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JSM Biology

Short Communication

Ex situ Bioremediation of a Tropical Soil Contaminated with Diesel

Fernando Reynel Fundora Tellechea[1], Marco Antonio Martins[1]*, Alexsandro Araujo da Silva[2], Andreia Francisca Riter Netto[1], and Meire Lelis Leal Martins[1]

[1]*Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil*
[2]*Universidade do Estado do Rio de Janeiro, Brazil*

***Corresponding author**
Marco Antonio Martins, Universidade Estadual do Norte Fluminense Darcy Ribeiro- UENF. Av. Alberto Lamego, 2.000. CEP 28013-602 Campos dos Goytacazes (RJ) Brazil, Tel: 55-22-27310004; Email: marco@uenf.br

Submitted: 16 December 2016

Accepted: 07 February 2017

Published: 09 February 2017

Keywords
- Bioreactor
- Sugarcane filter cake
- Petroleum
- Soil micro-organisms

Abstract

The aim of this study was to evaluate the use of sugarcane filter cake and NPK fertilization in the bioremediation of a soil contaminated with fuel diesel, under two conditions: with and without forced air supply. The soil was collected on the same day of the experiment, in an area close to the 101 Brazilian highway, where a truck involved in an accident leaked approximately 30,000 liters of diesel from a ruptured tank. Five treatments (uncontaminated soil -T1; soil contaminated with diesel - T2; soil contaminated with diesel and treated with 15% (wt) filter cake -T3; soil contaminated with diesel and treated with NPK fertilizer - T4; and soil contaminated with diesel and treated with 15% (wt) filter cake and NPK fertilizer - T5) and four time periods (1, 60, 120, and 180 days after the beginning of the experiment) were used according to a 5 x 4 factorial design to analyze colony forming units (CFU) and total petroleum hydrocarbons (TPH). The addition of filter cake and NPK increased the micro-organisms population in soil, under both conditions, with or without forced air supply. Significant TPH removal was observed on day 180, when compared with the initial value (time 1) in both air conditions. It was observed that the best TPH biodegradation of 96 and 87%, with or without air supply conditions, respectively, occurred in T5.

ABBREVIATIONS

NPK: Nitrogen, Phosphorus and Potassium; CFU: Colony Forming Units; TPH: Total Petroleum Hydrocarbons

INTRODUCTION

The growing demand in Brazil and in the world for oil and its derivatives associated to the environmental effects of its use, mainly in the activities of extraction, transportation and refinement, have contributed to the contamination of the earth around the planet [1]. Diesel is one the most commonly found hydrocarbons in the environment, consisting of alkanes and aromatic compounds which may be spilled during storage and transportation [2].

In Brazil, there are serious problems of soil contamination by diesel oil spills with increasingly frequent occurrences, affecting areas of agronomic and forest interest [3].

In order to maintain the ecological balance, the remediation of environments contaminated with hydrocarbons with biological agents is a technique considered efficient, safe and less expensive when compared to the traditional physical and chemical treatments [4,5]. A metabolic development of the micro-organisms requires several biotic and abiotic factors that can be controlled with bioreactors. Some authors emphasize the use of bioreactors as an interesting and promising alternative for the treatment of contaminated soils [6-8]. The bioreactors are closed systems that can assume different types of configurations, facilitate a greater contact of the microorganisms with the contaminants, nutrients and oxygen during shorter periods, facilitating the acclimatization of the microbiota as well as its development [9].

To increase the biodegradation of diesel in the soil, some strategies can be adopted, such as the use of combinations of fertilizers and structurants to improve the physical, chemical and biological properties of soils, activating the native flora in soil [10-12].

Filter cake is a carbon-rich precipitate left behind after sugar cane juice is filtered by rotary vacuum filters during refining. We believe that the main advantage of using filter cake in diesel-contaminated soil, in relation to the other organic amendments (manure, yard wastes, sewage sludge, food processing wastes), is that besides it can be considered as a structure capable of

improving the physical, chemical and biological properties of soils [13], it is also produced in large scale in sugar cane industry, which is a serious potential source to environmental contamination [14]. Each ton of milled sugarcane generates 30 Kg of filter cake [15]. Approximately 19.04 millions of tons of filter cake were generated in Brazil in 2015 [16]. However, this byproduct is still rarely used, most of the time due to the lack of information about its behavior in diesel-contaminated soil.

Thus, the objective of this work was to evaluate the use of filter cake and NPK fertilizers in bioremediation of a soil contaminated with diesel using ceramic reactors.

MATERIAL AND METHODS

The present study was carried out in the State University of Northern Fluminense Darcy Ribeiro (UENF) in the city of Campos dos Goytacazes, RJ, Brazil.

Soil samples (dark red podzol) were collected within a depth of 20 cm in an area of approximately 800,000 m^2 along a highway (BR101, km 88), district of Itibioca, Campos dos Goytacazes, RJ, Brazil, where at least 30,000 L of diesel was leaked from a fuel truck after an accident on the same day. Samples of uncontaminated soil nearby the contaminated area were also collected. All samples were treated following the procedures described by Melo and Azevedo [17].

