantages over petroleum diesel with respect to their environmental performance: it is less toxic to soil organisms, readily undergoes aerobic biodegradation, and some authors found that mixtures containing a greater amount of biodiesel usually facilitate biodegradation (Makareviciene and Janulis, 2003; DeMello, 2007; Prince, 2008; Demirbas, 2009). Biological degradation essentially consists in the oxidation of these compounds by heterotrophic soil bacteria in the respiratory metabolism, in order to obtain energy for their maintenance and growth.

The biodegradation process is of great importance in the degradation of hydrocarbons and other organic compounds in contact with the ground. The extent of soil bioremediation is dependent not only on the soil type, biological activity and the type of biota, but also depends on the longevity of the contaminants, toxicity, and water mobility in soil. Physical, chemical and biological soil characterization is essential to determine the remediation techniques that are viable as sanitation techniques. Furthermore it should be noticed that the bioavailability of contaminants varies with the type of oil and soil characteristics (Plaza, 2005).

Soil properties and activity of the indigenous microbial population affect the degree of bioremediation, so the site characterization is a priority to decide the most appropriate method for bioremediation (Bento, 2005). Doran, (1996) presented a number of properties that affect the functionality and quality of the soil, and consequently influence the rate of degradation of hydrocarbons in soil, that is conditioned by parameters such as temperature, pH, salt content, presence of nutrients, oxygen content, particle size and distribution, buffer capacity (Margesin and Shinner, 2001).

This work refers to the remediation of soils contaminated with biodiesel compared using different techniques: natural attenuation, organic biostimulation, inorganic biostimulation and bioaugmentation, in a pot trial. Simultaneously, the effect of such practices on some soil variables was evaluated.

MATERIALS AND METHODS

An essay in pots with a silt loam soil (Typic Argiudol), was performed in a completely randomized design (Table 1). The soil was placed in pots of 30 cm diameter by 40 cm depth, in open air. The design consisted of 5 treatments with 3 replications; soil without pollution (L), soil + biodiesel (LB), soil + biodiesel + inorganic fertilizer (LBF), soil + compost + biodiesel (LBC), soil + biodiesel + hydrocarbon degraders (LBM). The level of contamination with biodiesel was 20 % of the dry weight of the soil, applied in surface 20 cm. The fertilizer used as a nitrogen source was urea (800 mg / pot), in inorganic biostimulation. In treatments with organic biostimulation, manure compost was applied, in a dry basis, soil – compost ratio of 1: 0.3. Table 2 lists some of the relevant physical and chemical characteristics of the compost.

For bioaugmentation, a special formulation and reserved dose of a compound was used. Surface soil single samples were taken (0-20 cm). Sampling was performed at time T0 when soil was contaminated with biodiesel, T1 (1 month), T3 (3 months) , T6 (6 months) , and a year (T12). Edaphic variables studied were TPH (total petroleum hydrocarbons), determined by infrared (EPA 418.1), pH 1:2,5 soil: water (Page, 1982), organic carbon (% C), by Walkley and Black (Nelson y Sommers, 1982) , nitrate (NO3), using the method of diazotization with Snedd (Carole y Scarigelli, 1971) and extractable phosphorus (P) as Kurtz Bray Bray (1945).

Data were analyzed by ANOVA and Tukey test for comparison of means between treatments. INFOSTAT statistical software version 1.1 was used (Infostat, 2002).

Table 1.Some physical and chemical properties of the soil

Horizon	Clay (%)	Silt (%)	Sand (%)	C (%)	pH (1:2.5)	CE (ds/m)	CIC ($Cmol_c\ kg^{-1}$)
A	24,5	48	27,5	1,92	6,7	0,48	18,2

Table 2. Some physical and chemical properties of the compost

	C (%)	pH (1:2,5)	CE (ds/m)	C/N	P ppm	NO_3 mg $N\text{-}NO_3$/kg.
Compost	32	6,7	0,48	1,05	15	32

RESULTS AND DISCUSSION

I) Oxidizable carbon, pH , extractable phosphorus and nitrates

In Figure 1 mean values of oxidizable carbon (a), pH (b), nitrates (c) and extractable phosphorus (d), for all treatments and sampling time series are presented .

