

## Research Article

# The Effects of Natural Tropical Dry Season Conditions on the Rate of Bioremediation on Crude Oil Contaminated Soil

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**Citation:** Arjoon K, Speight JG (2019) The Effects of Natural Tropical Dry Season Conditions on the Rate of Bioremediation on Crude Oil Contaminated Soil. Arch Pet Environ Biotechnol 4: 157. DOI: 10.29011/2574-7614.100057

**Received Date:** 17 October, 2019; **Accepted Date:** 30 October, 2019; **Published Date:** 05 November, 2019

## Abstract

Treatment of oil contaminated soils is necessary to protect water supplies, human health and environmental quality. Bioremediation has proven to be one of the most cost effective treatment technologies for petroleum contaminated soils. But the success of biodegradation on the oil pollutants in soil depends on numerous environmental parameters and operational factors, all of which need to be optimized in order to achieve maximum contaminant treatment. This study shows bioremediation under the hot dry conditions of Trinidad and Tobago using heavy oil contaminants. The results will be beneficial to the petroleum industry because of the presence of heavy oil regions in the Southern basin area of Trinidad and the results being based on a two season type climate as opposed four season climate data.

**Keywords:** Bioremediation; Microorganisms; Oil Degradation; Tropical Climate

## Introduction

Soil is the material basis for the sustainable economic and social development and is one of the most valuable natural resources in each country. Therefore, soil pollution is of special importance because of its impact on surface, groundwater and air contamination and can easily spread and be consumed by humans. Pollution caused by crude oil is the most prevalent problem in the environment and the release of crude oil into the environment by oil spills is receiving worldwide attention due to the potential risks posed to the ecosystem. Petroleum products can be released into the soil environment via industrial and domestic accidents and negligence which adversely affect agricultural, residential or recreational land use. Therefore, it is very vital to control oil pollution in various environments and to remediate oil polluted sites as well. The characterization of the spilled oil and its derivatives is very important in order to predict the behaviour of oil and its long-term effects on the environment and to be able to select the proper cleaning methods.

It is also important to have remediation treatments that are both effective, and ecologically not harmful. As microbial treatments can enhance oil remediation in tropical settings, the success of a remediation approach will be determined based on the type and amount of oil, type of soil and/or sediment, microbial inoculants and the often changing physical, chemical and biological environmental conditions. In the tropical climates there are two seasons: a wet season and a dry season with the climates being relatively hot throughout the year with very intense sunlight. Thus, making microbes excellent players under these conditions as they are widely diverse organisms. They will adapt and grow at low temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. Secondly, all natural soils contain vast populations of microscopic plants and animals present in a state of dynamic equilibrium and changing balances. The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities, given a specific set of environmental and cultural conditions, has made them likely candidates for solving particularly difficult problems in the life sciences and other fields as well.

Soil remediation methods can be divided into three parts; biological, physical and chemical which can be done *ex situ* or *in situ* depending on the type of method [1,2]. Usually, the biological methods are environmentally friendly and retain the quality of environments (soil or water) during the remediation process. These methods are cheaper than physical and chemical techniques used for remediation. Therefore, making it a favorable, non-destructive, cost-effective clean up technology for treating contaminated soil.

Bioremediation is a complex process, with biological degradation taking place in the cells of microorganisms which absorb pollutant, where if they have specific enzymes, the degradation of pollutants and their corresponding metabolites will take place [3]. Hydrocarbons from oil are used as a source of nutrients and energy for microorganism growth, and at the same time, microorganisms decompose them to naphthenic acids, alcohols, phenols, hydro peroxides, carbonyl compounds, esters, and eventually to carbon dioxide and water [4,5].

Bioremediation uses microorganisms to reduce or break down hazardous organic material to harmless compounds, such as carbon dioxide and water. Bioremediation strategies range from encouraging natural biodegradation processes (bio stimulation), to supplementing the existing system with microorganisms able to degrade the contamination (bioaugmentation), to monitoring and verifying natural processes (natural attenuation). The microorganisms used to perform this function are known as bioremediators. The bioremediation method depends on having the right organisms with the environmental factors for optimum removal pollutant. It is determined by the interactions between three factors: substrate (pollutant), organisms, and the environment. The interactions among these factors affect the feasibility of bioremediation which determines whether the petroleum hydrocarbon can be degraded or not while the available microorganisms can degrade it. For bioremediation to be a viable mechanism, the environment needs to be habitable for organisms involved. These include nutrient availability, optimal pH, and availability of electron acceptors, such as oxygen and nitrate.

