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RESEARCH ARTICLE

Microbial Degradation of Waste Petroleum Contained in Soil and Water

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Abstract

In this research we have known to efficiency of both bacteria and fungi to degradation of petroleum like as petrol, diesel and petro-diesel mix contained soil and water, the petroleum substance a major parts of environment pollution cause to degrade fertility of soil and formation of surfactant upper surface of water. Improved to degradation of petroleum substances by microbial has using some parameter such as pH, Absorbance, and growth rate of microbial to remediation of soil fertility and remove surfactants from water surface**.**

Introduction

The Environmental pollution containing more use less substances as a parts of petroleum as hydrocarbon, polyaromatic hydrocarbon (B. Maliszewska-Kordybach, 1999) where increased pollution by waste petroleum material to more come way from industries, automobile work shop, who are discharged on soil and water (Beckley I., *et al.*2011). That formation of surfactants on upper surface of the water(Hilton B., *et al.* 2011) and also effect on soil fertility continues decreased, Petroleum contaminated soil contains various hazardous materials such as aromatic hydrocarbons and polycyclic aromatic hydrocarbons; they are potentially toxic, mutagenic, and carcinogenic (Jelena S. M. *et al.* 200**9**), because sample soil has been pH 6.6±0.2. Where petroleum oil like as petrol has pH < 6.6±0.2 and also diesel has pH >6.6±0.2. This way more acidic or alkaline soil has not been suitable for good fertility (Obire, O., *et al.* 2002;Zulfiqar, *et al.* 2012).

In this research for purpose to remediation ofsoil fertility and surfactant remove from waterby using microbials like as *Agrobacterium tumefaciens*, *Trichodermavirens*have much suitable properties of degradation of petroleum substance both soil and water they microorganism utilize to petroleum contained hydrocarbons as a nutrient substrate (Kishore Das, *et al.* 2006), The petroleum mixed soil was sample collected from an oil contaminated site, microbes isolated by enrichment technique (Eduardo, *et al.* 2009), and the applied that petroleum in various concentration percentages 10%, 20%, 30%, 40% and 50% contain with media YEMA and SDA incubated to optimal condition. The metabolically active of both *Agrobacterium tumefaciens* and *Trichodermavirens* microbial played the key role in the poly aromatic hydrocarbon degradation (A.Mrozik, *et al.* 2003;Zhu *et al.*, 2004; Anthony, 2006), After that observation analysis growth rates measured of Microorganisms by colony counting, pH and absorbance by UV-vis spectrophotometer at 310nm, observation with petroleum and there control, compared petroleum substances degradation efficiency between Bacteria and Fungi Figure No 1-5.

Materials and Methodology

Collection of sample

SamplesPetrol, Diesel, and Petro-Diesel contain soil collected from local city area of Betul District and the petroleum mixed soil wassample collected from an oil contaminated site, microbes isolated by enrichment technique in Biotechnology Laboratory of VivekanandVigyanMahavidhyalaya, Betul, MP, India.

Isolation and Characterizations of Microbial

Take 1gm petroleum contained soil well dissolved in 10ml distilled water and transfer a loopfull solution on YEMA solidified Media (Manitol-10 gm, KH2PO4- 0.5gm, MgSO4 -0.2gm, NaCl- 0.1gm, CaCo3- 4.0gm, Yeast extract- 1.0gm, 1% Congo red, Agar-20gm, Distilled water-1000ml pH 6.8-7.0showed in Figure No. A) and incubated at 28˚C ±2 for5-7 days pink color colony appeared of Agrobacterium tumefaciens after that characterization by gram staining in showed Photograph No3. and take a loopfullsolution spread on SDA (Peptone -10 gm, Dextrose 40 gm, Agar -15 gm in 100ml distilled water with pH 5.6 showed in Figure No. A) incubated at 28˚C ±2 for 3-5 days, white light greenish morphology show inculture plate of fungi*Trichodermavirens (*Mala Mukherjee, *et al.* 2007*)*after that characterization by lacto-phenol-cotton-blue mounting the slide take observation under the microscopy at 40X showed in Photograph No.4.

Microbial Degradation of Petroleum Detectionby pH meter

Take seventh clean test tubes add 8ml distilled water and 1ml media in each, adjust the pH value 7.0. Add in first two 1ml of petrol, second two 1ml diesel and third two 1ml of mixture of diesel and petrol shack well to each tubes and showing pH values with petrol pH 6.60, diesel pH 7.25 and mixture of petro-diesel pH 7.18, Add microorganisms, Bacteria culture in first three tubes and Fungi culture in second three tubes now recheck pH at zero time, very little changes of pH.Incubate to both culture contained tubes in incubator for 24hour check the pH values with petrol pH increased, diesel pH decreased and mixture of petrodiesel pH decreased. final observed pH value of data average show that bacteria has more fast maintained pH 7.0 approx. compared to fungi

Microbial Degradation of Petroleum Detection by UV-Vis Spectrophotometer

Prepared two replicates of 9ml samples of microbial culture add 1ml petroleum substances mix well and one samples centrifuge at 5000rpm for remove to microbial from sample take supernatant (Wael S. *et al.* 2009) forabsorbance at 310nm and another sample has been incubated at 28˚C for 24 hoursaftersamples centrifuge at same above rpm, remove to microbial from sample take supernatant for absorbance at 310nm, that the concentration decreased compared with zero time and 24 hours. Bacterial containing samples concentration low compared with fungi samples.

