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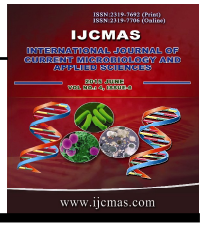

Original Research Article

A Strategy to Improve Efficiency of Hydrocarbon Degrading Microbial Flora through Biostimulation Approach

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Keywords

Hydrocarbon, Microbial degradation, Nutrient Fertilizer, Oil Sludge

A B S T R A C T

As in present scenario of world where accidental oil leakage or refineries made oil sludge is a common problem, these anthropogenic tasks introduced a large quantity of hydrocarbons in soil caused a enhance level of carbon in the soil composition. Therefore C: N, C: P, C: K ratio of the soil becomes very elevated. For proper growth and development of both flora and microbial flora an appropriate ratio of these constituents are required. The use of nutrient fertilizers (NPK fertilizers) may possibly be enormous assist to restore this ratio. The point of present study is to explore the role of NPK fertilizers to enhance microbial degradation of petroleum hydrocarbons in soil. Soil samples were analyzed for hydrocarbon degrading bacterial population and physiochemical properties of soil. The outcome of study showed that the hydrocarbon – degrading bacterial count increased from 3.76×10^4 to 2.9×10^6 cfu /g soil. So the result indicates that the applied fertilizer increased the degradation of hydrocarbons compared with the control.

Introduction

The worth of life strongly influenced by its environment which is degraded by various pollutants including hydrocarbon contaminants. Hydrocarbon compounds such as petroleum are vital elements of life. As petroleum based products are the major source of energy for industry and daily life. Wide scale production, transport, use and disposal of petroleum globally has made it a major contaminant Rahman *et al.* (2002). Due to large scale damage in ecosystem by these hydrocarbon compounds scientists have conducted research to develop new strategies for mitigation of this problem. Among the technologies available to deal with contaminated soils, bioremediation based on metabolic activity of microorganisms has certain advantages. Micro-organisms are nature's novel recyclers, converting toxic organic compounds into harmless products.

However, the efficacy of this emerging technology depends on a range of factors. By previous studies it is acknowledged that hydrocarbon biodegradation in soil can be limited by many factors, such as microorganism type, nutrients, pH, temperature, moisture, oxygen, soil properties, and contaminant concentration (Bradi *et al.,* 2000; Semple *et al.,* 2001; Sabate *et al.,* 2004; Ghazali *et al.,* 2004;

Walter *et al.*, 2005; Atlas and Bartha, 2006). One of the important factors is the bacterial quantity with catabolic activity in the polluted soil as these microbes are the key players of biodegradation. There are many other limiting factors which affect indirectly this process by providing appropriate conditions to microbial flora, as nutrient concentration. For global implementation of bioremediation approach, it is must to judge these factors for improvements (Jain *et al.*, 2011).

Nutrients are very significant ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and potassium (Cooney, 1984). According to Atlas oil spills cause increased supply of carbon in the environment and the accessibility of nitrogen, phosphorus and potassium became the limiting factor for oil degradation.

Under biostimulation plan, by adding nutrients (NPK) to soil to restore the appropriate ratio of these constituents, most appropriate conditions can be made for indigenous microbial flora. Thus by improving bacterial number in contaminated soil, enhanced hydrocarbon catabolism can be achieved.

Materials and Methods

Requirements

NPK fertilizers, pre- weighed soil samples, sterile distilled water etc.

Procedure

Commercial NPK fertilizer (19:19:19) was obtained from the local agriculture market. Different concentrations (10, 20, 40g /100g soil) of fertilizer were mixed in water (equal to 33% of the total WHC of respective soil samples). Water dissolved fertilizers were then added to the soil samples. The soil samples were than tested for their physiochemical and biological characteristics like total heterotrophic bacterial population (THBP) and petroleum degrading bacterial population (PDBP), soil respiration, carbon mineralized, soil pH, soil bicarbonate, chloride, etc.

Total heterotrophic and petroleum degrading bacterial population

Estimation of bacterial population (Benson, 1998)

To enumerate the viable bacterial count, serial dilution method is preferred. Petroleum degrading bacterial population in soil samples was counted by standard spread plate method with serial dilutions of oil sludge samples and defined selective growth medium (Minimum Salt Medium -) containing hydrocarbons as the sole source of carbon. Only those bacteria which can utilize oil constituents can grow in this medium.

The microbial status of soil samples was observed by enumerating the Total Heterotrophic counts and Petroleum degrading populations. These results were counterchecked by Soil Respiration experiment.