Oil biodegradation by bacteria can occur under both oxic and anoxic conditions (e.g., Zengler et al., 1999), albeit by the action of different consortia of organisms. In the subsurface, oil biodegradation occurs primarily under anoxic conditions, mediated by sulfate reducing bacteria in cases where dissolved sulfate is present (e.g., Holba et al., 1996), or methanogenic bacteria in cases where dissolved sulfate is low (e.g., Later et al., 2006, Bennett et al, 1993). The petroleum hydrocarbons present in oil spills are converted to carbon dioxide and water or are used as a primary food source by microorganisms, which use the energy to generate new cells. The most frequently used isolated hydrocarbon degraders in the bacterial genera are *Pseudomonas*, *Acinetobacter*,

*Flavobacterium*, *Brevibacterium*, *Corynebacterium*, *Arthrobacter*, *Mycobacterium*, and *Nocardia*, while the fungal genera are *Candida*, *Cladosporium*, *Rhodotorula*, *Torulopsis*, and *Trichosporium* [6].

Bioremediation is a scientifically intensive procedure, but will not always be suitable, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long and the residual contaminant levels achievable may not always be appropriate. Therefore, the questions that need to be answered before using the bioremediation process are as follows: are the contaminants biodegradable, is biodegradation occurring in the site naturally and whether the environmental conditions are appropriate for biodegradation or not.

## Materials and Methods

An extraction site for the soil sample was first located and a history on the site usage was conducted to ensure it met experimental requirements. The site location used for this extraction was in Trinidad, West Indies. It had not been subjected to any chemical or biological alteration for the past twenty-five years and there was no storage of anything that may have left traces of metals or chemicals in the soil to cause genetic modification of any living organisms in the soil. The site was covered entirely with grass that protected the soil from excessive drying that may have affected a proper representation of microorganisms in the topsoil.

Trays (1m x 1m) filled with virgin soil were covered with varying volumes of oil randomly spilled; this was done to simulate an oil spill. The experimental controls were heated in an oven to destroy any indigenous microbes that were in the soil so as to analyze the effects of natural conditions on the soil. The microorganisms present in the remaining trays were the indigenous microorganisms found in these soil samples, no additional microorganisms were added.

The microbes were tested by using an adaptation of Pour Plate Method (9215B) from Standard Methods for the Examination of Water and Wastewater 21st Edition. The pour plate method was used with an incubation temperature of 35 °C for 48 hours, using plate count agar. The composition of the soil sample was analyzed by the oven dry method, conductivity and pH analysis, UV/Visible Spectrometry and Infrared Spectrometry. Additional environmental parameters also measured were temperature and the presence of various gases.

## Results and Discussion

The pour plate method was used with an incubation temperature of 35 °C for 48 hours, using plate count agar. The composition of the soil sample was analyzed by the oven dry method, conductivity and pH analysis, UV/Visible Spectrometry and Infrared Spectrometry. Additional environmental parameters

also measured were temperature and the presence of various gases.

### Moisture Content of the Soil

The oven dry method is one of the most common methods of determining soil moisture content [7]. It is the standard method for the determination of water content in the laboratory. It consisted of taking a soil sample, determining its exact weight and drying the sample in an oven at a temperature of 110 centigrade for 24 hours, then weighing the sample and determining the moisture loss by subtracting the oven-dry weight from the moist weight. The moisture content was then expressed as a percentage of the oven-dry weight of the soil. The oven-drying method is the most accurate method of determining water content. The only disadvantage of the method is that it takes minimum 24 hours to know the test result.

