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BIOREMEDIATION (BIOSTIMULATION AND BIOAUGMENTATION) OF OIL SPILLS CONTAMINATED SOILS UNDER TROPICAL HUMID FOREST

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Abstract: Colombia has had large oil spills in old oil extraction areas and in oil infrastructure (associated to attacks and illegal valves) causing serious damages to natural ecosystems. In this study laboratory pilot tests were carried out on the bioremediation of oil contaminated soils from Silvania in Colombia. The objective of the current investigation is assessing the effect of Tween 80 surfactant in the degradation of TPH in the bioremediation (Biostimulation and Bioaugmentation) treatments in anaerobic conditions. Two Bioaugmentation treatments and three Biostimulation treatments were proposed: Bioaugmentation (B), Surfactant Bioaugmentation (SB), Biostimulation (Bs), Surfactant Biostimulation (SBs) and Surfactant Biostimulation with D-Limonene (SBsDL). For the Bioaugmentation treatments, the bacterial density was increased using composting and in Biostimulation treatments nutrients and amendments were applied. Additionally, Natural Attenuation treatment (NA) and Control treatment (C) were carried out. After 90 days the degradation of TPH the results for each treatment are Control (53.8%), NA (57.7%) B (65.4%), SB (69.2%), Bs (75.0%), SBs (76.9%) and SBsDL (76.9%). The results indicate that bioremediation treatments with surfactant have higher percentages of biodegradation of TPH, both B and Bs treatments show a decrease in the remaining TPH compared with the Natural Attenuation Treatment. The increase of the microbial population and the stimulation with nutrients in the bioremediation treatments under anaerobic conditions has a positive effect to decrease the number of hydrocarbons present in a contaminated site. The laboratory tests revealed that up to 76.9% of the oil contaminants were degraded within 90 days, indicating the feasibility of developing a bioremediation protocol on a large scale.

Key words: Soil contamination, Oil spill, Microbial consortia, Bioremediation, Anaerobic conditions.

1. INTRODUCTION

Soil pollution by oil in Colombia is a result of 2500 oil infrastructure attacks (1980 to 2015), installations of illegal valve and problems in abandoned wells.

According to Ecopetrol (2015), only the attacks caused the spill of 4 million barrels. Crude oil (petroleum) is a highly complex mixture of organic compounds that caused damages to superficial water, human health, plants and animals, reducing growth and interfering with normal develop (Li et al., 2010, 2015; Kauppi et al., 2011). Over 17000 organic compounds have been identified in crude oil, and subdivided into four main classes: the saturates, aromatics, asphaltenes and resins (Brooijmans et al., 2009; Marshall & Rodgers, 2004). In addition the crude oil is a compound of polar hydrocarbons as n-alkanes, branched and cycloalkanes, aromatics, resins and asphaltene which have low solubility in water, highly hydrophobic, absorbed on soil particles and persistent organic pollutants (Liang et al., 2014; Wu et al., 2015).

Some hydrocarbons like Polycyclic Aromatic Hydrocarbons (PAHs) have carcinogenic and mutagenic properties (Ambrosoli et al., 2005), for this reason PAHs are classified by the Environmental Protection Agency (EPA) as priority pollutants (Wu et al., 2015). In Colombia, Hydrocarbons are considered as toxic compounds by the Ministry of Environment and Sustainable Development (MESD). According to Xu & Lu (2010), bioremediation is an appropriate technology to clean-up contaminated soils, because it is efficient, environment friendly and an economical technology compared to chemical and physical methods.

Bioremedition using compost is one of the most-effective methods for simultaneously diminishing pollutant incidence increasing soil organic matter content and soil fertility, diminished possible impact of contaminants through time (Chen et al., 2015). Bioremediation strategies such as bioaugmentation and biostimulation have been applied in the world to cleanup soils, marine sediments, marsh sediments and water (Chang et al., 2008; Hatzikioseyian, 2010; Wu et al., 2015). In some case when oil is sequestered by sediments, the bioavailability can be severely reduced, significantly slowing down or even preventing biodegradation (Brooijmans et al., 2009). Nevertheless, surfactants are a promising form of bio stimulation, due to the increase of oil-surface area and with that the amount of oil actually available for attack by bacteria (Nikolopoulou & Kalogerakis, 2009). Traditionally the research about hydrocarbon biodegradation has focused on aerobic biodegradation, however several studies show that different electron acceptors like sulfate, nitrate, and ferric iron can degrade Aromatic Hydrocarbons (AH) under anaerobic conditions (Boopathy, 2003; Coates et al., 1997; Su et al., 2012). The objective of this study was to evaluate the effect of Tween 80 surfactant on the degradation of TPH in the treatments of biostimulation and bioaugmentation in anaerobic conditions for a chronically oil polluted soils in a tropical humid forest. To examine the efficiencies of biostimulation and bioaugmentation we used laboratory pilot plots with and without surfactant. We monitored changes in hydrocarbon concentration in soil and changes in soil microbial activity depending on degradation percentage.