Colonies/g of soil at a particular dilution =

$$\frac{\text{Average no. of colonies} \times \text{dilution factor}}{\text{Volume of dilution added to plates}}$$

Total colonies/ g of soil sample =

$$\frac{\text{Total no. of colonies at every dilution}}{\text{Total no of dilutions}}$$

Soil respiration (Dubey and Maheshwari, 2004)

Soil contains a variety of microorganism. The live microorganisms respire and evolve CO_2 from soil which can be measured and assessed as an index of microbial activity of the soil.

Results and Discussion

This study reports the effects of inorganic fertilizer (NPK agricultural fertilizer) on the microbial exploitation of petroleum hydrocarbon on contaminated soil. Different concentrations of NPK fertilizer were added to the polluted soil samples in order to determine the nutrient ratio that gives the best performance for remediation purposes.

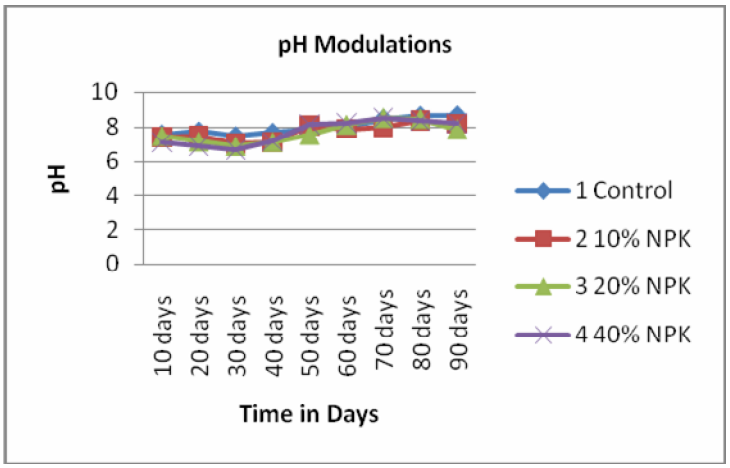
Effect of amendment of different concentrations of NPK fertilizers on pH, Bicarbonate, Chloride, Total Heterotrophic Bacterial Population (THBP) and Petroleum Degrading Bacterial Population (PDBP) were observed after every 10 days of incubation for 90 days. After the addition of fertilizers, pH of all soil samples was fluctuated during the whole experimental time. Initially pH of the sample was noticed decreased (towards neutrality) as compare to control soil, but with the time, soil became more alkaline.

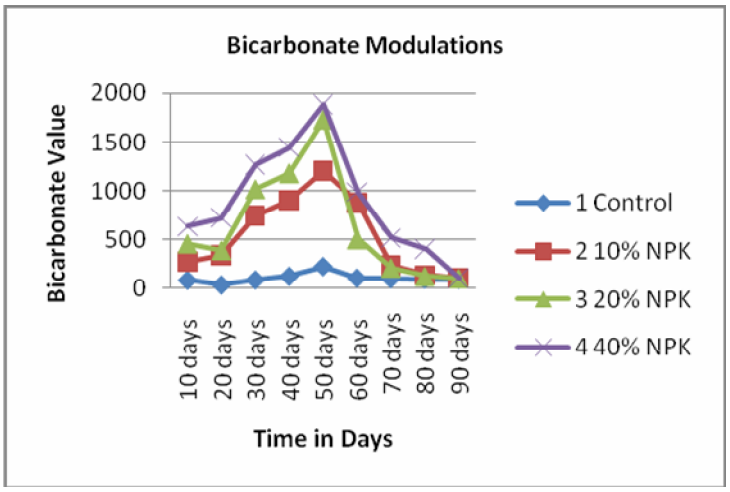
The important effect of NPK fertilizer on chloride and bicarbonates content in sample soil

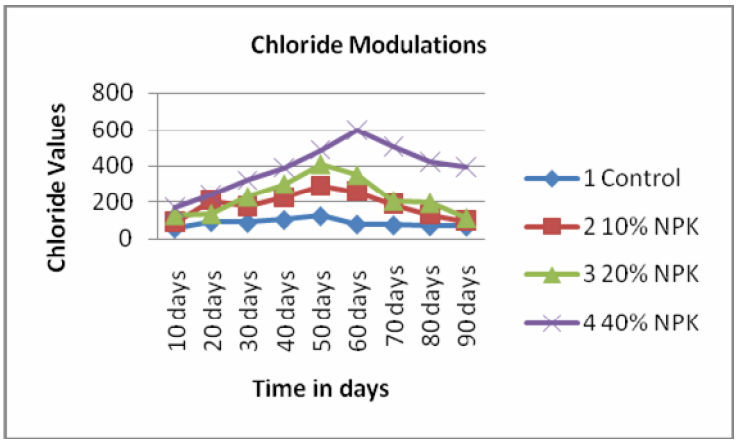
As increase the concentration of NPK and incubation time increase the chloride and bicarbonate content in soil. The maximum chloride and bicarbonate content at 40g/100g concentration and 40 and 50 days of incubation period.