Chemical composition and total bacterial counts of the uncontaminated soil and the filter cake were performed (Table 1,2), according to the methodology described in official Brazilian guidelines for soil analysis [18].

A completely randomized design was used to analyze data. A 4 x 5 factorial design was used to analyze colony forming units and total petroleum hydrocarbons (TPH), with five treatments (uncontaminated soil - T1; soil contaminated with diesel - T2; soil contaminated with diesel and treated with 15% (wt) filter cake - T3; soil contaminated with diesel and treated with NPK fertilizer - T4; and soil contaminated with diesel and treated with 15% (wt) filter cake and NK fertilizer - T5), and four time periods (1, 60, 120, and 180 days after the beginning of the experiment). All analyses were conducted in triplicate.

The experiment was carried out under two conditions: with and without forced air supply. A clay pot of 2.0 L capacity was used as a bioreactor. A multi-hole rubber tube was placed inside the base of the bioreactor and connected to an air compressor (40 $lbf.pol^2$) to inject air into the soil.

The treatment with NPK fertilizer was carried out using a N:P:K ratio of 10:1:1, and was based on the results of the analysis of contaminated soil [19], which indicated the need to add 1.07 g $container^{-1}$ of ammonium nitrate (NH_4SO_4) as nitrogen source and 2.87 g $container^{-1}$ of dibasic potassium phosphate ($KHPO_4$) as source of phosphor and potassium.

Heterotrophic bacteria counts were conducted using the plate count agar method (PCA), which is based on a non-selective, extremely nutrient rich culture medium suitable to grow mesophilic aerobic bacteria at pH 7.0. Results were expressed as colony forming units per gram of oil (CFU g^{-1}).

The hydrocarbons present in soil samples during the bioremediation process were extracted by continuous extraction in a Soxhelt apparatus using hexane as solvent for 4 h [20].

The extracts obtained were analyzed by high-resolution gas chromatography coupled with mass spectrometer (HRGC-MS) (456-GC, Bruker Daltonics Inc.) connected to a triple quadrupole mass spectrometer (Scion MS-TQ, Bruker Daltonics Inc.). The analyses were carried out following the procedures described by Tellechea et al. [21]. The data obtained were submitted to an analysis of variance, and means were compared using the Tukey test at 5% significance.

RESULTS AND DISCUSSION

The bacterial colony forming units (CFU) in T2 treatment decreased on the first day of the experiment, presenting 1.5 x 10^5 CFU (Table 3). Probably this reduced amount of micro-organisms is due to the toxic effect of diesel oil [22]. Bento et al. [23], studying the degradation of hydrocarbons concluded that depending on the toxicity of the contaminant and the microbiota present, the microbial growth in the first days can be inhibited. However, the addition of filter cake to soil contaminated with diesel oil (T3) increased the microbial population when compared to the other treatments at the initial time with forced air supply. This could be associated with the large number of micro-organisms present in the filter cake (5.9 x 10^8 CFU) as shown in Table (2). On the other hand, in this same treatment (T3), when comparing the initial and final time, decreases of 38% and 45% in the number of micro-organisms were observed with and without forced air supply, respectively. These decreases were also observed in studies

Table 1: Chemical and microbiological characteristics of the soil used in the experiment.

Sample	pH	P	K	Ca	Mg	CFU (g $soil^{-1}$)
		(mg kg^{-1})				
Contaminated soil	5.7		5	54.6	624	180	2.6 x 10^6
Uncontaminated soil	5.3		1	35.1	660	129.6	4.0 x 10^4

pH in water; P and K were analyzed using a Mehlich extractor; Ca and Mg were analyzed using KCl 1 mol L^{-1}; CFU: Colony forming units.

Table 2: Chemical and microbiological characteristics of the sugarcane filter cake used in the experiment.

	pH	C	OM	N	P	K	Ca	Mg	B	Zn	Cu	Fe	CFU (g $soil^{-1}$)
		(%)		(g kg^{-1})					(mg kg^{-1})				
Filter cake	8.4	23.8	41.3	1.2	7	3.6	18.7	2.6	44.6	8.4	40.5	129	1.2 x 10^9

pH in water; P and K were analyzed using a Mehlich extractor; Ca and Mg were analyzed using KCl 1 mol L^{-1}; OM: organic matter; CFU: Colony forming units.