The oxidizable carbon in organic biostimulation treatment (LBC) showed a statistically significant higher value than the remaining treatments, which did not differ from each other in T0. This increase is due to the incorporation of an organic compound with high content of oxidizable carbon.

However, in Table 4 statistical analysis of the carbon content is presented in T0 without the treatment of organic biostimulation, due to carbon elevated content of compost itself. It can be seen that contaminated biodiesel treatments have statistically significant higher than the control values, demonstrating that some of the hydrocarbons are considered in determining soil carbon.

In T1 organic biostimulation treatments (LBC) and bioaugmentation (LBM) had the highest values of oxidizable carbon, with statistically significant differences from each other. In the case of treatment of bioaugmentation the increase in carbon content of the soil is due to the degradation of the own biodiesel carbon chain, generating single strands that are considered in the measurement as soil carbon (Penet, 2006) .

In general it is observed that the content of oxidizable carbon at T1 and T3 sampling times for all remediation treatments have statistically significant higher values compared to the control treatment (L). This was explained in the previous paragraph. The same trend is shown in the oxidizable carbon content in T6 and T12 compared to T1 and T3. However the general content of oxidizable carbon tends to be less according to the sampling time as time goes by. This could be associated with the decrease of the content of soil HTP, corroborated by Chaineau, (2000) who found that initially pollution increased oxidizable carbon content in the soil and subsequently there was a significant decrease. Serrano, (2006) argued that there may be a moderate elevation in the levels of TPH after 40 days of contamination, because the degradation of the more complex hydrocarbons could generate degraded products.

Contamination with biodiesel produced a statistically significant decrease in pH in remediation treatments (LB, LBF, LBF, LBC, LBC) compared with the control (L) at T0 (Figure 1b). This decrease is due to the chemical properties of biodiesel (Table 3).

Treatment of organic biostimulation (LBC) produced the smallest decrease of soil pH, at different sampling times, this can be explained by the incorporation of a compound with high content of organic matter, which increases the buffer capacity of the soil (Frabrizzi, 1998). This same trend was maintained throughout the trial until T12. Martinez and Lopez (2001) and Romaniuk, (2007) reported that the pH did not change in the presence of different concentrations of other hydrocarbon types.

The extractable soil phosphorus at all sampling times T0, T1, T3, T6 and T12 showed no statistically significant differences between treatments (Figure 1. d) . This coincides with the results of Frick et al. (1999) , phosphorus is initially immobilized in the microbial biomass, and is available only a year later .

In T0 organic biostimulation treatments (LBC) and inorganic stimulation (LBF) had statistically significant higher values of nitrates in soil respect to other treatments (Figure 1. c). Obviously in the treatment of inorganic stimulation (LBF) this is due to incorporation of urea -based fertilizer nitrogen and in organic biostimulation treatment is due to the addition of nitrogenous substances rich compost.

In T1 and T3 content of nitrates in the soil of all treatments remediation were statistically significant lower than the control values situation. This could be related to the lowering of soil HTP used as carbon source for the microbial community, and in addition to the synthesis of nitrogen structures and cell multiplication.

Overall, the nitrate content in soil in T6 and T12 were statistically significant higher than those of T1 and T3. This could be due to the fact that low levels of PAH in soil generates a reduction of the microbial population due to the decrease in carbon sources, structures (Ciarlo and Palma, 2011).

Table 3.Some physical and chemical properties of the biodiesel

Properties	Unit	Content
Ester content	% (m/m)	96,5
Density 15 ° C	kg/m^3	900
Viscosity 40 ° C	mm^2/s	4
Cetane number		51,0
Water content	mg/kg	500
Acid value	mgKOH/g	0,5

II) Total Petroleum Hydrocarbons

Mean values of total petroleum hydrocarbons (TPH) (mg kg -1) for all treatments and sampling times, are shown in Figure 2.

The mean values of Total Petroleum Hydrocarbons (TPH) presented no statistically significant differences between treatments remediation at baseline (T0) and one month (T1) . However, in the following sampling instances organic treatments biostimulation (LBC) and bioaugmentation (LBM) presented statistically significant lower data compared to inorganic treatments biostimulation (LBF) and natural attenuation (LB), the last two presented no statistically significant differences between them.