Microbial Degradation of Petroleum Detection by direct on Petriplates

Preparation of Medias SDA and YEMAdivided into three replicates, contained with following percent's (10%, 20%, 30%, 40% and 50%) of petroleum like as petrol, diesel, and mix of both in media in showed Figure No. B,Applied 1 ml of the microbial culture in each plate and well mixed into 8 shaped.Incubated to plates at 28˚C ±2 and routinely observed after 24 hours to continue for 5days. Finally we have observed microbial growing into plates muchcompared to control plates.

Results and Discussion

Prepared samples into three replicates where adjust the pH value 7.0 when we contained petroleum like as diesel that the pH value are increased and petrol decreased the pH value when we applied the both mixture that time pH value are become to average of petrol and diesel. Final observed pH value of average showed that Bacteria has very fast maintained pH 7.0 approx. compared to Fungi's

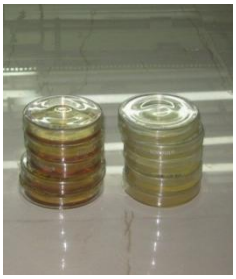
The petroleum substance has an optimum pH value when it containing to soil or other natural sources of water that changing to their pH values in acidic or alkali, where we present research investigation both petroleum materialhas approximates averages pH Values of three replicate samples as following Petrol (pH 6.60) and Diesel (pH 7.25) and there both mixture (pH 7.18). Collected soil in which contained petroleum substances there pH value as with petro-soil (pH6.70), Diesel-soil (pH 7.28), and Petro-Diesel-Soil (pH 7.17) this is effecting to soil fertility, when the applied microorganisms on petroleum contained soil that pH values remediated to as microbes-petrol (pH 6.92), microbes-diesel (pH 7.10) , and microbe-mixture (pH 6.94) remediation after 24 hours show in Figure No 5.

The present research investigation of petroleum substances degradation by using microorganisms Bacteria *Agrobacterium tumefeciens*And Fungi *Trichodermavirens,* Petroleum much degradation by bacteria growth with 20% petroleum at incubation periods 72 hours approximate numbers of colony 1760s presented show in Figure No2, while the fungi has maximum degradation by fungi growth with 40% petroleum at incubation periods 36 hours approximate cover the zone 22.0mm presented show in Figure No1 and measured the pH values contained with microorganisms at zero hour and after 24 hours, sample has highly maintained pH near to standard pH 7.0., UV-vis spectrophotometer improved of unknown to concentration of microbes contained petroleum samples at 320nm. Where bacteria contained samples at zero time has 3.820, after 24 hours to 0.480 concentration and fungi has zero time

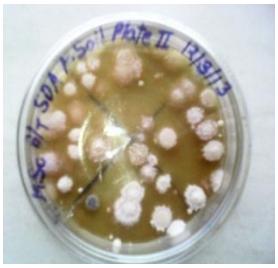
3.850, after 24 hours to 1.420 concentrations in culture media. When applied both bacteria and fungi with culture media that zero time 2.530, after 24 hours 2.270 less concentration compared to individual Microorganisms