2. MATERIALS AND METHODS

2.1. Soil sampling

The soil samples used for this study were taken in the site Silvania, Orito municipality, Putumayo region - Colombia (Fig. 1), January 2017. Sampled site is 310 m.a.s.l. The mean annual air temperature was 23.0°C; the annual mean precipitation was 3000-4000 mm.

This region has soil types ranging from soils with incipient development such as Fluventic Dystrudepts, to soils with a medium-low degree of weathering, such as Oxic Dystrudepts under alluvial and coluvio-alluvial megafan position (Instituto Geográfico Agustín Codazzi, 2014). Soils were well to poorly drained and depth, with high aluminum saturation and low to medium fertility, these soils have high rates of mineralization of organic matter. The predominant soil textures are sandy loam and loam. A detailed soil characteristics are showed in Table 1.

Figure 1. Study site

Surface soil samples were collected after removing the surface vegetation at 20 cm depth from the top. To ensure homogeneity of pollutants in samples, the soil was crushed and then passed through a 2 mm sieve. Soil properties were measured using standard methods for physically and chemically soil analysis. Soil texture was characterized using the Bouyoucos method (Bouyoucos, 1962). Organic matter (Walkley & Black, 1934), pH, electrical conductivity (Jackson, 1964), potassium and magnesium content (ammonium acetate 1M), ammonia, nitrate (Crosby, 1968) and phosphorus content (Bray & Kurtz, 1945) were assessed previously.

Table 1. Physical and chemical properties of contaminated soil samples from Orito (Putumayo, Colombia)

Parameter	Value
Soil texture	Sandy loam
Sand (%)	78
Silt (%)	12
Clay (%)	10
pH	5.9
Organic matter (%)	16.9
N Total (%)	0,29
K Total (mg/kg)	97,73
Mg Total (mg/kg)	461.89
P Total (mg/kg)	17

2.2. Experimental procedure

All experiments were conducted in the plastic pan in a shady conditions for 90 days, using a completely random statistical design of one factor corresponding to TPH and seven levels corresponding to treatments (Cavazzuti, 2013; Montgomery & Runger, 2003). Each replica has two kilograms of dry soil moistened up to 59% humidity (soil water saturation point), added 314g of KNO3 as electron acceptor. Seven treatments were replicated three times, the implemented treatments in this phase were: (C) Control with addition of hydrochloric acid 2M and Natural Attenuation (AT), Bioaugmentation (B), 10% of compost with Bioaugmentation and surfactant (SB), 10% of compost and Tween 80 CMC, (Bs) Biostimulation with molasses and Leonardite, (SBs) Biostimulation with surfactant, molasses, Leonardite and Tween 80 CMC, (SBsDL) Biostimulation with surfactant, D-Limonene, molasses, Leonardite and Tween 80 CMC.

The amount of nitrate used in this study was calculated using stoichiometric relationships established by the McCarthy method, Leonardite was applied according to Turgay et al., (2010). The bioaugmentation treatments have 10% of compost, it is the precise amount to not dilute the hydrocarbons in the microcosms according to Su et al., (2012). The bacterial count, redox potential (Ep), hydrogen potential (pH), electrical conductivity (EC) were monitored weekly for a 12 week period. Physicochemical characterization and quantification of TPH concentration were carried out at the beginning and the end of the experiment.

2.2.1. Chemical parameters.

The pH and the EC values were measured with a portable HQ40d (Hach oveland, CO, USA). Two grams of soil (dry weight) was mixed with 20ml of distilled water to a concentration of 1:10. The mixture was with a vibration stirrers Vortex (FALC®) for 90 seconds and the electrode was inserted for 1 minute in the solution to register the measurement. The Ep was measured directly in the microcosms according to Kolmans & Vásquez (1999) methodology .