This delay in the onset of biodegradation may be the result of an initial microbial acclimation, which rearranges the microbial community due to the microenvironmental change caused by biodiesel contamination, varying the relative proportions of the microbial populations without reaching an intense degradative activity until they present a new community equilibrium (Romaniuk, 2007). Trindade, (2005) observed that bacterial acclimatization, produced no HTP losses 15 days after contamination. In turn, Hutchison and Walecka and Walworth (2006) suggested that elevated levels of contamination can determine moderate initial inhibition of microbial activity.

III) Comparison between remediation treatments

Natural attenuation (LB) technique resulted less efficient on most sampling times (T3, T6 and T12). The same was corroborated by Romaniuk, (2007) comparing contaminated soils with different hydrocarbons. However, some authors have considered this remediation technique as "cost - effective and low risk" (Corona -Ramirez and Iturbe -Argüelles, 2005). Biostimulation organic treatments (LBC) and bioaugmentation (LBM) were more efficient (Table 4) in the degradation of biodiesel to 5 mg.g -1 established by the Dutch standards (Frank , 1999). The same was confirmed by Lee, (2008) comparing the efficiency of remediation through the use of inorganic fertilizer, fresh residues and compost: the incorporation of the latter substrate was the most efficient for remotion of hydrocarbons. However, Marja, (2005) reported that the addition of compost did not have a positive effect on the biodegradation of diesel. In agreement with the results of this study, Nuñez, (2002) obtained higher percentage of oil removal in less time with bioaugmentation techniques.
All treatments (LB, LBF, LBC and LBM) reached the remediation level in T12 (Table 4).

Table 4. Organic carbon content (%) at T0

	L	LB	LBF	LBM
% C	1,70 a	2,57 b	2,31 b	2,63 b

Different letters indicate significant differences (P <0.05) between treatments

CONCLUSIONS

Soil properties that were affected by contamination with biodiesel were soil pH and carbon content. All treatments showed a statistically significant decrease in pH and an increase in the carbon content.

Remarkable differences were obtained with the addition of compost (LBC). At baseline there were statistically significant differences of nitrate in soil, % C, and a buffer action in pH values.

Organic biostimulation treatments (LBC) and bioaugmentation (LBM) were the most effective treatments in the degradation of biodiesel, achieving the level of remediation at 6 months.

REFERENCES

Bento FM, Camargo FAO, Okeke BC and Frankenberger Jr WT. 2005. Comparative bioremediation of soil contamined with diesel oil by natural attenuation, biostimulation, and bioaugmentation. Biores. Techn. 96. 1049- 1055.

Bray RH y Kurtz LT. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59, 39-45

Cardona Villada W. Biocombustibles: verdades y mitos. 2007. www.chilecientifico.cl/index

Carole RS and Scarigelli FP. 1971. Colorimetric determination of nitrate after hydrazine reduction to nitrite. Microchemical Journal v.16, p.657-672.

Ciarlo E and Palma, M, Nitrógeno del suelo. 2011. Edafología bases y aplicaciones ambientales argentinas. Marta Conti-Lidia Giuffré Ed.s. Ed Fac de Agronomía-Orientación Gráfica Editora, Buenos Aires.

Corona-Ramírez L and Iturbe-Argüelles R. 2005. Atenuación natural en suelos contaminados con hidrocarburos. Ingeniería Investigación y Tecnología VI. 2. 119-126.

Chaıneau CH, Morel JL and Oudot J. 2000. Biodegradation of fuel oil hydrocarbons in the rhizosphere of Maize (Zea mays L.). J. Envir.l Qual. 29, 569–578.

DeMello JA, Carmichael CA, Peacock EE, Nelson RK, Arey JS and Reddy CM. 2007. Biodegradation and environmental behavior of biodiesel mixtures in the sea an initialstudy. Mar.Poll.Bull. 54,894–904.

Demirbas A. 2009. Political, economic and environmental impacts of biofuels: a review. Appl. Energy 86, 108–S117.

