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Research Article

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Effect of petroleum on seed germination in *Vigno radiata* and its degradation by *Pseudomonas fluorescens* and *Streptomyces* isolates

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ABSTRACT

Petroleum – degrading organisms has been isolated from a petroleum polluted site. Of the two isolates, the best degrader was selected on the basis of growth in liquid and soild media supplemented with petroleum as sole carbon source. The isolates were identified as a Pseudomonas fluorescens and *Streptomyces were further studied in batch culture for its petroleum degradation potential under aerobic condition and was observed for seven daya, where loss in petroleum constituent was up to 75%. Germination and growth of Vigno radiata was significantly enhanced in presence of the degrading microorganisms.*

Keywords: Pseudomonas fluorescens, Streptomyce, Vigno radiate, Seed germination, Biodegradation

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INTRODUCTION

Biodegradation is the natures way of recycling wastes or breaking down the organic matter into nutrients that can be carried out by various microorganisms. Soil pollution by petroleum products is also a wide spread petroleum and mineral oil hydrocarbons are the most frequently occurring environmental contaminants[1]. Biodegradation of petroleum hydrocarbon in oil and other minimal media has been reported by various scientist[2]. Oil degradation of subsurface does not require oxygen, it does required certain essential nutrients (Eg; nitrogen, phosphorus, potassium), which can be provided by dissolution of minerals in the water lake. The possibility of employing microorganisms for the degradation of petroleum and it derivatives in minimizing contamination due to oil leaks and spills has prompted a number of investigators to study the process in the laboratory [3].

Many bacterial species are involved in the degradation of petroleum hydrocarbon such as *Pseudomonas, Aeromonas, Moraxella, Corynebacteria, Streptomyces, Arthrobacter, Bacillus, Flavobacterium, Rhodococcus* and fungal species such as *Oomycetes, Zygomycetes, Basidomycota,* and microalgae such as *Porphyridium, Petalonia, Diatoms, Chlorella, Volvox, Chlamydomonas* [4].

Crude petroleum is made up of an integral mixed compounds of hydrocarbon and refined petroleum, the actual effective pollutant is a simpler mix of fewer components.Petroleum is a complicated mixture of several simple hydrocarbons showing again transportation by motor vehicles is the leading cause of this category being emitted into the air. Their evaporation during the refining of petroleum is also important. Chemotaxis towards pollutants by way of transport system. Protein is another important feature of *Pseudomonas* and other biodegrading bacteria. Another

important property of *Streptomyces* species capable of degrading organic solvents is the development of tolerance against them by alteration in the composition of their cytoplasmic and outer membranes/cell surface protein.

Keeping all these information, the present study was undertaken with the isolation of organisms from petroleum contaminated soil and their petroleum degrading ability and checking the impact of petroleum on seed germination and growth of *Vigno radiata.*

EXPERIMENTAL SECTION

Collection and processing of soil sample

100g of soil samples were collected from petroleum oil polluted soil at an auto mobile workshop in, Thiruvarur, Tamil Nadu, India. After sample collection, serial dilution was performed for isolating microbial growth from the collected of samples. The nutrient agar medium plates were inoculated with 10^5,10^6, 10^7 dilution for organisms and incubated at 37^0C for 24 hrs. Isolated organisms were identified based on morphological and biochemical characteristics [5].

Screening of petroleum degradation

The Starch agar medium was used for the assessment of petroleum degrading activity. The isolates were streaked on the starch agar medium and incubated at 37^0C for 48 hrs. After incubation, the colonies were observed for the clear zone.

Growth efficiency in petroleum as carbon source in liquid medium

Inoculum for *P.fluorescens* and *Streptomyces* were prepared as above, cells were inoculated into 250ml Erlenmeyar flask. Then 50ml of sterile nutrient broth with 2% petroleum was added. Immediately initial OD to obtain approximately10^7 cells/ml and then incubated at 37^0C under shaker condition at 120rpm for 7 days with one control. Control containing 50ml of medium with 2ml petroleum.

The growth parameters studied include cell turbidity was measured by determining the optical density at 540nm using colorimeter. Protein content of the cell biomass in the medium determined by Lowry's method [6] .

Biodegradation of petroleum by isolates

2ml culture suspension from the stock was added to a 250ml Erlenmeyar flask containing 100ml of nutrient broth and petroleum oil (2%) cells were allowed to grow for 7 days at 120rpm for 30^0C and then washed thrice in physiological saline. The final suspension was prepared in 10ml of nutrient broth to yield a cell concentration which was used as inoculums. Sampling was done on the first and seventh day to determine the amount of the initial and final concentration of petroleum and its hydrocarbon.

Gravimetric analysis of degradation

Petroleum extraction from test flask

100ml of culture broth containing petroleum was extremely difficult to filter the xylene water interphase through anhydrous Na_2So_4. Moreover significant loss of oil would be expected during this process. Thus, to enhance the efficiency of extraction the modified process was followed by medium with cell and the petroleum was centrifuged at the speed of 6000rpm for 15min the pellet was collected. The petroleum oil entrapped and sorbed on the biomass pellet was extracted by adding 2ml of xylene into flask. The procedure was repeated twice. The reduction in amount of petroleum due to microbial action and a biotic losses were determined.

Petroleum extraction from control flask

100ml of the flask medium was acidified with 0.1 to 0.2ml of concentrated Hcl was added and pH 2. 10ml xylene was added and then flask was placed on shaker at 120rpm for 20 min to enhance the mass transfer rate of petroleum from H_2O to xylene. This solution was then transferred in separating funnel, after mixed well and then aqueous xylene phase were allowed to separate. The lower layer of H_2O was drained back into the conical flask and the extraction was repeated with 10ml of solvent. The combined solvent extract was drained through a funnel containing 1g anhydrous Na_2So_4 in an ordinary filter paper, then completely evaporate the xylene.