2.3. Extraction and analysis of soil total petroleum hydrocarbons concentration

Total petroleum hydrocarbons (TPH) were extracted from each treatment two times, at the beginning and the end of the experiment. The extraction of TPH followed the procedure describes by Standard Method 5520f (Mathew, 2009). For the extraction of TPH, vials of polypropylene of 50 ml by centrifuge (Jouan serie MR22) were used. For subsamples of 1.0 g of soil (dry weight), 2.5 g of Na_2SO_4 anhydride, and then 5ml of dichloromethane were added and then put in the vortex (FALC®) for 90 seconds. The sub-samples were stirred at 7000 rpm for 20 min. This procedure was repeated twice. The supernatant was put in rotary evaporators (Heidolph Laborota 4003-control) at 40 C and pressure of 740 mmHg in order to separate the solvent and organic extract. A gas chromatograph (Agilent series 6890N) with Selective Mass Detector (Agilent 5973 Network), and an injector (Agilent 7683) besides column DB-TPH.123.1632 (Agilent Technologies) of 30m of longitude, 0,32mm of diameter and film packaging 0,25um were used for the qualification of TPH. All samples were analyzed at 60°C like initial temperature during 2 minutes, then temperature increase 8°C per minute until 300°C, this last temperature was maintained for 8 minutes. The temperature of the injector was 250°C while the temperature in the detector was 340°C. Hydrogen gas was used like carrier gas, constant flux was 2 ml per minute.

2.4. Statistical analysis.

Factorial analysis of variance (ANOVA) was used to determine the significance among the means of different treatments to compare whether applied bioremediation treatment differ significantly in TPH degradation ($p < 0.05$). The high value of F 29.21 indicates that surfactant and application of nutrients affect the rate of TPH degradation under different bioremediation treatments showing that there is significant difference between the means of the seven treatments. The significant differences between means were determined using Multiple range tests (Scheffé).

3. RESULTS AND DISCUSSION

All treatments start in moderately reducing conditions (this coincides with David (2017)) and Ep decreased over time except in the Control treatment. Figure 2 shows the Ep in all treatments. The Ep reduced as a result of the consumption of dissolved oxygen and onset the compound like ammonia and methane (Atlas & Bartha, 2002). The behavior of Control treatment may have been due to hydrochloric acid because it increases the redox potential (Santiago Jimenez, 1994). At the end of treatments Bioaugmentation have a Ep of 127,6 mV (B) and 86,8 mV (Bs), this behavior was due to low availability of high bioavailability carbon source than molasses, result in a slow consumption of dissolved oxygen in the microcosms. For Bioaugmentation treatments with 10% of compost (bulking agent enhance the oxygen transfer), Ep values at the end of Biostimulation treatments were -7,6 mV (BE), -48,6 mV (BES) and -22,7 mV (BESL). In the case of Biostimulation treatments with Leonardite and molasses have a large amount of carbon in their molecules, according to Atlas & Bartha (2002); Bia et al., (2012) this characteristic favors the occurrence of anaerobic conditions.

The Biostimulation treatments have the lowest Ep, for this reason, factorial analysis of variance and multiple range tests were performed. Figure 3 shows the Ep for Biostimulation treatments which were divided in six sections. According to the factorial analysis of variance sections 1 and 6 there is no significant difference between the means while sections 2, 3, 4 and 5 show a significant difference. The multiple range tests show that sections 2 and 3 have a significant difference between the treatments BES-BESL and BE-BESL, this result suggests that D-limonene exert an effect on the decrease in the Ep. A similar result was found in the sections 4 and 5 where there is significant difference between the treatments BE-BES and BE-BESL, suggesting that D-limonene and Tween 80 had effect in the decrease in the Ep in this period of time (47 to 77 days).