The moisture content of the given soil sample was less than one percent (Table 1) which may adversely affect microbial activity [8]. This is because soil salinity acts as a microbial stressor in certain environments. Salinity exerts a primary limitation on water availability, which is determined by soil composition and texture, and osmotic potential, which is controlled by the total ion concentrations. Soil salinity can also lead to high internal levels of ions that are toxic to metabolic activities and can denature the extracellular enzymes necessary for carbon and nutrient acquisition (Applied and Environmental Microbiology, May 2014).

Mass of clock glass/g	133.25
Mass of clock glass & sample before heating/g	283.98
Mass of clock glass & sample after heating/g	282.99
Mass of $H_2O$ lost/g	0.99
Moisture content of the soil/%	0.66

**Table 1:** Moisture Content of the Soil.

### Conductivity and pH of the Soil Sample

Soil Electrical Conductivity (EC) is a measure of the amount of salts in soil (salinity of soil). While the test for soil pH measures the acidity (pH less than 7.0) or alkalinity (pH greater than 7.0). Soil pH measurements are useful because it predicts the various chemical activities taking place. But, the determination of pH requires the presence of moisture, and given the fact that the moisture content in the soil was quite low, it may have led to the unavailability of nutrients. Soil pH also directly affects the solubility of many of the nutrients in the soil.

The pH of the soil sample indicated that the soil was acidic and may have hindered the microbial activity or even the presence of microorganism. Soil processes, including nutrient availability and microbial activity, are favoured by a soil pH range of 5.5 - 8. The growth of most bacteria in this experimental work is limited to a pH range of approximately 3, which is acidic. In very acid soils,

all the major plant nutrients (nitrogen, phosphorus, potassium, sulphur, calcium, manganese and the trace element molybdenum) may be unavailable, or only available in insufficient quantities.

The pH range classifications of micro-organisms include: acidophile (pH < 5.4), neutrophile (pH 5.4 - 8.5) and alkaliphile (pH 7.0 - 11.5). At very acid or alkaline pH levels, organic matter mineralization slows down or stops because of poor microbial activity linked to bacteria. The pH of this soil sample would allow the growth of acidophile (Table 2). Soil pH directly affects the activity of these microbes, as acid soil, particularly in the subsurface, restricts access to water and nutrients.

Conductance of the soil sample filtrate = 60.6 $\mu$ S K value = 0.351 $cm^{-1}$ Conductivity of the soil sample filtrate = 2.13 x 10-5 $\mu$ S $cm^{-1}$
pH of the soil sample filtrate = 3.27 Voltage produced = 205mV

**Table 2:** Conductivity and pH of The Soil.

### Infrared Analysis of the Soil

The solvent used for extraction was initially examined before analyzing the extracted material, so as to eliminate the peaks that may have appeared as a result of the presence of the solvent. Therefore, when these peaks were eliminated, four peaks were identified (Table 3). These could be identified as phosphorus functions, nitrogen functions and elements singly bonded to hydrogen.

Wavenumber/ $cm^{-1}$	Absorbance
3896.2	0.1146
3435.5	0.3607
2360.9	0.1459
1637.6	0.1962

**Table 3:** Soil Sample Peak Table.

### Ultraviolet–Visible spectrophotometry (UV/Vis) Analysis of the Soil

The UV/Vis analysis of the soil samples had verified the presence of nutrients which is the deciding factor in microbial metabolism. The results from the UV/Vis analysis in the soil used showed the concentration of phosphate present ranging between 0.01-1.0ppm initially. Optimum microorganism growth occurs when phosphorus levels exceed 7 ppm, while at least 1 ppm is required for growth. The results show an increase in phosphorus levels at the end of the trial period (Table 4). This is an indication of the presence of phosphorus solubilizing bacteria in the soil.

Sample Run	Absorbance Region / nm
At the Start of the Trial Period	190 – 344
At the End of the Trial Period	200 – 256

**Table 4:** UV/Vis Phosphate Results Obtained for Soil Sample Analysis.

Phosphorus creates phosphate, which is essential for the sustenance of plant and microorganisms. Phosphorus is found in both organic and inorganic forms in soils. The organic forms are found in humus and plant residues and in general, the inorganic forms of phosphorus are very slowly available because of their reactions with iron, aluminum, calcium or other elements. The results for nitrate analysis (Table 5) obtained for the soil sample analysis at beginning of biodegradation showed a concentration of 1.09 ppm, while at the end it increased to 4.41 ppm.