Photograph No.1: Collected Samples Dilutions in conical flask 10gm soil in 100ml

Photograph No.2: Culture Medias SDA and YEMA

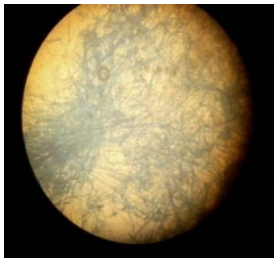


Photograph No.3: Culture Plates of SDAand YEMA

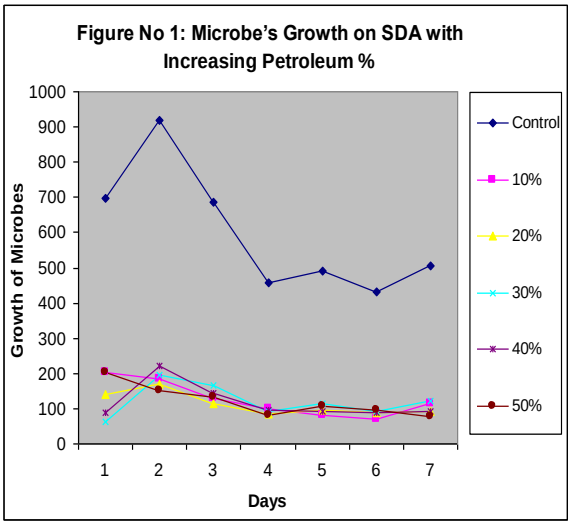


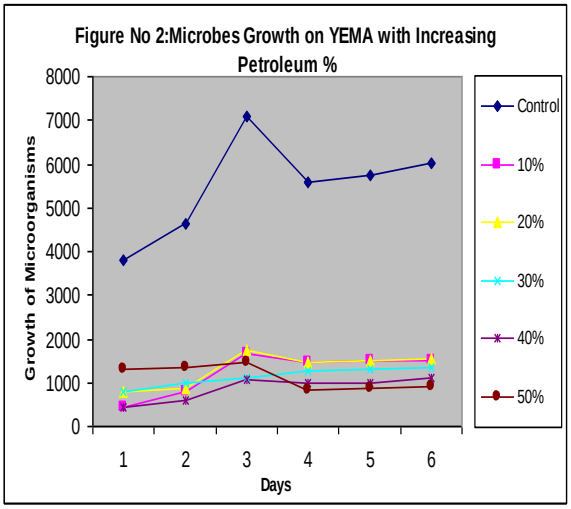
PhotographNo4: Culture of Fungi's with Petroleum substances (3days)

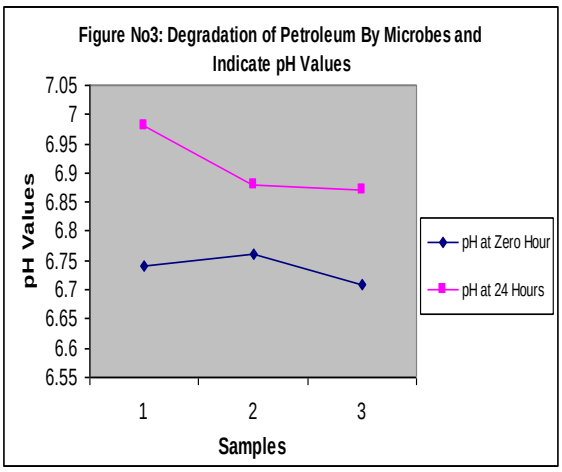
Photograph, No5: Culture of Agrobacterium (5 days)

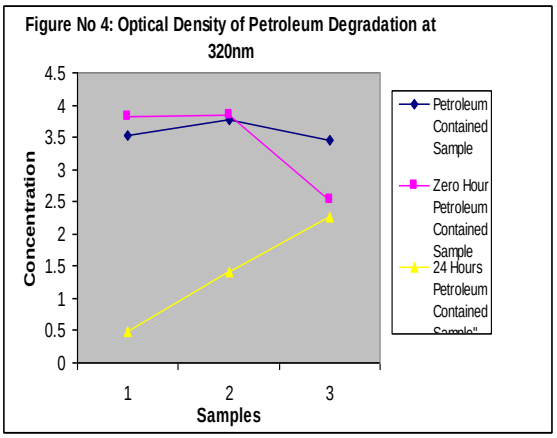


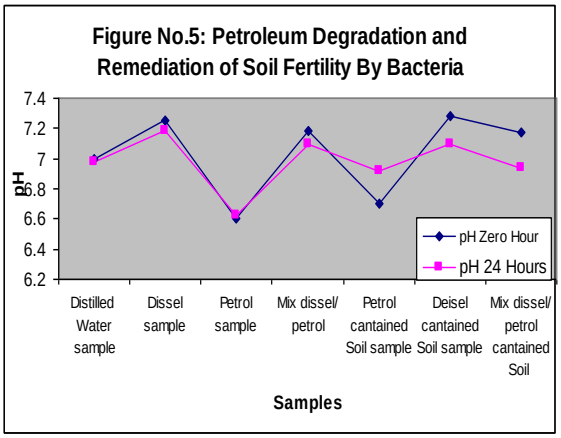
PhotographNo. 6: Microscopy view of Fungi at 40X











Conclusions

We have present research investigation that using to microorganisms like as Bacteria and Fungi play an important roles to degradation of petroleum substance from contained natural source soil and maintained to fertility of soil, we used various parameter in this research pH for determine the pH value of sources materials show in Figure No. 3 &5., UV-Vis spectrophotometer determine that concentration of sample increasing or decreasing by microorganisms show in Figure No. 4., and also individual effects of microbes to degradation of petroleum substances by broth media and solid media for Petri plate counting techniques to deterioration of microorganism on petroleum contained media show in Figure No. 1&2

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