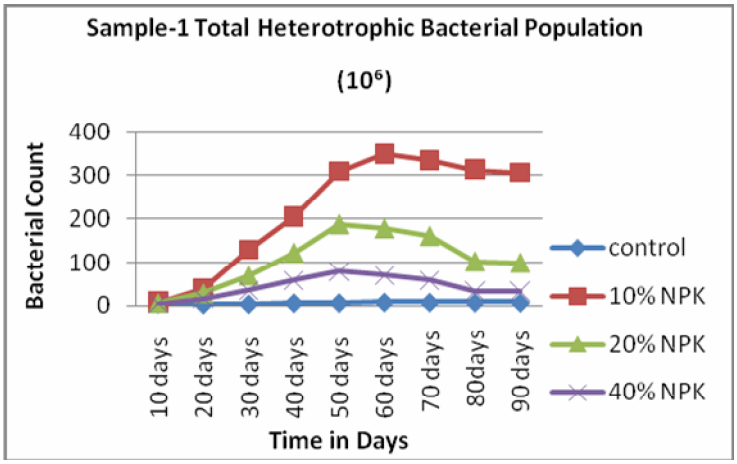
Inorganic nutrient amendments have been found to enhance hydrocarbon biodegradation rates. In present study addition of NPK fertilizers significantly enhanced the biodegradation process. Both total heterotrophic counts and petroleum degrading bacterial population increased. The increase in the bacterial counts was proportional to the concentration of NPK added. The maximum bacterial density was observed at NPK concentration of 10g/100g soil and significantly increases in CO_2 evolution and carbon mineralization also observed. So that optimum concentration of fertilizer is beneficial to increase the bacterial population and help the ingenuous microbes for degrading hydrocarbon.

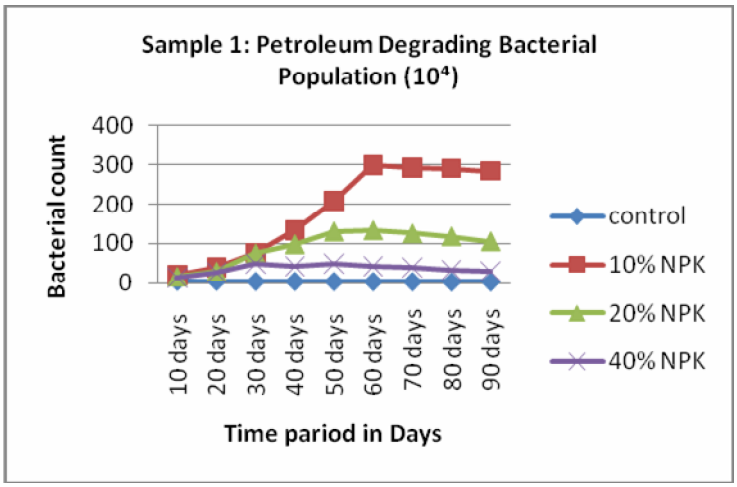
Effect of NPK fertilizers on soil properties