Sample Run	Absorbance at 220nm	Absorbance at 275nm
At the Start of the Trial Period	4	0.013
At the End of the Trial Period	4	-0.5

**Table 5:** UV/Vis Nitrates Results Obtained for Soil Sample Analysis.

Microbial cultures usually require at least five parts per million (ppm) nitrogen for acceptable growth. Nitrogen is found in the proteins, enzymes, cell wall components, and nucleic acids of microorganisms and is essential for microbial metabolism. Because only a few microorganisms can use molecular nitrogen, most microorganisms require fixed forms of nitrogen, such as organic amino nitrogen, ammonium ions, or nitrate ions. The presence of nitrogen fixing bacteria increases the required forms of nitrogen for microorganism use.

#### Micro-organism Analysis of the Soil Samples

The Pour Plate Method (9215B) from Standard Methods for the Examination of Water and Wastewater 21st Edition) was used to confirm that microorganisms were present in the soil and were available for biodegradation of the oil in the soil sample (Table 6). The increase in the amount of microorganism is a direct conformation that biodegradation has occurred. The oil sample provides a direct source of nutrient supply to the microorganisms for growth and metabolism. Waste products of this biologically catalyzed degradation are water and carbon dioxide.

Sample Description		Heterotrophic Plate Count (CFU/g)
Soil without oil	Soil Sample at Start of Experiment	25912
	Soil Sample at the End of Analysis	Too numerous to quantify
Soil with oil	Soil Sample mid-stream of Analysis	25,60,436
	Soil Sample at the End of Analysis	3,79,000

**Table 6:** Heterotrophic Plate Count for the Soil Samples.

Microorganisms tend to have temperature ranges at which their growth is optimized. Psychrophiles have an optimum temperature ranging of 15 °C to 20 °C while thermophiles can grow and prosper at temperatures in excess of what would denature key proteins in most organisms (i.e., typically 50 °C), up to and including the temperature of water boiling at sea level. Mesophiles are bacteria which have an optimal temperature ranging between 25 °C and 40 °C. The temperature of the environment in which the biodegradation took place was 29 °C -33 °C which would have permitted microbial growth and microbial activity of mesophiles (Table 7). The percentage of oxygen present, would allow aerobic metabolism on the surface of the soil while anaerobic metabolism in the subsurface. The presence of both aerobic and anaerobic microorganisms would assist in biodegradation of the oil sample.

#### Various Environmental Parameters for the Trial Period

Temperature/ °C = 29 - 32
O <sub>2</sub> /% = 20.9, CO/ppm = 0.00, H <sub>2</sub> S/ppm = 0.00, LEL /% = 0.00 - 0.01

**Table 7:** Temperature and Gas Recordings of the Area in Which the Experiment Occurred.

#### Conclusions

As the economy and the human population increases, the need for more crude oil has been explored and exploited worldwide to meet its demand. Despite improvements in technology and preventative measures and regulations the occurrence of oil spills are still seen globally. Therefore, to reduce its devastating environmental impact, bioremediation has been introduced into some oil spill accident scenes. These oil degrading indigenous microorganisms played a significant role in reducing the overall

environmental impact. But, the fate of all oil spills will depend upon a unique set of circumstances that will govern risk and impacts, including the volume of oil spilled the chemical nature of the oil, and the ecosystems with their specific environmental conditions impacted by the spilled oil.

Temperature is one of the most important environmental factors affecting the growth of microorganisms and also influences the petroleum degradation. The tropics environmental conditions are seen to be suitable based on the experimental data collected. The oil-degrading microbes were able to survive and increase in population under these given environmental conditions.

Bioremediation is an efficient and environmentally friendly technology for long-term restoration of sites contaminated with petroleum hydrocarbons and derivatives. However, the remediation of oil spill sites can be further increased by bioaugmentation and bio-enrichment in sites that lack significant microbial populations capable of degrading petroleum and its derivatives by using the environmental data collected.

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