Standardizing time required for evaporation of xylene
Removing xylene by evaporation in a heated oven is critical. To determine the time for complete evaporation of xylene, two beakers of 50ml capacity were weighed individually. In the first beaker, 20ml of pure xylene was taken while in other 20ml of xylene along with 1ml petroleum was taken and both was placed in an oven set at a constant temperature of 72ºC. The weight of both beakers was measured overtime until there be no further in weight. At 72ºC, 20ml of xylene is evaporated in 126min.

P.fluorescens
Effect of inoculum on germination of *Vigno radiata* in the presence of petroleum
Effects of petroleum on the growth of plant were analyzed by pot culture experiment. The following treatment was made for this study.

- Control – (sterile soil + seeds)
- Effect of petroleum –(soil + petroleum + seed)
- Degradation ability of microbes-(sterilesoil+ petroleum+ *P.fluorescens*+ seeds)
- Effect of microbes- (sterile soil + *P.fluorescens* + seeds)

RESULTS AND DISCUSSION

In this study, petroleum degrading microorganisms were isolated from the petroleum contaminated soil sample. The isolates were identified and used for biodegradation of petroleum by various methods.

Identification of Microorganisms
The isolated organisms was identified as a gram negative, motile, rod shaped organisms using Gram's staining and hanging drop techniques. The isolates showed to the biochemical test, the isolated organisms were identified as *Pseudomonas fluorescens* and *Streptomyces* and also has been screened for the petroleum degrading organisms.

Growth efficiency in petroleum as carbon source in liquid medium
The growth parameters were studied include cell turbidity by determining the optical density at 540nm using colorimeter and Protein content of the cell biomass in the medium determined by Lowry's method [7]. Cell biomass concentration was determined by optical density using colorimeter. A liquid medium (50ml) with 2% concentration petroleum was prepared and 0.1ml of *P.fluorescens* and *Streptomyces* was inoculated respectively. The maximum growth of *P.fluorescens* was observed (0.14) than Streptomyces (0.11). After 7 days of incubation, the optical density was noted for *P.fluorescens* (0.89) than *Streptomyces* (0.50) and Protein content was estimated in *P.fluorescens* (3.40 ± 0.9) and *Streptomyces* (2.12 ± 0.72) by using colorimeter at 680 nm [Tale-1].

Gravimetric Analysis of Petroleum Degradation
A fungal strain which showed high potentiality to adsorb and degrade crude petroleum oil has been isolated. Nutritional and environmental factor affecting petroleum degradation have been evaluated by applying Placketl – Burman design, where K_2 HPO_4, inoculum size and pH were most significant variables. The degradation of hydrocarbon was achieved at 5% oil concentration and proved a maximum petroleum oil degradation of 98.8% [8]. Petroleum degrading ability was measured by gravimetric analysis. Flask containing liquid medium of petroleum, 0.1ml of *P.fluorescens* and *Streptomyces* culture. After the incubation, petrol was extracted by using xylene. The petroleum containing xylene was evaporated using oven. Petroleum degrading ability in control (2ml), *P.fluorescens* (0.7ml) and *Streptomyces* (1ml) [Table-2].

Pot Culture Experiment
Ten pots of oil contaminated soil was prepared with tomato plants of *Pseudomonas putida*, to test on microbial community at various time intervals. The hypothesis that the *Pseudomonas putida* promotes the rhizosphere bioremediation of oil contaminated soil by increasing the composition of the microbial community. The symbiotic relationship between the soil microbes and in *Pseudomonas* spp., may be responsible for the degradation of oil contaminates. The Alfalfa sample with *Pseudomonas putida* worked best at removing hydrocarbon from the soil [9]. Seed germinations were observed in pot culture, the petroleum treated seed showed only 55% germination and petroleum along with *P.fluorescens* showed considerable raising seed germination of 75% when compared with control. Seeds treated with *P.fluorescens* were seen equal germination of 100% with control. Shoot length and leaf length analysis showed that 50% reduced level in petroleum treated seeds and 75% in petroleum along with

microbes treated seeds and there are slightly variation in microbes compared with control. The values of seed germination, shoot length and leaf length were tabulated [Table-3].

Table-1: Protein content of the biomass grown in presence of petroleum

Test organisms	Concentration of protein (µg)
Pseudomonas fluorescens Streptomyces	3.40 ± 0.90 2.12 ± 0.72

Values are expressed Mean ± Standard deviation

Table-2: Degradation of petroleum

Organisms	1st day in petroleum concentration (ml) (initial conc)	7th day in petroleum concentration (ml) (final conc)
Control	2	2
Pseudomonas fluorescens	2	0.7
Streptomyces	2	1

Table-3: Germination and growth of *Vigno radiata* after 7 days

Growth ability	Control	Petroleum + seeds	Petroleum +Seed+ *P. fluorescens*	*P.fluorescens* + seeds
Germination(%)	93	55	75	100
Shoot length	5.7 ± 0.76	2.8 ± 0.57	2.9 ± 0.70	5.9 ± 1.6
Leaf length	4.8 ± 0.37	2.3 ± 0.17	2.8 ± 0.5	3.9 ± 1.0

Values are expressed Mean ± Standard deviation

CONCLUSION

Finally concluded that petroleum contaminated soil were treated by using *Pseudomonas fluorescens*, it give a better yield for cultivation process. Hence, the petroleum contaminated soil was degraded by *Pseudomonas fluorescens* , then the soil was reused for cultivation process.

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