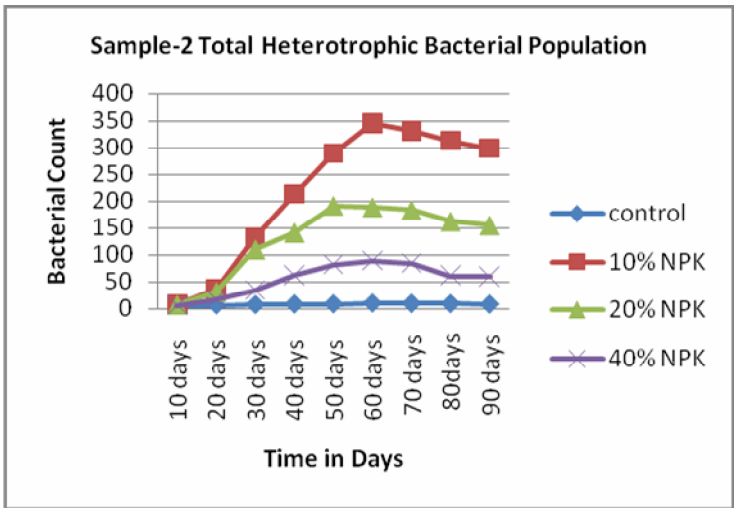


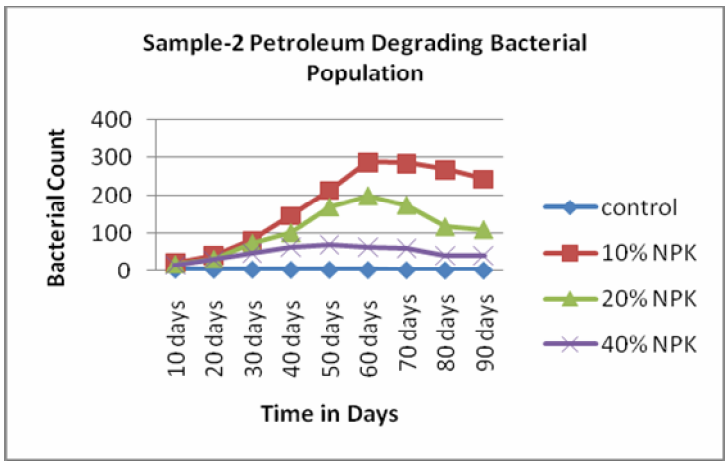


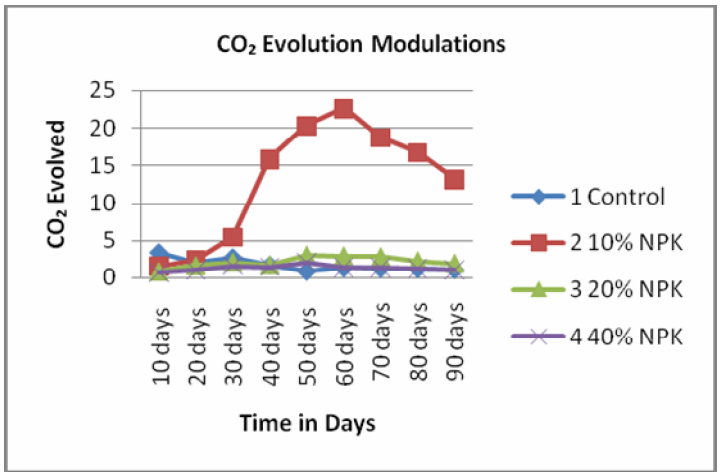


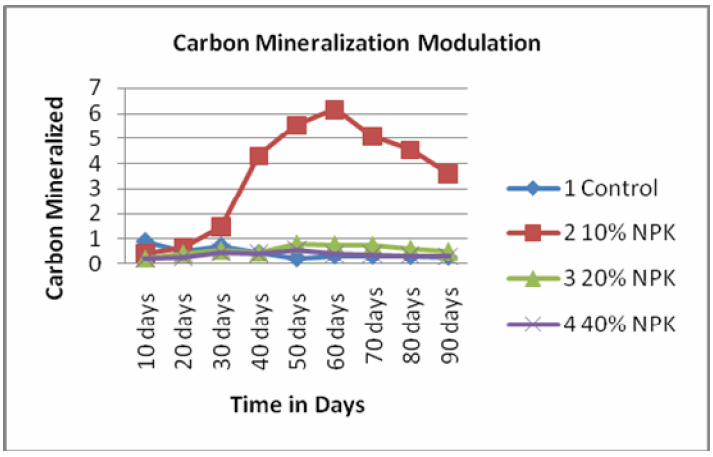

Sample-1 Total Heterotrophic Bacterial Population
(10⁶)
Bacterial Count
400
300
200
100
0
10 days
20 days
30 days
40 days
50 days
60 days
70 days
80days
90 days
Time in Days
control
10% NPK
20% NPK
40% NPK


Sample 1: Petroleum Degrading Bacterial Population (10⁴)
Bacterial count
400
300
200
100
0
10 days
20 days
30 days
40 days
50 days
60 days
70 days
80 days
90 days
Time pariod in Days
control
10% NPK
20% NPK
40% NPK


Sample-2 Total Heterotrophic Bacterial Population
Bacterial Count
400
350
300
250
200
150
100
50
0
10 days
20 days
30 days
40 days
50 days
60 days
70 days
80days
90 days
Time in Days
control
10% NPK
20% NPK
40% NPK







The enhanced carbon in mineralization and co_2 evolution confirmed the increase in bacterial population. Enhanced co_2 evolution is attributed to the release of co_2 by chemical decarboxylation of organic contaminant matters (Schinner *et al.*, 1996; Vashishtha Abhishek, 2009).

Nature has the incredible ability to heal itself, but it often takes a great deal of time for things to return to normal after the environment has been disrupted. Introduction of inorganic fertilizers recover soil's physiochemical parameters and construct optimal environment for bacterial population. As a result high concentrations of microbes within the contaminated area were achieved which help to accelerate the natural remedial process.

Therefore, based on the present study, it may be concluded that microbial degradation of petroleum hydrocarbons can be enhanced by the use of NPK fertilizers under the biostimulation strategy. This study supports the fact that nutrient supplementation enhances biodegradation and favors the use of 10g NPK agricultural fertilizer for soil quality similar to the one used for this study.

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