Table 3: Number of colony forming units in soil (CFU g^{-1} of soil)/

TRAT	Treatment period (days)							
	With air supply				Without air supply			
	1	60	120	180	1	60	1vv20	180
T1	31,67 B a*	17,07 B a	10,98 C b	6,39 B b	13,79 C a	12,60 C a	7,51 B a	83,59 B a
T2	15,01 C b	47,03 A a	47,03 A a	40,54 A a	40,95 A a	33,39 B a	26,37 A a	24,68 A a
T 3	62,58 A a	42,69 A b	23,69 B c	24,20 A c	58,89 A a	41,79 A a	38,92 A b	27,05 A b
T 4	41,42 B a	43,09 A a	37,56 A a	27,77 A a	30,23 B a	36,58 A a	39,65 A a	40,73 A a
T 5	43,55 B a	48,38 A a	42,32 A a	24,21 B b	41,77 A a	52,25 A a	43,39 A a	41,80 A a

Table 4: Total petroleum hydrocarbons (TPH) in soil.

TRAT	Treatment period (days)					Treatment period (days)				
	1	60	120	180	[1]Biodegradation (%)	160	120	180	[1]Biodegradation (%)	
	With air supply					Without air supply				
T1	0.00E+00	0.00E+000	00.00E+00	00.00E+00	**00**	0.00E+00	0.00E+00	0.00E+00	0.00E+00	**00**
T2	3.84E+08	3.13E+08	1.51E+08	1.89E+08	**51**	7.85E+08	5.34E+08	2.06E+08	3.15E+08	**60**
T 3	5.13E+08	2.45E+08	5.60E+07		6.08E+07	**88**	8.57E+08	5.34E+08	1.64E+08	1.53E+08	**82**
T 4	4.87E+08	3.15E+08	2.10E+08		1.80E+08	**63**	7.61E+08	2.10E+08	1.49E+08	1.97E+08	**74**
T 5	6.03E+08	2.63E+08	7.52E+07		2.20E+07	**96**	8.23E+08	2.35E+08	1.16E+08	1.08E+08	**87**

T1: uncontaminated soil; T2: soil contaminated with diesel fuel; T3: soil contaminated with diesel fuel and treated with filter cake; T4: soil contaminated with diesel fuel and treated with NPK fertilizer; T5: soil contaminated with diesel fuel and treated with filter cake and NPK fertilizer.
[1]Difference of TPH remains in soil between period 1 and 180 days

with soils contaminated with petroleum, probably related to the decrease in the capacity of some micro-organisms to degrade the contaminant [24,25].

In the 60-day period with forced air supply, 120 and 180 days without forced air supply, treatments with contaminated soil did not present a significant difference. These results suggest that the most labile compounds were consumed earlier (initial time) and those more resistant to microbial degradation, were consumed later (120 and 180 days). In addition, the consumption occurred faster in the treatments in which forced air was used, since the oxygenation of the environment favored the microbial activity, accelerating the degradation of the pollutant.

Regarding the TPH levels, the treatments led to a reduction in the values as a function of time (Table 4). The natural attenuation (T2) led to a lowest biodegradation rates, namely 51% and 60%, under forced and not forced air supply, respectively. The NPK edition (T4) increases the biodegradation rates to 63% and 74%, under forced and not forced air supply, respectively. However, the highest biodegradation rates were observed when filter cake was added to soil (T3 and T5), namely 96% and 87%, under forced and not forced air supply, respectively. These results indicate that sugarcane filter cake induced the biodegradation of TPHs, possibly due to the greater microbial community (biostimulation) expressed as CFU (Table 3). Since micro-organisms are the first agents of the degradation of organic contaminants in soil, the application of organic matter can be successfully used to accelerate contaminant degradation, as it n increases not only the microbial density, but also provides nutrients and readily degradable organic [26]. Though diesel is the source of carbon to the microorganisms, it does not provide other nutrients such as nitrogen and phosphorus, reducing significantly the bioremediation rates. The lack of these nutrients affects severely the microbial metabolism in soil, because an inappropriate nutrient ratio may retard or inhibit microbial activity [27,28].

Several studies have reported the potential of composted materials in bioremediation of TPH pollutants. Koshlaf et al. [12], reported that the addition of pea straw in a diesel contaminated soil reduced the TPH in 96%, after 12 weeks. Tellechea et al. [21], reported significant biodegradation of TPH after 180 days of a soil contaminated with diesel after addition of filter cake and nutrients. Also, Llado et al. [29], studying the bioremediation of heavy oil contaminated soil observed that treatments based on biostimulation (nutrient addition) and bioaugmentation (addition of micro-organisms) achieved between 30% and 50% total petroleum hydrocarbon (TPH) biodegradation after 200 days.

CONCLUSION

Sugarcane filter cake in association with NPK fertilizer can be successfully used to remediate areas contaminated with hydrocarbons from fuel diesel since it increases the microbiota (biostimulation) and removes TPHs of the contaminated soil. The air supply promotes a greater bioremediation of soil diesel. Further studies are required to evaluate the use of sugarcane filter cake in diesel contaminated soil under field condition (*"in situ"*).

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grants of the first author and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the financial support.

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Cite this article

Fundora Tellechea FR, Martins MA, da Silva AA, Riter Netto AF, Martins L (2017) Ex situ Bioremediation of a Tropical Soil Contaminated with Diesel. JSM Biol 2(1): 1007.