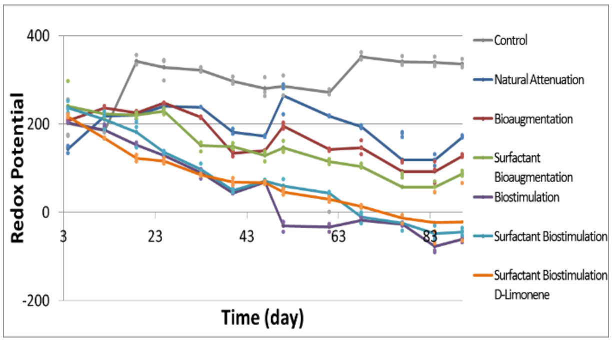

Figure 2. Redox Potential (Ep) throught treatments

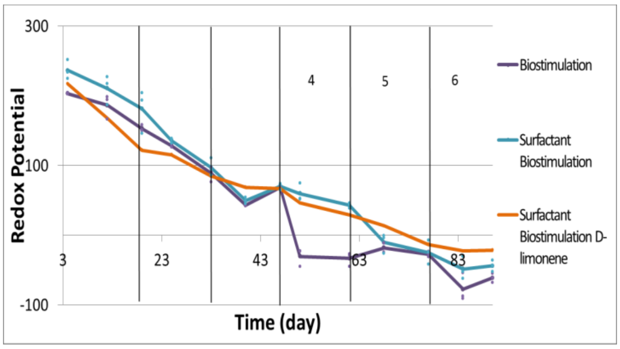

Figure 3. Redox Potential (Ep) in Biostimulation treatments

The pH in the Bioaugmentation treatments fluctuate between 7,2 to 7,3, this results are according to (Terrón, 1992), who reports a gradual variation of pH as a result the buffer effect of compost. The Biostimulation treatments reach values of 8,8 to 9,0. This result coincides with Dell'Anno et al., (2009), who attributes that response to hydrolysis of urea and increase of anaerobic breathing, or Leonardite's pH (8,46) conditions. Figure 4 shows the percentage of degradation in all treatments. The control treatment has the lowest percentage of degradation (53.8%) this result is associated with the hydrochloric acid because it was the only substance added in the treatment furthermore there was not count of bacteria in the petri dish during the experiment. The Natural Attenuation had a percentage degradation of 57.7% which is associated with native bacteria. Natural Attenuation had a maximum amount of bacteria of 3.77×10^7 UFC/g in treatment. The highest degradation percentages correspond to the Biostimulation treatments (76.9%). Because of the proximity of the percentage degradation in treatments, multiple range tests were used for determining whether there is the significant difference between treatments. The result showed no significant difference between SBsDL–SBs treatments, however between surfactant and without surfactant exist significant differences, see Table 2 Biostimulation with surfactant (SBs) showed values 76.9% higher than the other experimental essays being the best treatment.

Table 2. Degradation percentages applying the Scheffé test.

Treatment	Cases	Mean	Homogeneous group
NA	3	57.7	A
B	3	65.4	B
SB	3	69.2	C
Bs	3	75.0	D
SBsDL	3	76.9	E
SBs	3	76.9	E

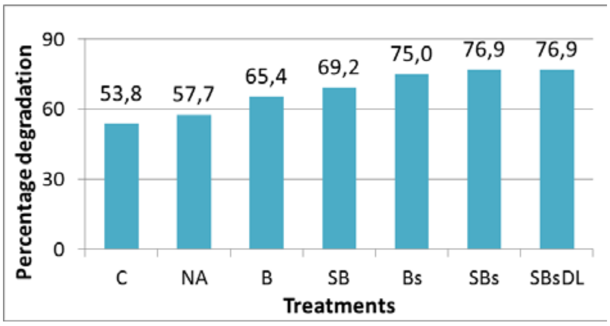

Figure 4. All treatments Biodegradation

The treatments with surfactant showed a higher percentage of biodegradation, because of Tween 80 surfactant solubilize the hydrocarbons in the soil increasing bioavailability, and stimulate the microorganism present in the substrate to use TPH as carbon source. These results are according to Atagana et al., (2003) and Zhang & Zhu (2012) Tween 80 surfactant research. Studies with methyl-β-cyclodextrin (MCD) realized by Sun et al., (2014) showed high PAH biodegradation comparing to control. According to Sun et al., (2014), this response is related to higher bioaccessibility of PAH or MCD associated to better microenvironmental conditions for microorganisms populations, resulting in major PAH degradation. The better results for Biostimulation treatments were conjoint with another carbon source (help to increase bacterial density), responsible to greater biodegradation percentages, probably the carbon source help to increase the bacterial density, thus leading to have less TPH in the soil. Researches than Su et al., (2012), Liang et al., (2014) and Ambrosoli et al., (2005) have shown the same results. Studies of natural attenuation without surfactant and compost than carbon source realized by

Ambrosoli et al., (2005) showed the lowest percentage of degradation.

The increase in 18.18% in Bioaugmentation respect to 57.7% in Natural Attenuation treatment can be explained for addition of 10% compost favored bacterial density. This results are according to Su et al., (2012) who added 10% of spent mushroom (SMC) compost with similar results. The cited authors attribute increasing degradation of PAH to SMC have substantial nutrients and abundant different kind of bacteria. Bia et al. (2012) found that in Ep at anoxic level close to anaerobic conditions, the denitrification and decomposition of organic compound can be improve, nevertheless, at the end of this research Biostimulation treatments have lower level Ep and less concentration of TPH. A detailed description of biodegradation percentages between treatments are showed in the Figure 4.

4. CONCLUSIONS

The bioremediation treatments with surfactant have higher percentages of biodegradation of TPH comparing with treatments without Tween 80. The Biostimulation with surfactant (SBs) and Biostimulation with surfactant and D-limonene (SBsDL) treatments did not present a difference in the percentage of biodegradation. The Bioaugmentation and Biostimulation treatments showed a decrease in the remaining TPH compared with the Natural Attenuation treatment. The stimulation with nutrients under anaerobic conditions in the bioremediation treatments have a positive effect in the decrease of hydrocarbons present in a contaminated site. This methodology is a cheaper possibility for soil bioremediation, environmental friendly that could be applied in different scales. Nevertheless, it is necessary to study in depth the interrelation between microorganism population diversity, amount and mechanism improved the nutrient contents of soil, carbon degradation and reduced the soil contaminant content.

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