Doran JW and Parkin TB. 1996. Quantitative indicators of soil quality: a minimum data set. In: Doran JW, Jones AJ (Eds.).Methods for Assessing Soil Quality. SSSA Special Pub. 49, Soil Sci. Soc. Am., Madison, WI, pp. 25-37.

EPA. 1978. Method 418.1. Test method for evaluating total recoverable petroleum hydrocarbon (spectrophotometric, infrared). United States Government Printing Office, Washington, D. C., 9 pp.

Frabizzi K, Picone L, Berardo A and Garcia F. 1998. Efecto de la fertilización nitrogenada y fosforada en las propiedades químicas de un Argiudol Típico. Ciencia del Suelo 16; 71-76.

Frank, AS. 1999. Risk-Based Assessment of Soil and Groundwater Quality in the Netherlands: Standards and Remediation Urgency. Risk Anal. 19: 1235-1249.

InfoStat. 2002. versión 1.1. Manual del Usuario. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Primera Edición, Editorial Brujas Argentina.

Lee SH, Oh BII, and Kim J. 2008. Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity. Biores. Techn. 99 2578-2587.

Makareviciene V and Janulis P. 2003. Environmental effect of rapeseed oil ethyl ester. Renew. Energy 28, 2395–2403.

Margesin R and Shinner F. 2001. Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Appl. Environ. Microbiol. 67: 3127 - 3133.

Marja RTP, John P and Jaako AP. 2005. Phytoremediation of subarctic soil contamined with diesel full. Biores. Techn. 99 221- 228.

Martínez MV and López S. 2001. Efecto de los hidrocarburos en las propiedades físicas y químicas de un suelo arcilloso. Terra 19:9-17.

Nelson DW and LE Sommers. 1982. Total carbon, organic carbon and organic matter. Pp. 539-579 en AL Page (ed.). Methods of soil analysis. Part 2. American Society of Agronomy, USA.

Núñez RE, Ortiz EL, Fonseca O, Barban V and Almazán E. 2002. Biorremediación de hidrocarburos de los sedimentos de la bahia de La Habana. Revista Contribución a la Educación y la Protección Ambiental 12 (6): 45-60

Page AL. 1982. Ed. Methods of soil Analysis. Part 2. Chemical and microbiological properties. 2nd Edition. Madison, Wisc. USA, American Society of Agronomy (Agronomy Series no. 9).

Penet S, Vendeuvre C, Bertoncini F, Marchal R and Monot F. 2006. Characterisation of biodegradation capacities of environmental microflorae for diesel oil by comprehensive two-dimensional gas chromatography. Biodegradation 17:577–585.

Plaza G, Natecz-Jawaki G, Ulgih K and Bridmon RL. 2005. The application of bioassays as indicators of petroleum-contaminated soil remediation. Chemosphere 59, 289–296.

Prince RC, Haitmanek C and Lee CC. 2008. The primary aerobic biodegradation of biodiesel B20. Chemosphere 71, 1446-1451.

Romaniuk R, Brandy JF, Rios RP and Giuffré L. 2007. Atenuación natural y remediación inducida en suelos contaminados con hidrocarburos. Ciencia del Suelo (Argentina). 25(2): 139-149.

Serrano A, Gallego M, González JL and Tejada M. 2006. Natural attenuation of diesel aliphatic hydrocarbons in contaminated agricultural soil. Environ.l Pollut. 151 494-502.

Trindade PVO, Sobral LG, Rizzo ACL, Leite SGF and Soriano AU. 2005. Bioremediation of a weathered and recently oil-contaminated soils from Brazil: a comparison study. Chemosphere 58 (2005) 515–522.

Walecka-Hutchison CM and Walworth JL. 2006. Assessment of C:N Ratios and Water Potential for Nitrogen Optimization in Diesel Bioremediation. Bioremediation Journal Vol 10.

Zhuo E and Crawford R. 1995. Effects of oxygen, nitrogen, and temperature on gasoline biodegradation in soil. Biodegradation 6. 127-140.

Zitrides T. 1983. Biodecontamination of spill site. Poll. Eng. 15, 25- 27.